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Venous Thrombosis and Cancer: from Mouse Models to Clinical Trials

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Abstract

Cancer patients have a ~4 fold increased risk of venous thromboembolism (VTE) compared with the general population and this is associated with significant morbidity and mortality. This review summarizes our current knowledge of VTE and cancer from mouse models to clinical studies. Notably, risk of VTE varies depending on the type and stage of cancer. For instance, pancreatic and brain cancer patients have a higher risk of VTE than breast and prostate cancer patients. Moreover, patients with metastatic disease have a higher risk than those with localized tumors. Tumor-derived procoagulant factors and growth factors may directly and indirectly enhance VTE. For example, increased levels of circulating tumor-derived, tissue factor-positive microvesicles may trigger VTE. In a mouse model of ovarian cancer, tumor-derived IL-6 and hepatic thrombopoietin has been linked to increased platelet production and thrombosis. In addition, mouse models of mammary and lung cancer showed that tumor-derived granulocyte colony-stimulating factor causes neutrophilia and activation of neutrophils. Activated neutrophils can release neutrophil extracellular traps (NETs) that enhance thrombosis. Cell-free DNA in the blood derived from cancer cells, NETs and treatment with cytotoxic drugs can activate the clotting cascade. These studies suggest that there are multiple mechanisms for VTE in patients with different types of cancer. Preventing and treating VTE in cancer patients is challenging; the current recommendations are to use low molecular weight heparin. Understanding the underlying mechanisms may allow the development of new therapies to safely prevent VTE in cancer patients.

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Keywords

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Introduction

Venous thromboembolism (VTE) causes significant morbidity and mortality in cancer patients and the incidence has increased in the past 2 decades [1]. Various studies have reported the absolute risk (cumulative incidence) of VTE in cancer patients to be 1-8% [2]. It is estimated that cancer patients have a 4 to 7-fold increased risk of VTE compared with the general population [2, 3]. VTE also reduce the quality of life of cancer patients and potentially interrupt or delay anti-cancer treatments.

The association between cancer and thrombosis was first described in 1823 by Jean-Baptiste Bouillard [4]. Later in 1865 a French physician, Armand Trousseau, reported the association between gastric cancer and thrombosis [5]. However, it was not until 1935 that thrombosis was reported as a first sign of an occult cancer [6]. Current estimates are that 20% to 30% of VTEs are associated with cancer [2]. Hospitalized cancer patients with acute medical illness or immobility have an increased risk of VTE and receive thromboprophylaxis. In ambulatory cancer patients, the overall risk of VTE is lower and no routine thromboprophylaxis is recommended. Two recent clinical trials reported the rate of VTE in ambulatory patients with solid tumors and receiving chemotherapy was 3.4 and 3.9% [7, 8]. This rate is too low to consider routine thromboprophylaxis. However, the risk of VTE is increased with certain types of cancer and these patients might benefit from thromboprophylaxis [2, 9]. For instance, pancreatic (5.3-26.0%) and brain (1.6-26.0%) cancer have the highest rates of VTE, whereas breast (0.4-8.1%) and prostate (0.5-1.4%) cancer have the lowest rates of VTE [2, 10]. In addition, patients with metastatic cancer have higher rates of VTE than patients with localized cancer [2, 9]. One recent study observed a 12.6% rate of VTE (ranging from 8.2% in bladder cancer to 19.2% in pancreatic cancer) in high-risk ambulatory cancer patients (lung, ovary, pancreatic and gastric) 3-12 months after chemotherapy versus 1.4% in non-cancer patients [11].

Cancer patients with VTE have reduced survival compared with patients without VTE [12-14]. One study by Khorana and colleagues has the provocative title “thromboembolism is a leading cause of death in cancer patients receiving outpatient chemotherapy” [15]. Indeed, many papers use this study to support the statement that thrombosis is the second leading cause of death in cancer patients. This prospective observational study analyzed 4466 cancer patients of which 141 (3.2%) died. As expected the leading cause of death was the cancer itself (70.9%) followed by thrombosis (9.2%) and infection (9.2%). Only 5 of the 13 deaths attributed to thrombosis were VTE. However, this study may underestimate VTE in cancer patients. In a Swedish autopsy study of in-hospital deaths involving 23796 between 1970 and 1982 23% of the patients had pulmonary embolism (PE) and 10% of the population had a fatal PE [16]. Strikingly, 42% of the pancreatic cancer patients had PE [16]. This study indicates a high burden of VTE in cancer patients.

There are many factors that likely contribute to VTE in cancer patients, such as on tumor characteristics, treatment and patient characteristics (Figure 1). The presence of the tumor may affect the host coagulation system [17]. Anti-cancer treatments also increase the risk of VTE in cancer patients (Figure 1). For instance, surgery increased the risk of VTE in cancer patients 2-fold compared with non-cancer patients [18]. Chemotherapy treatment of cancer patients has been estimated to further increase the risk of VTE to 6.5-fold compared with the general population [2, 19, 20]. The risk of VTE in cancer patients would also be expected to be influenced by patient characteristics, such as history of VTE and age (Figure 1). In addition, cancer patients with prothrombotic variants, such as factor V Leiden and prothrombin G20210, or deficiencies in antithrombin, protein C or protein S, would be expected to increase the propensity to develop VTE [21]. Some cancers may increase the number of platelets or leukocytes that will increase the risk of VTE. This may explain the different rates of VTE associated with different cancers.

This review will summarize the multiple approaches that have been taken to study VTE and cancer. They range from basic science studies using mouse models to clinical trials with cancer patients and have elucidated a variety of pathways that may contribute to VTE in cancer patients (Figure 2). Importantly, information from the different sources needs to be integrated into a comprehensive picture of cancer-associated VTE.

Biomarkers of a hypercoagulable state in cancer patients

There are a number of biomarkers that have been reported in cancer-associated VTE [22]. We will initially discuss biomarkers of a hypercoagulable state [23, 24]. Activation of the coagulation system in cancer patients means that any insult that triggers clotting is more likely to result in pathological thrombosis. Biomarkers of a hypercoagulable state include thrombin-antithombin complexes (TATc), prothrombin fragment 1 + 2 (F1 + 2) and factor VIII. In addition, D-dimer is a fibrin degradation product that is used as a biomarker of ongoing activation of coagulation. The Pabinger group has measured many different biomarkers of a hypercoagulable state in patients with different types of cancer enrolled in the Vienna Cancer and Thrombosis Study (CATS) to determine which ones can be used to predict VTE [25].

D-dimer is probably the most robust measure of a hypercoagulable state and is used clinically in the work-up of a suspected VTE. One problem with D-dimer is that the specificity is decreased in older individuals and there are a number of different commercial assays with varying sensitivities and specificities [26]. Kodama and colleagues measured plasma D-dimer in 267 gynecologic cancer patients 3 days after surgery and found that the median value of D-dimer for VTE-positive patients (7.1 μg/mL) was significantly higher than that for VTE-negative patients (4.7 μg/mL) ($P = 0.009$) [27]. Stender and colleagues measured the preoperative plasma D-dimer level of 176 colorectal cancer patients and found that patients with a D-dimer >0.3 μg/mL (43% of the patients) had a significantly higher risk of deep vein thrombosis (DVT) than patients D-dimer <0.3 μg/mL ($P = 0.009$) [28]. In the Vienna CATS that analyzed many different types of cancers a D-dimer ≥1.44 μg/mL was associated with a higher incidence of VTE [29].

One study found that pre-surgery levels of TATc in the plasma of abdominal cancer patients undergoing surgery correlated with postoperative DVT [30]. In the Vienna CATS, an elevated level of F1 + 2 was an independent risk factor for VTE in cancer patients (>358 pmol/L) [29]. Furthermore, patients with elevated levels of both D-dimer and F1 + 2 had the highest hazard ratio for VTE [29]. FVIII activity was also identified as an independent risk factor for VTE in cancer patients [31]. During a 6 month follow up, 14% patients with elevated levels of FVIII activity had a VTE compared with 4% in those with normal FVIII activity ($P = 0.001$). These studies indicate that biomarkers of a hypercoagulable state are associated with VTE in patients with different types of cancer.

Tumor cell tissue factor and tumor-derived tissue factor-positive MVs

Many tumors express tissue factor (TF) and release TF^+ microvesicles (MVs) in the circulation (Figure 2) [32, 33]. MVs are small membrane vesicles that are derived from the plasma membrane. Tumors derived from epithelial tissue often express very high levels of TF, particularly pancreatic adenocarcinomas [34-36]. We found that mice bearing TF-expressing human pancreatic tumors but not TF-negative tumors had systemic activation of coagulation that was abolished by inhibition of tumor-derived human TF [37]. Consistent with the notion that tumor cell TF contributes to thrombosis, a high level of TF expression in biopsy samples from ovarian, pancreatic and brain cancer patients is associated with an increase in the rate of VTE [35, 38, 39]. However, a more recent study did not find that TF expression in brain tumors was associated with future VTE [39].

Cultured TF-expressing cancer cells release TF^+ MVs into the culture supernatant and TF-expressing tumors release TF^+ MVs into the blood [37, 40-42]. One study using the mouse pancreatic adenocarcinoma cell line Panc02 found that tumor-derived MVs accumulate at sites of vascular injury, and that tumor-bearing mice had reduced occlusion times in mesenteric vessels injured with ferric chloride [43]. We found that injection of TF^+ MVs from a human pancreatic cell line increased venous thrombosis in mice [37], and that this enhancement was abolished by inhibition of TF (Geddings and Mackman, unpublished data).

MV TF activity has been measured the plasma in a variety of cancer patients. These in-house assays should be viewed as semiquantitative. One general conclusion from these studies is that pancreatic cancer patients have the highest levels of MV TF activity [33]. For instance, one study measured levels of MV TF activity in pancreatic, brain, gastric and colorectal cancer patients because these cancers are associated with a high risk of VTE [44]. The highest levels of MV TF activity were observed in pancreatic cancer patients (mean value of MV TF activity: pancreatic 0.10 pg/mL; stomach 0.07 pg/mL; colorectal 0.05 pg/mL; brain 0.04 pg/mL). A second observation is that MV TF activity in pancreatic cancer patients is associated with VTE but not other types of cancers [33]. At present, it is unclear why the highest levels of MV TF activity are observed in pancreatic cancer patients, and that this activity correlated with VTE in pancreatic cancer patients but not other types of cancers. This may reflect a high level of TF expression in pancreatic tumors relative to other tumors, and that pancreatic cancer is often detected at a later stage than other cancers. Taken together, these studies suggest that MV TF plays a role in VTE in pancreatic cancer patients.

We speculate that TF^+ MVs may contribute to VTE in cancer patients via both direct and indirect pathways (Figure 3). For instance, TF^+ MVs may directly trigger VTE by binding to activated endothelium or activate other cells, such as platelets. Recently, we found that TF^+ MVs from two human pancreatic cell lines bind to platelets and activate them by generating thrombin (Geddings J and Mackman N, unpublished data). The notion that tumor-derived TF^+ MVs may activate platelets was proposed in a recent review [45]. Further studies are needed to determine the mechanisms by which tumor-derived TF^+ MVs enhance VTE in mouse models and in cancer patients.

Role of platelets in VTE in cancer patients

Platelets have been shown to play a role in cancer by increasing tumor growth, metastasis and thrombosis [46, 47]. Cancer patients often have low platelet counts due to the chemotherapy. However, in a subset of patients the presence of the tumor appears to increase the number of platelets. Khorana and colleagues were the first group to describe an association between a high platelet count and VTE in ~3000 ambulatory cancer patients beginning chemotherapy [48]. The rate of VTE in patients with platelet counts ≥350,000/μL (21.7% of the patients) measured prior to chemotherapy was 4% compared with 1.2% in patients with a platelet count <200,000/μL ($P = 0.0003$). Similarly, patients in the Vienna CATS with a high platelet count (>443,000/μL) (5% of the patients) had a 1 year cumulative rate of VTE of 34.3% compared with 5.9% in all other patients [49]. Finally, the Tromsø group measured platelet counts in people prior to the development of cancer and found that cancer patients with a platelet count greater than the 80^{th} percentile (>295,000/μL) had a 2-fold increased risk of VTE compared with cancer patients with platelet counts below the 40^{th} percentile (235,000/μL) [50]. This indicates that people with naturally high platelet counts that develop cancer have a higher risk of VTE. In a mouse study, it was reported that ovarian tumors induced thrombocytosis by releasing IL-6 that stimulates hepatic thrombopoietin expression [51]. Interestingly, increased levels of plasma von Willebrand factor have been reported in patients with ovarian, bladder, and colon cancers [52]. These studies suggest that platelets contribute to the pathophysiology of VTE in cancer patients.

Biomarkers of platelet activation

Several different groups have measured a variety of biomarkers of platelet activation in cancer patients [47]. These biomarkers include P-selectin, soluble CD40 ligand, platelet factor-4, thrombospondin-1 and β-thromboglobulin. Most of these biomarkers were found to be elevated in a variety of cancer patients [47]. However, few studies have determined if elevated levels of these platelet biomarkers predict VTE in cancer patients.

P-selectin is a transmembrane adhesion molecule expressed on activated platelets and endothelial cells [53]. In the Vienna CATS, an elevated level of soluble P-selectin (sP-selectin) was identified as a risk factor for VTE in cancer patients [54]. Patients with a high level of sP-selectin (> 53.1 ng/mL) had a 2.6-fold increase in risk of VTE compared with cancer patients with a low level of sP-selectin (< 53.1 ng/mL) ($P = 0.003$) [55]. Another study found that the odd ratio for the development of VTE was ~3 in pancreatic cancer patients with high platelet factor-4 levels [56]. A recent study measured a variety of platelet

activation markers in 1779 patients with different types of cancer [57]. The study confirmed that sP-selectin was associated with VTE in cancer patients but no association was found for soluble CD40 ligand, thrombospondin-1 and platelet factor-4 [57]. These differences could be due to the sensitivity of the different assays and/or that endothelial cells are a source of plasma sP-selectin in cancer patients. These studies provide further evidence suggesting that platelets are activated in cancer patients and likely contribute to VTE, although the relative risk for VTE associated with these markers is relatively low.

Role of leukocytes in VTE in cancer patients

Leukocyte counts are frequently elevated in cancer patients [58]. Khorana and colleagues first described a relationship between leukocytosis at the time of diagnosis and VTE in cancer patients [59]. They found a 2.2-fold increased risk of VTE in patients with a high leukocyte count ($>11 \times 10^9$ cells/L) compared with patients with a low leukocyte count. In the Vienna CATS, leukocyte count was also identified as an independent risk factor for thrombosis in cancer patients [60]. Leukocytosis is also associated with VTE in brain tumor patients [61]. Finally, a study by Blix and colleagues in Tromsø found that cancer patients with a high pre-cancer leukocyte count ($\geq 8.6 \times 10^9$ cells/L) (20% of the patients) had a 2.4-fold higher risk of VTE than those with a lower leukocyte count ($<6.4 \times 10^9$ cells/L) [62]. Leukocytosis is also related to poor prognosis in cancer patients [63]. Connolly and colleagues performed a multicenter observational study evaluating the relationship between leukocytosis, VTE and mortality in cancer patients starting chemotherapy. The mortality rates of patients with VTE only or leukocytosis only were 2.9% or 6.9%, respectively, whereas those with both leukocytosis and VTE had a mortality rate of 20% [63]. These studies indicate that cancer patients with a high leukocyte count due to either their genetics or the presence of the tumor have a higher risk for VTE.

Leukocytes have been shown to play a role in animal models of venous thrombosis but do not appear to be necessary for hemostasis [64]. Leukocytes activate the coagulation cascade as part of the innate immune response to infection to prevent the spread of a pathogen. However, unlike hemostasis that must occur rapidly, leukocyte-induced activation of coagulation may take several hours. For instance, the maximal levels of TF are observed in monocytes 6 hours after LPS stimulation. In venous thrombosis, leukocytes appear to be activated by host factors in the absence of a pathogen. Neutrophils are the most abundant white blood cell and levels increase during infection. Release of the neutrophil proteases elastase and cathepsin G has been shown to activate platelets by cleavage of Par4, and to activate the coagulation cascade by inactivation of the anticoagulant protein tissue factor pathway inhibitor [65]. In a mouse model of venous thrombosis both neutrophils and monocytes were recruited at the site of thrombosis and both cell types appeared to express TF [64]. Importantly, depletion of neutrophils reduced thrombosis in this model [64]. Taken together, these studies suggest that leukocytes actively contribute to VTE in cancer patients.

Neutrophil extracellular traps (NETs)

Highly activated neutrophils release neutrophil extracellular traps (NETs) via a process called NETosis [66, 67]. This process requires the enzyme peptidylarginine deiminase 4

(PAD4), which converts arginine residues to citrulline on the histone tail. Therefore, the best biomarker of NETs is hypercitrullinated histone H3. NETs are composed of extracellular DNA fibers, histones and granular proteins that have been shown to play a role in innate immunity by enhancing the proteolysis and killing of bacteria [68, 69].

Recently, it was reported that NETs enhance clot formation in vitro and in vivo [70-72]. NETs promoted erythrocyte-rich thrombus formation and platelet aggregation in vitro [70]. In addition, NETs contribute to venous thrombosis in a mouse model [71, 72]. Importantly, mice lacking the ability to make NETs due to a deficiency in PAD4 had smaller thrombi compared with controls [73]. Finally, NETs have been observed in human VTE [74].

Do NETs contribute to cancer-associated thrombosis? Demers and colleagues investigated the role of NETs in lung thrombosis in two mouse tumor models: 4T1 mammary tumors grown in BALB/c mice and Lewis lung carcinoma tumors grown in C57Bl/6 mice [75]. They found that both types of tumors were associated with an increase in the number of peripheral blood neutrophils, and that the neutrophils in tumor-bearing mice were more prone to NETosis. In the 4T1 model, tumor-derived granulocyte colony-stimulating factor (G-CSF) was responsible for the neutrophilia and sensitization of the neutrophils to NETosis [75]. There was also an increase in plasma DNA that appears to originate, in part, from activated neutrophils. Finally, hypercitrullinated histone H3 was detected in thrombi in the lungs of tumor-bearing mice. These results suggest that NETs contribute to thrombosis in mouse models of cancer (Figure 2) [76, 77]. Although high levels of cell-free DNA in the plasma of cancer patients has been well described for many years, the levels of NETs in cancer patients or its participation in coagulation have not been reported. Therefore, further studies are needed to define the role of neutrophils and NETs in VTE in cancer patients. One study concluded that “the current enthusiasm about NETs in cancer is not yet supported by clinical data” [60].

Cell-free DNA

Neutrophils are not the only source of cell-free DNA in plasma. Tumor cells release various nucleic acids, such as DNA, mRNA and microRNA, and cancer patients have an elevated level of cell-free DNA in their blood [78]. Levels of cell-free DNA in the blood also increase with tumor burden and malignancy. DNA and RNA have been reported to activate factor XII, although this remains somewhat controversial (Figure 2) [79]. In addition, administration of chemotherapy may increase levels of cell-free DNA in the blood. For instance, Swystun and colleagues found that administration of various chemotherapeutic agents to mice increased levels of cell-free DNA in the plasma and activated coagulation [80]. In addition, they detected elevated levels of cell-free DNA in breast cancer patients 24 hours after administration of chemotherapy. Although Swystun and colleagues showed that cell-free DNA induced thrombin generation in an FXIIa-dependent manner in vitro, no studies were performed to determine the role of FXII in the activation of coagulation in mice treated with chemotherapeutic agents [80]. These results suggest that cell-free DNA in the blood of cancer patients is a prothrombotic stimulus that contributes to VTE.

Risk assessment models

Risk assessment models have been developed to predict the risk of VTE in the general population. One of these models is the Padua Prediction Score, which was developed to determine which hospital inpatients would benefit from thromboprophylaxis [81]. Clinical indicators are given a different number of points depending on their relative importance in the prediction of future VTE. Importantly, active cancer ("patients with local or distant metastasis and/or in whom chemotherapy or radiotherapy had been performed in the previous 6 months") is considered a high risk factor. Khorana and colleagues developed a risk assessment model for predicting VTE in ambulatory cancer patients that is known as the "Khorana score" [59]. There are five clinically available factors that are assessed: site of cancer, erythropoiesis stimulating agents, platelet count, leukocyte count, and body mass index. Based on the different rates of VTE associated with different cancer types, the Khorana score designates pancreas and stomach as "very high risk", whereas lung, lymphoma, gynecologic cancers and genitourinary (excluding prostate) are designated "high risk". Breast and colorectal cancer are considered low risk and brain cancer was not included in the analysis. The score is used to divide patients into 3 risk categories for a predicted rate of VTE over a 2.5 month period: low (0 points) (0.3-0.8% VTE), intermediate (1-2 points) (1.8-2.0% VTE), and high (≥ 3 points) (6.7-7.1% VTE). It should be noted that the percentage of patients in the "high-risk" group was 10.9% and 12.6% for the validation and derivative cohorts, respectively. Interestingly, thrombocytosis is also included in a risk assessment model for VTE in medical inpatients [82].

Recently, Ay and colleagues extended the Khorana score by adding brain cancer to the very high risk cancer site and the biomarkers D-dimer and sP-selectin and called this the Vienna score [54]. Of note s-P-selectin is not available to most clinicians. A total of 819 cancer patients were analyzed for 6 months for VTE and divided into 4 categories based on their score: 0 points (1.5% VTE), 1 point (3.8% VTE), 2 points (9.6% VTE), ≥3 (17.7% VTE). Again, it should be noted that only 11.4% of the patients were at "high-risk". A risk assessment model has also been developed to assess the risk of recurrent VTE in cancer patients called the Ottawa score [83]. This model uses 4 patient characteristics that are proposed to increase the risk of VTE: female sex, history of VTE, cancer type and late stage. The rate of VTE in patients with a score of 1-3 was 13.8-17.5% compared with 3-5.6% for those with a score of 0. These risk assessment models are designed to identify ambulatory cancer patients at high risk for primary and recurrent VTE that would benefit from thromboprophylaxis.

Use of the Khorana score has been recommended by the American Society of Clinical Oncology to guide VTE prophylaxis in cancer patients [84, 85]. However, only ~10% of the ambulatory cancer patients receiving chemotherapy are categorized as "high-risk" using the Khorana score and within this group only ~7% of the patients develop VTE within the first 2.5 months. This may explain why clinical oncologists are not screening their patients for risk of VTE.

Prevention of VTE in cancer patients

Low molecular weight heparin

Low molecular weight heparins (LMWH) are a mainstay of anticoagulant therapy. In addition to their anticoagulant activity, they have been proposed to have anti-tumoral activity. This has led to several clinical trials that determined the effect of various LMWHs on survival of cancer patients. This topic has been recently reviewed by Franchini and Mannucci [86]. We will focus on those studies that investigating LMWH for prevention of VTE in cancer patients receiving chemotherapy as outpatients. The PROTECHT study enrolled 1150 ambulatory patients with lung, gastrointestinal, pancreatic, breast, ovarian and head and neck cancers. Patients were randomized to receive placebo (n = 779) or the LMWH nadroparin (n = 387) daily for 4 months. Nadroparin significantly reduced the rates of VTE from 3.9% to 2.0% but did not prolong survival [7]. The SAVE-ONCO study enrolled 3212 patients with locally advanced or metastatic cancer and randomized them to receive placebo (n = 1604) or the LMWH semuloparin (n = 1608) daily for a median duration of 3.5 months. Similar to the PROTECT study, semuloparin significantly reduced VTE from 3.4% to 1.2% (p = 0.001) but again did not affect survival [8]. Thromboprophylaxis with semuloparin did not increase major bleeding. In TOPIC I and TOPIC II patients with disseminated metastatic breast cancer or stage III/IV non-small-cell lung cancer received the LMWH certoparin daily for 6 months but this did not change the rate of VTE or survival [87]. One study randomized 123 patients with advanced pancreatic cancer to receive either placebo or the LMWH dalteparin for 3 months and found a striking reduction in VTE in the patients receiving dalteparin (23% versus 3.4%, p = 0.02) [88]. Despite the reduction in VTE dalteparin did no prolong survival of the pancreatic cancer patients, which is likely due to the fact that the study was underpowered to show an effect on survival [88]. Finally, PROSPECT-CONKO 004 was a prospective, randomized trial to assess the effect of the LMWH enoxaparin on VTE in advanced pancreatic cancer patients [89]. Enoxaparin reduced VTE from 14.5% (22/152 patients) to 5.0% (8/160 patients) with no increase in major bleeding or survival. It should be mentioned that higher doses of dalteparin and enoxaparin were used for primary thromboprophylaxis in the latter two studies. Taken together, these results indicate that LMWHs are effective in reducing VTE in cancer patients, particularly in high-risk cancer patients. Although these trials do not report an increase in bleeding in the cancer patients receiving LMWH this would be a concern in this group of patients [90]. VTE has been estimated to be the cause of death of ~3.3% of patients [15]. Therefore, it is not surprising that reducing VTE in cancer patients did not improve survival, although post mortem autopsy studies are needed to more reliably quantify VTE as an underlying cause of death.

Anti-platelet drugs

Traditionally, platelets have not been thought to play a major role in VTE and antiplatelet drugs are not used to prevent or treat VTE. However, recent basic and clinical studies have shown that platelets contribute to VTE. For instance, a meta-analysis of two large clinical trials showed that low dose aspirin significantly reduces recurrent VTE when administered after the completion of a 6-18 month period of oral anticoagulation with a vitamin K antagonist (VKA) [91]. In mouse models depletion of platelets or a deficiency of von

Willebrand factor significantly reduced the size of venous thrombi suggesting that platelets may contribute to VTE in patients [64, 92]. Two retrospective studies analyzed the effect of aspirin on VTE in breast and ovarian cancer patients and found no difference in breast cancer and a "marginally significant reduction" with ovarian cancer patients ($P = 0.053$) [93, 94]. However, low dose aspirin was found to be as effective as LMWH in reducing VTE in multiple myeloma patients treated with either thalidomide or lenalidomide [95, 96]. In a mouse model of pancreatic cancer inhibition of platelet activation prevented accumulation of tumor-derived MVs at the site of thrombosis [97]. We found that inhibition of platelet activation with clopidogrel abolished the ability of exogenous tumor-derived TF+ MVs to enhance venous thrombosis in mice (Geddings and Mackman, unpublished data). These data suggest that anti-platelet drugs may reduce VTE in cancer patients. A recent review on the role of platelets in cancer-associated thrombosis concluded that "while platelet inhibitory agents might be considered in the prevention of VTE, there is no obvious indication for platelet inhibition in the treatment of VTE" [47].

Statins

Statins have been proposed to reduce VTE. However, meta-analysis of 22 trials did not find a significant effect of statins on VTE in the general population [98]. Two studies have determined the effect of statins on VTE in cancer patients. The first analyzed retrospectively 740 cancer patients and found that 21% of the patients who were not on a statin had a VTE (n = 116) whereas only 8% of the patients taking statins had a VTE [99]. A less dramatic difference was observed in a prospective study that included 729 cancer patients; VTE was observed in 8.1% of the patients without statins versus 3.5% of the patients with statins [100]. It is unclear if the differences are large enough to warrant randomized clinical trials of statins for primary thromboprohylaxis in cancer patients.

Treatment of VTE in cancer patients.

Standard treatment of acute VTE event in the general population consists of initial treatment with fast acting LMWH together with a VKA. Once the VKA is in the therapeutic range, the LMWH can be stopped. Direct oral anticoagulant drugs (DOACs) that target either factor Xa or thrombin are fast acting and can be used without overlapping LMWH treatment. Lee and Peterson recently reviewed the treatment of VTE in cancer patients [101]. Interestingly, meta-analysis of open-label, randomized controlled trials showed improved efficacy of different LMWHs versus VKAs for the treatment and prevention of recurrent VTE in cancer patients. It is unclear why LMWHs are more effective than VKAs in preventing recurrent VTE in cancer patients but suggests that different thrombotic mechanisms may be in play. Analysis of the recent DOAC clinical trials showed that they were comparable to VKAs in preventing recurrent VTE [102]. However, randomized controlled trials are needed to determine the effectiveness and safety of DOACs compared with LMWHs in preventing recurrent VTE in cancer patients.

Conclusion

Cancer-associated thrombosis increases morbidity and mortality in patients. The occurrence of VTE is likely triggered by multiple pathways in different cancer patients. Further studies

are needed to determine the relative contributions of these different pathways in VTE associated with different cancer types. The National Heart, Lung and Blood Institute and the National Cancer Institute held a Cancer and Thrombosis Workshop in August 2014 to discuss cancer-associated thrombosis (http://www.nhlbi.nih.gov/research/reports/national-heart-lung-and-blood-institute-nhlbi-national-cancer-institute-nci-cancer-and-thrombosis). This joint initiative between two National Institutes of Health Institutes should stimulate new research into the mechanisms of cancer-associated thrombosis and the prevention and treatment of thrombosis in cancer patients. These and other studies may identify new targets that can be used to develop novel antithrombotic drugs that can be safely used in cancer patients.

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References

1. Stein PD, Beemath A, Meyers FA, Skaf E, Sanchez J, Olson RE. Incidence of venous thromboembolism in patients hospitalized with cancer. Am J Med. 2006; 119:60–8. [PubMed: 16431186]
2. Timp JF, Braekkan SK, Versteeg HH, Cannegieter SC. Epidemiology of cancer-associated venous thrombosis. Blood. 2013; 122:1712–23. [PubMed: 23908465]
3. Horsted F, West J, Grainge MJ. Risk of venous thromboembolism in patients with cancer: a systematic review and meta-analysis. PLoS Med. 2012; 9:e1001275. [PubMed: 22859911]
4. Bouillard JB, Bouillaud S. De l'Obliteration des veines et de son influence sur la formation des hydropisies partielles: consideration sur la hydropisies passive et general. Arch Gen Med. 1823; 1:188–204.
5. Trousseau A. Phlegmasia alba dolens. Clinique Medicale de l'Hotel-Dieu de Paris. 1865:654–712.
6. James TI, Matheson N. Thrombophlebitis in cancer. Practitioner. 1935; 134:683–84.
7. Agnelli G, Gussoni G, Bianchini C, Verso M, Mandala M, Cavanna L, Barni S, Labianca R, Buzzi F, Scambia G, Passalacqua R, Ricci S, Gasparini G, Lorusso V, Bonizzoni E, Tonato M, Investigators P. Nadroparin for the prevention of thromboembolic events in ambulatory patients with metastatic or locally advanced solid cancer receiving chemotherapy: a randomised, placebo-controlled, double-blind study. Lancet Oncol. 2009; 10:943–9. [PubMed: 19726226]
8. Agnelli G, George DJ, Kakkar AK, Fisher W, Lassen MR, Mismetti P, Mouret P, Chaudhari U, Lawson F, Turpie AG, Investigators S-O. Semuloparin for thromboprophylaxis in patients receiving chemotherapy for cancer. N Engl J Med. 2012; 366:601–9. [PubMed: 22335737]
9. Falanga A, Russo L, Verzeroli C. Mechanisms of thrombosis in cancer. Thromb Res. 2013; 131(Suppl 1):S59–62. [PubMed: 23452745]
10. Khorana AA, Connolly GC. Assessing risk of venous thromboembolism in the patient with cancer. J Clin Oncol. 2009; 27:4839–47. [PubMed: 19720906]
11. Khorana AA, Dalal M, Lin J, Connolly GC. Incidence and predictors of venous thromboembolism (VTE) among ambulatory high-risk cancer patients undergoing chemotherapy in the United States. Cancer. 2013; 119:648–55. [PubMed: 22893596]
12. Young A, Chapman O, Connor C, Poole C, Rose P, Kakkar AK. Thrombosis and cancer. Nat Rev Clin Oncol. 2012; 9:437–49. [PubMed: 22777060]
13. Sorensen HT, Mellemkjaer L, Olsen JH, Baron JA. Prognosis of cancers associated with venous thromboembolism. N Engl J Med. 2000; 343:1846–50. [PubMed: 11117976]

14. Chew HK, Wun T, Harvey D, Zhou H, White RH. Incidence of venous thromboembolism and its effect on survival among patients with common cancers. Arch Intern Med. 2006; 166:458–64. [PubMed: 16505267]
15. Khorana AA, Francis CW, Culakova E, Kuderer NM, Lyman GH. Thromboembolism is a leading cause of death in cancer patients receiving outpatient chemotherapy. J Thromb Haemost. 2007; 5:632–4. [PubMed: 17319909]
16. Ogren M, Bergqvist D, Wahlander K, Eriksson H, Sternby NH. Trousseau's syndrome - what is the evidence? A population-based autopsy study. Thromb Haemost. 2006; 95:541–5. [PubMed: 16525584]
17. Falanga A, Marchetti M, Russo L. The mechanisms of cancer-associated thrombosis. Thromb Res. 2015; 135(Suppl 1):S8–S11. [PubMed: 25903541]
18. Agnelli G, Bolis G, Capussotti L, Scarpa RM, Tonelli F, Bonizzoni E, Moia M, Parazzini F, Rossi R, Sonaglia F, Valarani B, Bianchini C, Gussoni G. A clinical outcome-based prospective study on venous thromboembolism after cancer surgery: the @RISTOS project. Ann Surg. 2006; 243:89–95. [PubMed: 16371741]
19. Edwards RL, Klaus M, Matthews E, McCullen C, Bona RD, Rickles FR. Heparin abolishes the chemotherapy-induced increase in plasma fibrinopeptide A levels. Am J Med. 1990; 89:25–8. [PubMed: 2368789]
20. Weitz IC, Israel VK, Waisman JR, Presant CA, Rochanda L, Liebman HA. Chemotherapy-induced activation of hemostasis: effect of a low molecular weight heparin (dalteparin sodium) on plasma markers of hemostatic activation. Thromb Haemost. 2002; 88:213–20. [PubMed: 12195692]
21. Pabinger I, Ay C, Dunkler D, Thaler J, Reitter EM, Marosi C, Zielinski C, Mannhalter C. Factor V Leiden mutation increases the risk for venous thromboembolism in cancer patients - results from the Vienna Cancer And Thrombosis Study (CATS). J Thromb Haemost. 2015; 13:17–22. [PubMed: 25381723]
22. Hanna DL, White RH, Wun T. Biomolecular markers of cancer-associated thromboembolism. Crit Rev Oncol Hematol. 2013; 88:19–29. [PubMed: 23522921]
23. Rickles FR, Falanga A. Molecular basis for the relationship between thrombosis and cancer. Thromb Res. 2001; 102:V215–24. [PubMed: 11516455]
24. Levi M. Cancer-related coagulopathies. Thromb Res. 2014; 133(Suppl 2):S70–5. [PubMed: 24862149]
25. Pabinger I, Thaler J, Ay C. Biomarkers for prediction of venous thromboembolism in cancer. Blood. 2013; 122:2011–8. [PubMed: 23908470]
26. Schouten HJ, Geersing GJ, Koek HL, Zuithoff NP, Janssen KJ, Douma RA, van Delden JJ, Moons KG, Reitsma JB. Diagnostic accuracy of conventional or age adjusted D-dimer cut-off values in older patients with suspected venous thromboembolism: systematic review and meta-analysis. BMJ. 2013; 346:f2492. [PubMed: 23645857]
27. Kodama J, Seki N, Masahiro S, Kusumoto T, Nakamura K, Hongo A, Hiramatsu Y. D-dimer level as a risk factor for postoperative venous thromboembolism in Japanese women with gynecologic cancer. Ann Oncol. 2010; 21:1651–6. [PubMed: 20129998]
28. Stender MT, Frokjaer JB, Larsen TB, Lundbye-Christensen S, Thorlacius-Ussing O. Preoperative plasma D-dimer is a predictor of postoperative deep venous thrombosis in colorectal cancer patients: a clinical, prospective cohort study with one-year follow-up. Dis Colon Rectum. 2009; 52:446–51. [PubMed: 19333044]
29. Ay C, Vormittag R, Dunkler D, Simanek R, Chiriac AL, Drach J, Quehenberger P, Wagner O, Zielinski C, Pabinger I. D-dimer and prothrombin fragment 1 + 2 predict venous thromboembolism in patients with cancer: results from the Vienna Cancer and Thrombosis Study. J Clin Oncol. 2009; 27:4124–9. [PubMed: 19636003]
30. Iversen LH, Okholm M, Thorlacius-Ussing O. Pre- and postoperative state of coagulation and fibrinolysis in plasma of patients with benign and malignant colorectal disease--a preliminary study. Thromb Haemost. 1996; 76:523–8. [PubMed: 8902990]
31. Vormittag R, Simanek R, Ay C, Dunkler D, Quehenberger P, Marosi C, Zielinski C, Pabinger I. High factor VIII levels independently predict venous thromboembolism in cancer patients: the

cancer and thrombosis study. Arterioscler Thromb Vasc Biol. 2009; 29:2176–81. [PubMed: 19778945]

32. Falanga A. Thrombophilia in cancer. Semin Thromb Hemost. 2005; 31:104–10. [PubMed: 15706482]
33. Geddings JE, Mackman N. Tumor-derived tissue factor-positive microparticles and venous thrombosis in cancer patients. Blood. 2013; 122:1873–80. [PubMed: 23798713]
34. Callander NS, Varki N, Rao LV. Immunohistochemical identification of tissue factor in solid tumors. Cancer. 1992; 70:1194–201. [PubMed: 1381270]
35. Khorana AA, Ahrendt SA, Ryan CK, Francis CW, Hruban RH, Hu YC, Hostetter G, Harvey J, Taubman MB. Tissue factor expression, angiogenesis, and thrombosis in pancreatic cancer. Clin Cancer Res. 2007; 13:2870–5. [PubMed: 17504985]
36. Kakkar AK, Lemoine NR, Scully MF, Tebbutt S, Williamson RC. Tissue factor expression correlates with histological grade in human pancreatic cancer. Br J Surg. 1995; 82:1101–4. [PubMed: 7648165]
37. Wang JG, Geddings JE, Aleman MM, Cardenas JC, Chantrathammachart P, Williams JC, Kirchhofer D, Bogdanov VY, Bach RR, Rak J, Church FC, Wolberg AS, Pawlinski R, Key NS, Yeh JJ, Mackman N. Tumor-derived tissue factor activates coagulation and enhances thrombosis in a mouse xenograft model of human pancreatic cancer. Blood. 2012; 119:5543–52. [PubMed: 22547577]
38. Uno K, Homma S, Satoh T, Nakanishi K, Abe D, Matsumoto K, Oki A, Tsunoda H, Yamaguchi I, Nagasawa T, Yoshikawa H, Aonuma K. Tissue factor expression as a possible determinant of thromboembolism in ovarian cancer. Br J Cancer. 2007; 96:290–5. [PubMed: 17211468]
39. Thaler J, Preusser M, Ay C, Kaider A, Marosi C, Zielinski C, Pabinger I, Hainfellner JA. Intratumoral tissue factor expression and risk of venous thromboembolism in brain tumor patients. Thromb Res. 2013; 131:162–5. [PubMed: 23084660]
40. Yu JL, Rak JW. Shedding of tissue factor (TF)-containing microparticles rather than alternatively spliced TF is the main source of TF activity released from human cancer cells. J Thromb Haemost. 2004; 2:2065–7. [PubMed: 15550054]
41. Yu JL, May L, Lhotak V, Shahrzad S, Shirasawa S, Weitz JI, Coomber BL, Mackman N, Rak JW. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. Blood. 2005; 105:1734–41. [PubMed: 15494427]
42. Davila M, Amirkhosravi A, Coll E, Desai H, Robles L, Colon J, Baker CH, Francis JL. Tissue factor-bearing microparticles derived from tumor cells: impact on coagulation activation. J Thromb Haemost. 2008; 6:1517–24. [PubMed: 18433463]
43. Thomas GM, Panicot-Dubois L, Lacroix R, Dignat-George F, Lombardo D, Dubois C. Cancer cell-derived microparticles bearing P-selectin glycoprotein ligand 1 accelerate thrombus formation in vivo. J Exp Med. 2009; 206:1913–27. [PubMed: 19667060]
44. Thaler J, Ay C, Mackman N, Bertina RM, Kaider A, Marosi C, Key NS, Barcel DA, Scheithauer W, Kornek G, Zielinski C, Pabinger I. Microparticle-associated tissue factor activity, venous thromboembolism and mortality in pancreatic, gastric, colorectal and brain cancer patients. J Thromb Haemost. 2012; 10:1363–70. [PubMed: 22520016]
45. van Es N, Sturk A, Middeldorp S, Nieuwland R. Effects of cancer on platelets. Semin Oncol. 2014; 41:311–8. [PubMed: 25023347]
46. Connolly GC, Phipps RP, Francis CW. Platelets and cancer-associated thrombosis. Semin Oncol. 2014; 41:302–10. [PubMed: 25023346]
47. Riedl J, Pabinger I, Ay C. Platelets in cancer and thrombosis. Hamostaseologie. 2014; 34:54–62. [PubMed: 24305775]
48. Khorana AA, Francis CW, Culakova E, Lyman GH. Risk factors for chemotherapy-associated venous thromboembolism in a prospective observational study. Cancer. 2005; 104:2822–9. [PubMed: 16284987]
49. Simanek R, Vormittag R, Ay C, Alguel G, Dunkler D, Schwarzinger I, Steger G, Jaeger U, Zielinski C, Pabinger I. High platelet count associated with venous thromboembolism in cancer patients: results from the Vienna Cancer and Thrombosis Study (CATS). J Thromb Haemost. 2010; 8:114–20. [PubMed: 19889150]

50. Jensvoll H, Blix K, Braekkan SK, Hansen JB. Platelet count measured prior to cancer development is a risk factor for future symptomatic venous thromboembolism: the Tromso Study. PLoS One. 2014; 9:e92011. [PubMed: 24642868]
51. Stone RL, Nick AM, McNeish IA, Balkwill F, Han HD, Bottsford-Miller J, Rupairmoole R, Armaiz-Pena GN, Pecot CV, Coward J, Deavers MT, Vasquez HG, Urbauer D, Landen CN, Hu W, Gershenson H, Matsuo K, Shahzad MM, King ER, Tekedereli I, Ozpolat B, Ahn EH, Bond VK, Wang R, Drew AF, Gushiken F, Lamkin D, Collins K, DeGeest K, Lutgendorf SK, Chiu W, Lopez-Berestein G, Afshar-Kharghan V, Sood AK. Paraneoplastic thrombocytosis in ovarian cancer. N Engl J Med. 2012; 366:610–8. [PubMed: 22335738]
52. Shavit JA, Motto DG. Coagulation and metastasis--an unexpected role for von Willebrand factor. J Thromb Haemost. 2006; 4:517–8. [PubMed: 16460433]
53. Vandendries ER, Furie BC, Furie B. Role of P-selectin and PSGL-1 in coagulation and thrombosis. Thromb Haemost. 2004; 92:459–66. [PubMed: 15351841]
54. Ay C, Dunkler D, Marosi C, Chiriac AL, Vormittag R, Simanek R, Quehenberger P, Zielinski C, Pabinger I. Prediction of venous thromboembolism in cancer patients. Blood. 2010; 116:5377–82. [PubMed: 20829374]
55. Ay C, Simanek R, Vormittag R, Dunkler D, Alguel G, Koder S, Kornek G, Marosi C, Wagner O, Zielinski C, Pabinger I. High plasma levels of soluble P-selectin are predictive of venous thromboembolism in cancer patients: results from the Vienna Cancer and Thrombosis Study (CATS). Blood. 2008; 112:2703–8. [PubMed: 18539899]
56. Poruk KE, Firpo MA, Huerter LM, Scaife CL, Emerson LL, Boucher KM, Jones KA, Mulvihill SJ. Serum platelet factor 4 is an independent predictor of survival and venous thromboembolism in patients with pancreatic adenocarcinoma. Cancer Epidemiol Biomarkers Prev. 2010; 19:2605–10. [PubMed: 20729288]
57. Riedl J, Hell L, Kaider A, Koder S, Marosi C, Zielinski C, Panzer S, Pabinger I, Ay C. Association of platelet activation markers with cancer-associated venous thromboembolism. Platelets. 2015:1–6.
58. Shoenfeld Y, Tal A, Berliner S, Pinkhas J. Leukocytosis in non hematological malignancies--a possible tumor-associated marker. J Cancer Res Clin Oncol. 1986; 111:54–8. [PubMed: 3949851]
59. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. Blood. 2008; 111:4902–7. [PubMed: 18216292]
60. Pabinger I, Posch F. Flamethrowers: blood cells and cancer thrombosis risk. Hematology. 2014; 2014:410–7. [PubMed: 25696887]
61. Thaler J, Ay C, Kaider A, Reitter EM, Haselbock J, Mannhalter C, Zielinski C, Marosi C, Pabinger I. Biomarkers predictive of venous thromboembolism in patients with newly diagnosed high-grade gliomas. Neuro Oncol. 2014; 16:1645–51. [PubMed: 24987133]
62. Blix K, Jensvoll H, Braekkan SK, Hansen JB. White blood cell count measured prior to cancer development is associated with future risk of venous thromboembolism--the Tromso study. PLoS One. 2013; 8:e73447. [PubMed: 24023876]
63. Connolly GC, Khorana AA, Kuderer NM, Culakova E, Francis CW, Lyman GH. Leukocytosis, thrombosis and early mortality in cancer patients initiating chemotherapy. Thromb Res. 2010; 126:113–8. [PubMed: 20684071]
64. von Bruhl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, Khandoga A, Tirniceriu A, Coletti R, Kollnberger M, Byrne RA, Laitinen I, Walch A, Brill A, Pfeiler S, Manukyan D, Braun S, Lange P, Riegger J, Ware J, Eckart A, Haidari S, Rudelius M, Schulz C, Echtler K, Brinkmann V, Schwaiger M, Preissner KT, Wagner DD, Mackman N, Engelmann B, Massberg S. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med. 2012; 209:819–35. [PubMed: 22451716]
65. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol. 2013; 13:34–45. [PubMed: 23222502]
66. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007; 176:231–41. [PubMed: 17210947]

67. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. Blood. 2014; 123:2768–76. [PubMed: 24366358]
68. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532–5. [PubMed: 15001782]
69. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH, Kubes P. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med. 2007; 13:463–9. [PubMed: 17384648]
70. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr. Wrobleski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci U S A. 2010; 107:15880–5. [PubMed: 20798043]
71. Gould TJ, Vu TT, Swystun LL, Dwivedi DJ, Mai SH, Weitz JI, Liaw PC. Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms. Arterioscler Thromb Vasc Biol. 2014; 34:1977–84. [PubMed: 25012129]
72. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, Bhandari AA, Wagner DD. Neutrophil extracellular traps promote deep vein thrombosis in mice. J Thromb Haemost. 2012; 10:136–44. [PubMed: 22044575]
73. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, Hu J, Wang Y, Wagner DD. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. Proc Natl Acad Sci U S A. 2013; 110:8674–9. [PubMed: 23650392]
74. Savchenko AS, Martinod K, Seidman MA, Wong SL, Borissoff JI, Piazza G, Libby P, Goldhaber SZ, Mitchell RN, Wagner DD. Neutrophil extracellular traps form predominantly during the organizing stage of human venous thromboembolism development. J Thromb Haemost. 2014; 12:860–70. [PubMed: 24674135]
75. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, Scadden DT, Wagner DD. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc Natl Acad Sci U S A. 2012; 109:13076–81. [PubMed: 22826226]
76. Demers M, Wagner DD. Neutrophil extracellular traps: A new link to cancer-associated thrombosis and potential implications for tumor progression. Oncoimmunology. 2013; 2:e22946. [PubMed: 23526174]
77. Demers M, Wagner DD. NETosis: a new factor in tumor progression and cancer-associated thrombosis. Semin Thromb Hemost. 2014; 40:277–83. [PubMed: 24590420]
78. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011; 11:426–37. [PubMed: 21562580]
79. Gailani D, Renne T. The intrinsic pathway of coagulation: a target for treating thromboembolic disease? J Thromb Haemost. 2007; 5:1106–12. [PubMed: 17388803]
80. Swystun LL, Mukherjee S, Liaw PC. Breast cancer chemotherapy induces the release of cell-free DNA, a novel procoagulant stimulus. J Thromb Haemost. 2011; 9:2313–21. [PubMed: 21838758]
81. Barbar S, Noventa F, Rossetto V, Ferrari A, Brandolin B, Perlati M, De Bon E, Tormene D, Pagnan A, Prandoni P. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction Score. J Thromb Haemost. 2010; 8:2450–7. [PubMed: 20738765]
82. Zakai NA, Callas PW, Repp AB, Cushman M. Venous thrombosis risk assessment in medical inpatients: the medical inpatients and thrombosis (MITH) study. J Thromb Haemost. 2013; 11:634–41. [PubMed: 23336744]
83. Kyrle PA. Predicting recurrent venous thromboembolism in cancer: is it possible? Thromb Res. 2014; 133(Suppl 2):S17–22. [PubMed: 24862139]
84. Lyman GH, Khorana AA, Kuderer NM, Lee AY, Arcelus JI, Balaban EP, Clarke JM, Flowers CR, Francis CW, Gates LE, Kakkar AK, Key NS, Levine MN, Liebman HA, Tempero MA, Wong SL, Prestrud AA, Falanga A, American Society of Clinical Oncology Clinical P. Venous thromboembolism prophylaxis and treatment in patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013; 31:2189–204. [PubMed: 23669224]

85. Mandala M, Falanga A, Roila F, Group EGW. Management of venous thromboembolism (VTE) in cancer patients: ESMO Clinical Practice Guidelines. Ann Oncol. 2011; 22(Suppl 6):vi85–92. [PubMed: 21908511]

86. Franchini M, Mannucci PM. Thrombin and cancer: from molecular basis to therapeutic implications. Semin Thromb Hemost. 2012; 38:95–101. [PubMed: 22314607]

87. Haas SK, Freund M, Heigener D, Heilmann L, Kemkes-Matthes B, von Tempelhoff GF, Melzer N, Kakkar AK, Investigators T. Low-molecular-weight heparin versus placebo for the prevention of venous thromboembolism in metastatic breast cancer or stage III/IV lung cancer. Clin Appl Thromb Hemost. 2012; 18:159–65. [PubMed: 22275397]

88. Maraveyas A, Waters J, Roy R, Fyfe D, Propper D, Lofts F, Sgouros J, Gardiner E, Wedgwood K, Ettelaie C, Bozas G. Gemcitabine versus gemcitabine plus dalteparin thromboprophylaxis in pancreatic cancer. Eur J Cancer. 2012; 48:1283–92. [PubMed: 22100906]

89. Riess H, Pelzer U, Deutschinoff G, Opitz B, Stauch M, Reitzig P, Hahnfeld S, Hilbig A, Stieler J, Oettle H. A prospective, randomized trial of chemotherapy with or without the low molecular weight heparin (LMWH) enoxaparin in patients (pts) with advanced pancreatic cancer (APC): Results of the CONKO 004 trial. J Clin Oncol. 2009; 27(suppl) abstr LBA4506.

90. Chee CE, Ashrani AA, Marks RS, Petterson TM, Bailey KR, Melton LJ 3rd, Heit JA. Predictors of venous thromboembolism recurrence and bleeding among active cancer patients: a population-based cohort study. Blood. 2014; 123:3972–8. [PubMed: 24782507]

91. Simes J, Becattini C, Agnelli G, Eikelboom JW, Kirby AC, Mister R, Prandoni P, Brighton TA, Investigators IS. Aspirin for the prevention of recurrent venous thromboembolism: the INSPIRE collaboration. Circulation. 2014; 130:1062–71. [PubMed: 25156992]

92. Brill A, Fuchs TA, Chauhan AK, Yang JJ, De Meyer SF, Kollnberger M, Wakefield TW, Lammle B, Massberg S, Wagner DD. von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. Blood. 2011; 117:1400–7. [PubMed: 20959603]

93. Shai A, Rennert HS, Rennert G, Sagi S, Leviov M, Lavie O. Statins, aspirin and risk of thromboembolic events in ovarian cancer patients. Gynecol Oncol. 2014; 133:304–8. [PubMed: 24631448]

94. Shai A, Rennert HS, Lavie O, Ballan-Haj M, Bitterman A, Steiner M, Keren S, Rennert G. Statins, aspirin and risk of venous thromboembolic events in breast cancer patients. J Thromb Thrombolysis. 2014; 38:32–8. [PubMed: 24154915]

95. Palumbo A, Cavo M, Bringhen S, Zamagni E, Romano A, Patriarca F, Rossi D, Gentilini F, Crippa C, Galli M, Nozzoli C, Ria R, Marasca R, Montefusco V, Baldini L, Elice F, Callea V, Pulini S, Carella AM, Zambello R, Benevolo G, Magarotto V, Tacchetti P, Pescosta N, Cellini C, Polloni C, Evangelista A, Caravita T, Morabito F, Offidani M, Tosi P, Boccadoro M. Aspirin, warfarin, or enoxaparin thromboprophylaxis in patients with multiple myeloma treated with thalidomide: a phase III, open-label, randomized trial. J Clin Oncol. 2011; 29:986–93. [PubMed: 21282540]

96. Larocca A, Cavallo F, Bringhen S, Di Raimondo F, Falanga A, Evangelista A, Cavalli M, Stanevsky A, Corradini P, Pezzatti S, Patriarca F, Cavo M, Peccatori J, Catalano L, Carella AM, Cafro AM, Siniscalchi A, Crippa C, Petrucci MT, Yehuda DB, Beggiato E, Di Toritto TC, Boccadoro M, Nagler A, Palumbo A. Aspirin or enoxaparin thromboprophylaxis for patients with newly diagnosed multiple myeloma treated with lenalidomide. Blood. 2012; 119:933–9. quiz 1093. [PubMed: 21835953]

97. Mezouar S, Darbousset R, Dignat-George F, Panicot-Dubois L, Dubois C. Inhibition of platelet activation prevents the P-selectin and integrin-dependent accumulation of cancer cell microparticles and reduces tumor growth and metastasis in vivo. Int J Cancer. 2015; 136:462–75. [PubMed: 24889539]

98. Rahimi K, Bhala N, Kamphuisen P, Emberson J, Biere-Rafi S, Krane V, Robertson M, Wikstrand J, McMurray J. Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. PLoS Med. 2012; 9:e1001310. [PubMed: 23028261]

99. Khemasuwan D, Divietro ML, Tangdhanakanond K, Pomerantz SC, Eiger G. Statins decrease the occurrence of venous thromboembolism in patients with cancer. Am J Med. 2010; 123:60–5. [PubMed: 20102993]

100. Lotsch F, Konigsbrugge O, Posch F, Zielinski C, Pabinger I, Ay C. Statins are associated with low risk of venous thromboembolism in patients with cancer: a prospective and observational cohort study. Thromb Res. 2014; 134:1008–13. [PubMed: 25234407]

101. Lee AY, Peterson EA. Treatment of cancer-associated thrombosis. Blood. 2013; 122:2310–7. [PubMed: 23843493]

102. Carrier M, Lazo-Langner A, Shivakumar S, Tagalakis V, Gross PL, Blais N, Butts CA, Crowther M. Clinical challenges in patients with cancer-associated thrombosis: Canadian expert consensus recommendations. Curr Oncol. 2015; 22:49–59. [PubMed: 25684988]

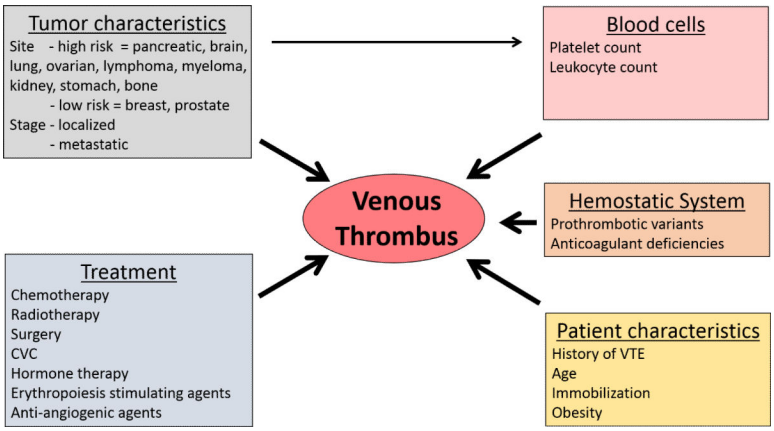

Figure 1. Risk factors of cancer-associated thrombosis

Tumor characteristics and treatment can contribute to VTE in cancer patients. The tumor may also release growth factors that elevate the platelet and leukocyte counts contributing to increased risk of thrombosis. Finally, patient characteristics and prothrombotic mutations could combine with tumor-specific factors to increase the risk of venous thrombosis.

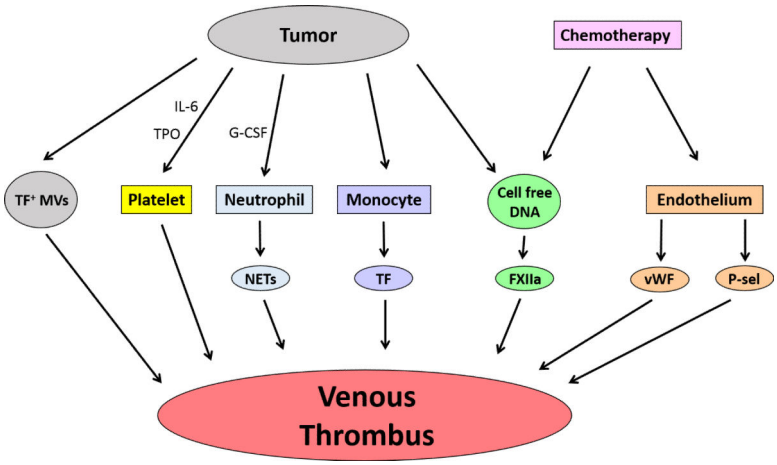

Figure 2. Mechanisms of cancer-associated thrombosis

Tumors release cell-free DNA, procoagulant factors and growth factors that enhance venous thrombosis. These include tissue factor (TF)-positive microvesicles, IL-6, thrombopoietin (TPO) and granulocyte colony-stimulating factor (G-CSF). Release of neutrophil extracellular traps (NETs) from neutrophils and tissue factor expression by monocytes enhance thrombosis. Chemotherapy and other drugs used to treat cancer patients may damage the endothelium and increase levels of cell-free DNA in the plasma.

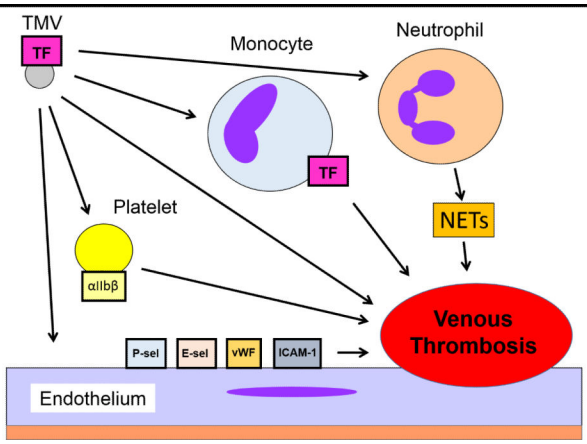

Figure 3. Tissue factor (TF)-positive tumor microvesicles may enhance venous thrombosis via different pathways

Tumor microvesicles (TMV) may bind to activate the endothelium bind to activate endothelium. TMV may bind to platelets and activate them in a thrombin-dependent manner. TMV may bind to monocytes and induce TF expression. Finally, TMV may enhance the release of neutrophil extracellular traps (NETs) from neutrophils.