

## Pathogenesis and Diagnosis of Venous Hypercoagulable States in Cancer

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### Abstract

An hypercoagulable state frequently accompanies malignancy, due to the ability of tumor cells to activate the coagulation system, which leads to thrombosis that is responsible for the second cause of mortality in cancer patients. A four-fold increase of thrombotic risk occurs in cancer, and the increase is even greater during chemotherapy.

A clinically detectable venous thromboembolism was detected in 15% of all cancer patients. Some cancers are more likely to be prothrombotic (prevalence is 27-28%, in pancreas and lung, 13% in stomach, but only 3% in colon cancer).

**Keywords:** Cancer; Venous hypercoagulable

### Introduction

Venous thrombosis being a well-known complication of cancer, healthy patients presenting with a venous thrombo-embolism episode should be investigated for cancer.

The thrombotic risk depends on tumor type, the stage or extent of the cancer, and treatment with antineoplastic agents. Furthermore, age, surgery, immobilization, and other comorbid features will also influence the thrombotic complications. Treatment for cancer (such as surgery, hormonal therapy, chemotherapy) also contributes to increasing the thrombotic risk in cancer patients [1].

### Pathogenesis of Hypercoagulable State in Cancer

The hypercoagulable state in cancer is multifactorial.

#### Tissue factor expressed by tumor cells, endothelial cells and monocytes in cancer

Tissue factor (TF) initiates a cascade of interactions that lead to clot formation.

1. In the 1st step, plasma factor VII binds to TF. When it binds to TF, factor VII becomes FVIIa, almost a thousand times more active. FVIIa is detectable in trace amounts in normal plasma (less than 1% of the total amount)
2. In the 2nd step, FVIIa-TF make a specific cut in factor X, changing it into an active form (FXa).
3. Then Factor Xa induces thrombin generation, which induces the coagulation of fibrinogen into fibrin that forms the fibrous structure of the clot.

In this cascading process, a few molecules of TF and factor VII can activate many copies of factor X, which, in generating thrombin, induces clot formation.

#### Tumor cells express TF and cancer procoagulant factor

TF is expressed by tumor cells and contributes to a variety of pathologic processes, including

1. Thrombosis: Tumor cells release TF-procoagulant microparticles into the circulation that may trigger VTE in patients with cancer. Indeed, elevated levels of tissue factor-bearing microparticles were associated VTE in cancer patients. In cancer patients presenting without VTE, a retrospective analysis revealed a 1-year cumulative incidence of VTE of 34.8% in patients with tissue factor-bearing microparticles versus 0% in those without detectable tissue factor-bearing microparticles [2].
2. Metastasis: TF on circulating tumor cells leads to the coating of the cells with fibrin that traps them within the microvasculature and facilitates hematogenous metastasis.
3. Tumor growth, and tumor angiogenesis: In addition to activation of coagulation, it was evidenced that TF-VIIa implicates signaling by protease activated receptors PAR2 in tumor cells, TF-VIIa-PAR2 inducing proangiogenic and immune modulating cytokines, chemokines and growth factors promoting cancer progression [3].

TF expression is increased by heparanase, which is up-regulated in almost all tumors (see chapter heparanase).

Tumor cells also express a cancer procoagulant (CP), a cysteine protease activating factor X, independently of factor VII [4]. CP is expressed in 85% of cancer patients [5].

#### Tumor cells produce and secrete proinflammatory cytokines responsible for TF expression on endothelial cells.

Tumor cells secrete proinflammatory cytokines such as TNF- $\alpha$ , responsible for TF expression on endothelial cells [6].

TNF- $\alpha$  also down-regulates the expression of thrombomodulin, the surface receptor for thrombin, at the surface of endothelial cells [7].

### **Tumor cells, by interacting with monocytes-macrophages, also induce TF expression by these cells**

The production of tissue factor by monocyte-macrophages in response to exposure to some tumor cells (or tumor cell products) may represent a mechanism whereby blood coagulation is activated in malignancy [8].

### **Tumor cells express and release the receptor for protein C**

Protein C (PC) is a serine protease that serves as a major regulator of the coagulation process on endothelial cells: Protein C bound to its endothelial cell receptor (EPCR) is activated in activated protein C (APC) by the thrombin-thrombomodulin complex. APC, in combination with its cofactor protein S, limits the amplification and progression of the coagulation cascade by degrading factors Va and VIIIa.

EPCR was evidenced in many cancer cells (e.g., in ovarian (90%), breast (60%), lung (80%) and colon (65%) cancer biopsies) [9] as well as in the 6 leukemic cell lines tested [10].

EPCR can be released from cancer cells by the pro-inflammatory cytokines IL1- $\beta$  and TNF- $\alpha$  [11], and the released EPCR (sEPCR) might be responsible for thrombotic events in cancer, since

1. The soluble form of EPCR (sEPCR) circulates in plasma and inhibits APC anticoagulant activity. Soluble EPCR (sEPCR) retains its affinity for both protein C and APC, but inhibits APC anticoagulant activity by blocking the interaction of protein C and APC with negatively charged surfaces, an interaction that is necessary for efficient inactivation of factors Va and VIIIa [12].
2. sEPCR released from malignant cells could serve as a "trap" for protein C, preventing its binding to EPCR on the surface of endothelial cells [10].

In a retrospective study performed in leukemic patients, it was found that the frequency of thrombotic episodes occurring in these patients depends on plasma sEPCR level: 8.8% in patients with plasma sEPCR <100 ng/ml; 13% in patients with plasma sEPCR between 100 and 200 ng/ml and 40% in patients with plasma sEPCR >200 ng/ml [10].

### **Heparanase expressed by tumor cells**

Heparanase is an endo- $\beta$ -D glucuronidase present in platelets and in activated leukocytes. It is up-regulated in almost all solid tumors as well as in hematological malignancies such as myeloma, leukemia and lymphoma [13].

Heparanase has multiple effects in cancer [13]:

**Heparanase enzymatic activity:** It cleaves heparin sulfate side chains of heparin sulfate proteoglycans present on cell surfaces and in the extra cellular matrix. Because its optimal pH is low (around 6), it acts at sites of ischemia and in the center of a tumor.

Due to its enzymatic activity, heparanase facilitates angiogenesis by releasing heparin sulfate (HS)-bound VEGF and b-FGF.

**Heparanase non-enzymatic pro-coagulant activity in cancer:** Nadir et al. [12] showed that an over-expression of heparanase in tumor-derived cell lines resulted in a 2-fold increase in TF expression levels that involves activation of the p38 signaling pathway. They also showed that addition of exogenous heparanase to endothelial or

tumor-derived cells resulted in enhanced TF expression and activity. As TF expression was also induced by inactive heparanase, it was suggested that this effect was independent of heparanase enzymatic activity [13,14].

Secreted heparanase interacts with tissue factor pathway inhibitor (TFPI) on the cell surface, leading to dissociation of TFPI from the cell membrane of endothelial and tumor cells. Since TFPI binds to FVIIa and to FXa, inhibiting the pro-coagulant activity of the TF-FVIIa-FXa complex, the release of TFPI from the cell membrane results in increased cell surface coagulation activity [15].

### **Consequences of activation of coagulation in cancer progression**

The thrombin generated

1. Activates PAR-1, which is involved in proliferation and cell motility in cancer cells [16].
2. Is involved in tumor cell resistance to chemotherapy (hematological diseases and solid tumors) [17] and in tumor invasion and metastasis: thrombin receptors on cancer cells, when occupied, mediate enhanced adhesion of the tumor cells to endothelium and subendothelial matrix, thus promoting cancer invasion and metastasis [18].
3. Activates angiogenesis: thrombin upregulates the expression of integrin  $\alpha\beta_3$ , the marker of the angiogenic phenotype of endothelial cells. Thrombin has chemotactic effects on endothelial cells and upregulates the expression of the vascular endothelial growth factor (VEGF) receptors (VEGFR-1 and VEGFR-2). Thus, thrombin synergizes with the key angiogenic factor VEGF in endothelial cell proliferation. Furthermore, thrombin enhances the secretion of VEGF by cancer cells [19].
4. Induces a rapid expression of P-selectin on the surface of both endothelial cells and platelets that is responsible for the adhesion of tumor cells expressing P-selectin receptor to endothelium or to platelets, thus contributing to metastasis [20,21].

### **Diagnosis of hypercoagulable states in cancer**

It is well established that an increase in plasma D-dimer is generally a useful marker to detect the formation of a thrombus that has been degraded by plasmin. However, the level of D-dimer is not a good marker of thrombotic disorders in cancer patients, since many tumor cells possess a strong procoagulant activity promoting local activation of the coagulation system generating peritumoral fibrin. Fibrin is then partially degraded by plasmin due to local activation of plasminogen by urokinase (u-PA), both being bound to their receptors present on the tumor cell membrane. Fibrin degradation products generated locally pass into the bloodstream. We found that the level of fibrin degradation products (D-dimer) in patients with ovarian cancer, before they receive chemotherapy, is correlated with the tumor load [22].

It thus emerges that D-dimer cannot be used to detect an activation of coagulation in cancer patients. Therefore, in cancer patients, when a deep vein thrombosis or a pulmonary embolism is suspected, only imaging enables one to confirm or to refute the diagnosis.

A novel and promising approach to group cancer patients according to their risk of venous thromboembolism is the use of risk scoring models.

The first score was developed by Khorana et al. to predict chemotherapy-associated thrombosis in ambulatory in cancer patients [23]. The thrombotic risk is based on a collection of five predictive variables present before initiation of chemotherapy-primary site of cancer, body mass index (BMI) and complete blood count (platelet, leukocyte, hemoglobin). Each variable in the score is assigned a value of Risk score (Table 1).

Cancer site	Risk score
-very-high-risk" site of cancer (stomach, pancreas, brain)	2
- "high risk" site of cancer (lung, kidney, lymphoma, and myeloma)	1
- all other cancer sites	0
Platelet count $\geq$ 350,000/ $\mu$ l	1
Hemoglobin less than 10 g/dL and/or use of erythropoiesis-stimulating agent	1
Leukocyte count more than $11 \times 10^9$ /L	1
Body Mass Index (BMI) of 35 kg/m <sup>2</sup> or more	1

**Table 1:** Rates of VTE were 0.3% in low-risk (score 0), 2% in intermediate-risk (score 1-2), and 6.7% in high-risk (score >3) over a median of 2.5 months.

The cumulative VTE probability in this model after 6 months was 17.7% in patients with the highest risk score ( $\geq$ 3), 9.6% in those with score 2, 3.8% in those with score 1, and 1.5% in those with score 0 (Table 2).

Cancer site	Risk score
Soluble P-selectin $\geq$ 53.1 ng/mL	1
D-Dimer $\geq$ 1.44 ng/mL	1

**Table 2:** The 2nd score was a score modified by Ay et al. [24]; he included the above parameters of Khorana Score plus 2 other biomarkers.

In this expanded risk model, the cumulative VTE probability after 6 months in patients with the highest score ( $\geq$ 5) was 35.0% and 10.3% in those with an intermediate score (score 3) as opposed to only 1.0% in patients with score 0.

### Clinical therapeutical consequences of hypercoagulability in cancer

Hypercoagulability is commonly found in patients with cancer. This hypercoagulability was reported as early as 1865 by Armand Trousseau, who first described the clinical association between thromboembolic disease and malignancies.

When a deep vein thrombosis or a pulmonary embolism occurs in patients without any risk factor (i.e., stasis, inherited risk factors..), it can be the first symptom of cancer. Up to 10% of patients with unexplained thrombosis will be diagnosed with cancer within one year. Therefore, in patients with idiopathic thrombosis, it can be proposed to perform a check-up for early cancer detection, including a medical history, physical examination, chest X-ray, determination of several tumor markers in blood.

In cancer patients presenting a venous thromboembolism disease, treatment with low molecular weight heparin (LMWH) has been shown to be more effective than warfarin. Use of LMWH for at least the first 3-6 months of long-term treatment is now considered the standard of care for patients with cancer and is recommended in numerous guidelines.

The effectiveness of new oral anticoagulants (NOAC) in patients with cancer is not yet clearly defined. For some authors, Rivaroxaban might be equally effective and just as safe as vitamin K antagonist LMWH in prevention of thromboembolism in cancer patients [24,25]. In contrast, for other authors an eventual use of NOACs in cancer patients should be restricted only to patients presenting with contraindications for low molecular weight heparins, fondaparinux, or for vitamin K antagonists, because of the elimination of NOACs by the liver and renal pathway as well as because of their pharmacological interactions with drugs which are frequently used in cancer patients [26].

Considering thromboprophylaxis, most hospitalized patients with cancer require thromboprophylaxis throughout hospitalization, but the treatment is not routinely recommended for outpatients with cancer. It may be considered only for selected high-risk patients, such as 1) patients with multiple myeloma receiving antiangiogenesis agents with chemotherapy, and 2) patients undergoing major cancer surgery, thromboprophylaxis starting before surgery and continuing for at least 7 to 10 days. Extending prophylaxis up to 4 weeks should be considered in those with high-risk features [27].

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