Venous thromboembolism, coagulation and cancer
van Doormaal, F.F.

Citation for published version (APA):
CHAPTER

Cancer and thrombosis: from molecular mechanisms to clinical presentations

H.R. Büller, F.F. van Doormaal, G.L. van Sluis, P.W. Kamphuisen

J Thromb Haemost. 2007;5 Suppl 1:246-54
Chapter 2

SUMMARY
Although the bidirectional association between cancer and venous thromboembolism (VTE) has been known for almost two centuries, recent advances in our understanding of the clinical, laboratory and epidemiological aspects of this association have created a renewed interest in this topic. This review consists of two parts. The first discusses the occurrence, determinants and significance of VTE in those with cancer, as well as the risk of developing and the possible need to detect cancer in those presenting with VTE. The second part reviews the role of hemostatic constituents (coagulation and fibrinolytic proteins and platelets) in promoting growth and progression of cancer, as well as the effects and possible mechanisms of the (low-molecular-weight) heparins (LMWH) in this process.

CANCER, THROMBOSIS AND CANCER
Historically, Armand Trousseau, the French physician (1801-1867), is often considered to have been the first scientist who described the association between cancer and venous thrombosis. This is incorrect for both the relationship of cancer with subsequent complicating VTE as well as for occult cancer in thrombosis patients. A careful literature analysis revealed that already in 1823 Bouillaud described three cancer patients with deep venous thrombosis [1;2]. He speculated in his monogram that the peripheral edema in the legs of these cancer patients was the result of obstruction of the veins by “caillot fibrineux” (fibrin clots) induced by the cancer process. Trousseau was 22 years old at that time. In the frequently quoted book by Trousseau, published in 1865, he indeed described in detail the relationship between cancer and VTE, but that was 42 years after Bouillaud [3].

Trousseau is also often quoted for the link between venous thrombosis and occult cancer. Close reading of his papers reveal that all his patients described with VTE already had extensive evidence of cancer at the time of the diagnosis of thrombosis [4;5]. The honor of the first description of a patient with deep venous thrombosis and the manifestation of a gastric cancer several months later goes to Illtyd James and Matheson [6]. They published their paper in 1935. Interestingly, the first proper cohort study in patients with VTE to assess the incidence of occult cancer was only published in 1982 [7]. These authors coined the question whether an extensive search for cancer at the time of VTE diagnosis was indicated.
The focus in this part of the review will be on deep-vein thrombosis of the leg (DVT) or pulmonary embolism (PE) and the two-way association with cancer, with special emphasis on the epidemiological aspects, the magnitude and consequences. Other manifestations of venous thrombosis, such as superficial phlebitis, arm vein thrombosis and catheter related thrombosis, as well as the prevention and treatment of DVT and PE are outside the scope of this contribution.

Cancer and venous thromboembolism
In cancer patients VTE complications are common and the second leading cause of death [8-10]. Of every seven patients with cancer who die in hospital, one dies of pulmonary embolism [11]. Compared to non-cancer patients the risk of developing symptomatic VTE is 6 to 7 times higher in cancer patients with similar risks for the components of VTE, i.e. DVT and PE [12;13]. The precise incidence and time course of VTE complications among cancer patients have until recently largely been unknown [14]. Initially, small cohort studies estimated the incidence among patients with various types and stages of cancer to be approximately 4% [14]. Subsequent larger epidemiological studies found incidences of clinically manifest VTE of 110-120 events per 10,000 patients [15;16]. Recently, a large population-based study determined the incidence of VTE among patients diagnosed with specific types and stages of cancer [14]. Among 235,149 cancer cases, confirmed symptomatic VTE events were diagnosed within 2 years in 1.6%. Twelve percent of these events occurred at the time cancer was diagnosed and the remaining 88% were observed subsequently. In another recent large population-based study the duration between the diagnosis of cancer and the occurrence of VTE was determined [13]. In the first 3 months the risk of VTE was the highest, with an odds ratio of 54, whereas in the period between 3 and 12 months following the cancer diagnosis, the odds ratio of VTE was 14, and between 1 and 3 year this figure was 4 [13].

Table 1 details the determinants for the risk of symptomatic episodes of VTE in patients with cancer. In risk-adjusted models Chew and colleagues found metastatic disease at the time of cancer diagnosis the strongest predictor of VTE [14]. The risk was 4 to 13 times higher as compared to cases with localized disease. In patients with metastatic-stage disease the highest incidences (expressed as VTE events per 100 patient-years) were observed for pancreatic (20.0), stomach (10.7), bladder (7.9), uterine (6.4), renal (6.0) and lung (5.0) cancer. Most of the VTE complications occurred in the first year of follow up [14]. Blom and colleagues also determined the significance of distant
metastases for the risk of VTE and found an odds ratio of 20 as compared to patients without distant metastases [13].

Table 1 - Determinants for the risk of venous thromboembolism in cancer patients

- Tumor stage
- Tumor type
- Anticancer therapy
  - Chemotherapy
  - Hormonal therapy
  - Angiogenesis inhibitors
  - Supportive therapy
- Surgery
- Prothrombotic abnormalities

All hematological and solid tumor types have been associated with VTE. However, conflicting data exists about the relative risk of VTE according to tumor histology and site of primary location [8]. It is, however, clear that the VTE risk is not equal among the various types of cancer. Adjusted for age and sex, Blom et al. observed, in a population based study, the highest risk of VTE among patients with hematological malignancies (odds ratio 28), followed by lung cancer (odds ratio 22) and gastro-intestinal cancer (odds ratio 20) [13]. In another population based study, Heit and colleagues noted that pancreatic cancer, lymphoma and brain cancer have relative risks of VTE greater than 25, followed by liver cancer, leukemia and other gastro-intestinal (esophagus, gallbladder) and gynecological cancer, with a relative risk above 17 [17]. In a study of hospitalised patients the highest incidence of VTE occurred among women with ovarian cancer followed by brain and pancreas cancer and lymphoma [15;16].

The third and probably at present the most changing determinant for the risk of VTE is anticancer therapy (Table 1). Chemotherapy is a well recognized independent risk factor for VTE and the annual incidence has been estimated to be 10-20% depending on the type of drugs given [8;18]. However, one of the most prominent reasons for the increasing importance of anticancer therapy as a determinant for the thrombosis risk is that in addition to chemotherapy, other frequently combined therapies to treat the cancer, such as hormonal therapy, angiogenesis inhibitors and supportive therapy,
carry their own risk to induce a hypercoagulable state [8]. Probably the best studied influence of chemotherapy on the risk of thrombosis is in patients with breast cancer. In early stage breast cancer the risk of VTE is similar to that in the general population (approximately 0.2%). With chemotherapy or hormonal treatment this increases to 1-2%. When in stage I-II chemo and hormonal therapy are combined the incidence rises to 5-7% [8;19;20]. For patients with stage IV breast cancer these rates may be as high as 18% with the combined treatments [8]. Other chemotherapeutic agents, such as cisplatin (reported VTE rates of 8-18%), L-asparaginase (reported VTE rates in adults of 4-14%) and fluorouracil (reported VTE rates of 15-17%), often in combination with other forms of chemotherapy, have been clearly associated in retro and prospective studies with an increased risk of VTE [8]. Thalidomide, particularly in combination with dexamethasone or doxorubicin, may induce VTE rates as high as 20 to 40% [8;21]. Whether the newer thalidomide analogues (IMiDs) really have lower VTE complication rates remains to be demonstrated, in particular when combined with other forms of chemotherapy. Initially, angiogenesis inhibitors (such as bevacizumab), again in combination with chemotherapy were reported to induce higher rates of VTE, but more recent comparisons (with and without bevacizumab) suggest that the contribution of angiogenesis inhibitors may be marginal [8;22]. Finally, supportive therapies associated with an increased risk of VTE include erythropoietin, hematopoietic colony stimulating factors and high dose corticosteroids.

Cancer patients undergoing surgical procedures have an approximately two fold higher risk to develop VTE as compared to non-cancer patients [12;23]. Incidences of symptomatic VTE within 91 days after the procedure in cancer patients were as high as 3-4% for radical cystectomy, nephrostomy, brain surgery and total hip replacement [23].

Other determinants of the VTE risk in cancer patients include the classical risk factors such as immobility, age and also prothrombotic abnormalities. Carriers of the Factor V Leiden mutation who also had cancer had a 12-fold increased risk to develop VTE as compared to individuals with the mutation but no cancer [13]. This is in agreement with earlier observation and also applies to other genetic thrombophilias, such as the prothrombin mutation [13;24;25].

Cancer patients who have developed VTE have an approximately 3-fold higher risk to experience a recurrent thrombosis in the first 12 months as compared to VTE patients without cancer [12;15;26]. Prandoni and colleagues observed recurrent VTE in
6.8% of patients without cancer, whereas in those with cancer the incidence was 20.7% [26].

Developing VTE predicts a worse prognosis in cancer patients [15;27]. The one-year survival was 12% in patients diagnosed with cancer and VTE at the same time, as compared to 36% in cancer patients without VTE in a population based study [27]. Figure 1 shows the mortality rates in the first 180 days in patients with cancer and VTE, which is more than two-fold higher relative to cancer patients without VTE [27].

![Graph showing mortality rates](image.png)

**Fig. 1.** Probability of death within 183 days of initial hospital admission [15]. Reproduced with permission of the publisher.

**Venous thromboembolism and cancer**

In patients with symptomatic VTE, the prevalence of concomitant cancer, i.e. cancer not known before the diagnosis of VTE and discovered by routine investigation, at the time of VTE diagnosis, varies in the larger studies between 4-12% [28]. The cancer stage in patients with concurrently diagnosed VTE is often advanced [27;29]. Furthermore, the risk of concomitant cancer is 3-4-fold increased in patients with idiopathic VTE as
compared to secondary VTE [28]. The risk of occult cancer, i.e. cancer that becomes clinically apparent during follow-up, is also increased in patients with VTE. Summarising 17 cohort and 2 population-based studies, Otten and Prins calculated an incidence of new cancer in patients with idiopathic VTE of 4-10%, diagnosed within 3 years after the thrombotic event [28]. Again, this incidence was higher in idiopathic VTE patients than in patients with secondary VTE.

The incidence of cancer is the highest shortly after VTE has been diagnosed. During the first year, the standardized incidence ratio for cancer in patients with VTE is 2.1-4.6, while after this period the risk gradually decreases [30]. In the large Californian registry, White et al. showed that the incidence of cancer was the highest during the first 60 days after an unprovoked episode of VTE (Figure 2), with a gradual decline afterwards [29]. In the first 4 months after VTE diagnosis, the standardized incidence ratio was twofold higher than the expected incidence, whereas the observed and expected incidences of cancer 4 to 12 months after VTE were virtually the same. Also the occurrence of metastasized disease was the highest in the first 4 months after an unprovoked VTE. This indicates that the risk of cancer is only increased in the first months to a year after a VTE, while many patients already have metastases.

Fig. 2. Timing of diagnosis of unprovoked venous thromboembolism events relative to the cancer diagnosis date, using 2-month (61-day) increments [29]. Reproduced with permission of the publisher.
The risk of developing overt cancer after VTE is also influenced by the type of cancer. In the large Danish registry, Sørensen and colleagues showed that around 15% of the patients with VTE and cancer within one year had lung cancer, followed by prostate (11.4%), pancreas (7.9%), colon (7%), and breast (4.3%) cancer [27]. Leukemia and non-Hodgkin lymphoma were found in 2.5% of the patients [27]. The Californian cancer registry, White et al. revealed the highest standardised incidence ratios for acute myelogenous leukemia (4.2), followed by ovarian cancer (2.8), non-Hodgkin lymphoma (2.7), pancreatic (2.6), renal cell (2.5), stomach and lung cancer (1.8) [29].

Considering the high incidence of cancer in the first months after VTE, screening for an underlying malignancy may be clinically relevant. The literature is however not concordant whether extensive screening for occult malignancy is indicated. Retrospective studies indicated that a careful medical history, physical examination and laboratory testing detected most occult cancers in patients with VTE [31-33], suggesting that routine examination at the time of VTE diagnosis is sufficient to detect most underlying malignancies and that additional testing would not be required. Prospective studies seem to indicate that extensive screening is able to detect more cancers than routine examination alone. The six prospective studies that systematically evaluated the added value of more extensive screening compared to routine examination are summarized in Figure 3 [34-39]. Routine examination was in most studies defined as a combination of careful medical history taking, thorough physical examination, laboratory testing, and chest X-ray. In most studies, extensive testing included specific tumor markers, such as prostate-specific antigen, carcinoembryonic antigen or cancer antigen-125 levels, and abdomino-pelvic ultrasonography. Upon indication this screening was extended with CT-scanning of chest, abdomen or pelvis, gastro- colono- or sigmoidoscopy, mammography, or sputum cytology. In the six studies routine followed by more extensive screening was associated with a 1.8 fold (95% CI 1.28-2.51) higher probability of finding occult cancer, compared to routine testing alone (Figure 3). The only randomized trial by Piccioli et al., however, found no additional value of extensive screening [38]. Furthermore, after routine screening the incidence of cancer showed a large variation in the different studies. This is likely due to differences in patient characteristics, but also to the type of routine screening itself. A predefined protocol focusing on the possibility of occult cancer that clearly describes history taking, physical examination and laboratory testing is a prerequisite to reliably assess the additional value of extensive screening. In addition, another potential source of variation is the definition of extensive screening, which is not
well defined. Finally, if extensive screening is advocated, early detection of cancer must lead to a benefit in survival, a matter that has not yet been adequately addressed.

Fig. 3. Detection of occult malignancy in patients with venous thromboembolism by routine screening alone or by routine screening followed by more extensive screening.

An ongoing study may soon provide more information. This multicenter study directly compares routine and extensive testing for occult malignancy in patients with VTE (The Trousseau study). Centers that only used routine testing, consisting of medical history, physical examination, basic laboratory examination and chest X-ray, are compared to matched centers that additionally perform thoracic and abdominal CT, plus mammography in women. Thus far 444 patients have been included. The planned total number of patients is 1200. An interim analysis reveals that in the routine testing arm, 16 of 176 had a suspected cancer after the initial assessment, which was confirmed in 1 patient (0.6%, 95% CI; 0.2-3.1%). During a median follow up of 12 months 6 new malignancies were diagnosed (incidence 3.4%, 95% CI; 1.3-7.3%). In routine and additional screening arm, 30 of 268 patients were thought to have a malignancy after the initial assessment. In 11 patients a malignancy was diagnosed (4.1%, 95% CI; 2.1-7.2%). Of these 268 patients, 214 underwent the additional screening tests. Follow up diagnostic tests were ordered in 49 patients. In 4 a malignancy was found. During a median follow up of 12 months, 6 new neoplasms were diagnosed (incidence 2.4%, 95% CI; 0.87-5.1%) (abstract ISTH).

Based on these preliminary data the additional value of CT screening appears to be limited in patients with idiopathic VTE. A standard initial assessment seems effective enough for diagnosing cancer in these patients.
CANCER, HEMOSTASIS AND HEPARINS

This second part first describes the way cancer cells utilise hemostatic constituents for their growth and progression. Recently, a rise in the interest in the association of cancer and thrombosis was driven by the discovery of potential anti-cancer effects of anticoagulants in particular heparins. The clinical effects of (LMW) heparins in cancer patients are summarized and the potential mechanisms of the anti-cancer effects of heparins are discussed. Other anticoagulants are outside the scope of this review.

Hemostasis and cancer

Multiple pathways result in cancer growth and progression. Cancer cells gain capacity to proliferate, migrate and induce proteolysis and permeability. They have a reduced sensitivity to apoptosis and develop functions to increase motility and adhesion [40]. It is now well established that the essential actors of hemostasis (e.g. coagulation and fibrinolytic proteins, and platelets), apart from their procoagulant effects, directly play a role in these processes. However, fibrin formation itself is essential as a supportive scaffold for angiogenesis that ensures the tumors oxygen supply [41-43]. The effects and possible pathways through which the individual actors and their complexes work are summarized in Table 2.

Tissue factor levels are associated with clinical progression of cancer and elevated levels are an unfavourable prognostic indicator. Tissue factor gene silencing can inhibit tumor growth and metastasis in vivo [44-46]. The importance of tissue factor for invasive growth is nicely illustrated by the fact that without tissue factor there can be no embryonic development [47]. The aberrant expression of tissue factor in cancer and vascular endothelial cells is stimulated by the tumor as a result of activation of the k-ras oncogene and loss of p53 tumor suppressor gene, as well as through inflammatory signals such as interleukine-1β and tumour necrosis factor-α [48]. These cytokines also influence leukocyte, platelet and endothelial cell function resulting in decreased thrombomodulin expression on the endothelial cell surface, impairment of the protein C anticoagulant pathway and enhanced expression of adhesion molecules [46].

Furthermore, tissue factor (isolated as well in complex with factor VIIa) induces vascular derived growth factor (VEGF) expression, downregulation of thrombospondin and further tissue factor expression [49-52]. Together, this stimulates angiogenesis, adhesion and migration [53;54]. The TF-FVIIa complex induces also survival of cells that have been directed towards apoptosis [55;56].
Table 2 - The role of hemostatic constituents (coagulation and fibrinolytic proteins and platelets) in the growth and progression of cancer.

<table>
<thead>
<tr>
<th>Haemostasis</th>
<th>Pathway/mecanism</th>
<th>Effects on cancer growth and progression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific interactions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue factor</td>
<td>VEGF expression↑</td>
<td>Angiogenesis↑, permeability↑</td>
</tr>
<tr>
<td>TF-FVIIa</td>
<td>VEGF↑ TSP↓ TF↑</td>
<td>Angiogenesis↑, cell survival↑, apoptosis↓</td>
</tr>
<tr>
<td>Factor Xa</td>
<td>Cancer procoagulant</td>
<td>Proliferation↑</td>
</tr>
<tr>
<td>Thrombin</td>
<td>TF, VEGF, VEGFR, bFGF, MMP-2</td>
<td>Altered cell shape, proliferation↑, permeability↑, migration↑, cell survival↑, proteolysis↑</td>
</tr>
<tr>
<td></td>
<td>Platelets activation, growth factors release</td>
<td>Angiogenesis↑, apoptosis↓</td>
</tr>
<tr>
<td></td>
<td>Mobilisation of adhesion molecules</td>
<td>Motility↑</td>
</tr>
<tr>
<td><strong>Pro-coagulant interactions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF-FVIIa</td>
<td>Thrombin/fibrin formation</td>
<td>Matrix for angiogenesis</td>
</tr>
<tr>
<td>TF-FVIIa-FXa</td>
<td>Thrombin formation</td>
<td>Feedback: TF expression↑</td>
</tr>
<tr>
<td><strong>Fibrinolytic proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>u-PA, t-PA</td>
<td>Proteolysis/fibrinolysis</td>
<td>Invasion, proliferation</td>
</tr>
<tr>
<td>PAI-1, PAI-2</td>
<td>Fibrin matrix conservation</td>
<td>Angiogenesis↑</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>Release of growth factors (VEGF, PDGF, bFGF)</td>
<td>Angiogenesis↑, permeability↑, apoptosis↓</td>
</tr>
<tr>
<td></td>
<td>P-selectin expression</td>
<td>Tumour cell adhesion↑, migration↑</td>
</tr>
<tr>
<td></td>
<td>NK-cell protection (coating)</td>
<td>Tumour cell survival↑</td>
</tr>
<tr>
<td></td>
<td>COX-2 ↑</td>
<td>Apoptosis↓</td>
</tr>
</tbody>
</table>

Cancer procoagulant, a cystein protease which is expressed on the surface of many cancer cells, can directly induce activation of factor X independent of FVIIa. Hereby, vascular cell proliferation is promoted [46].

Thrombin enhances the expression of tissue factor, VEGF, VEGF-receptor, basic fibroblast growth factor and matrix metalloproteinase through signalling via the protease activated receptors on the cell surface of cancer cell and/or vascular endothelial cells [57-61]. This leads to changes in endothelial cell shape and proliferation, increased vascular permeability, migration and enhanced survival of cancer cells [54;62;63].

The factors mentioned above are all well known for their classic role in the hemostatic system, which naturally occurs at the same time. It appears that activating the coagulation system has advantages for cancer growth and progression, since fibrin is an excellent matrix for angiogenesis. The fibrin matrix also protects against proteolysis. Furthermore, fibrin formation leads to proliferation and migration and in reverse to tissue factor expression, which is probably signalled through the pro-angiogenetic cytokine II-8 [46;64;65]. The complex of tissue factor-FVIIa-FXa stays active because of impaired TFPI binding, which results in prothrombin conversion and thrombin formation [43].

Cancer cells can express many fibrinolytic proteins (uPA, tPA, PAI-1, PAI-2) and both promotion as well as inhibition occurs [66]. Cancer cells overall impair the fibrinolytic system, which results in fibrin matrix conservation, tumor invasion, cell proliferation and metastasis.

Activation of platelets by thrombin leads to release of growth factors such as VEGF, platelet derived growth factor and basic fibroblast growth factor. These growth factors contribute to angiogenesis and inhibit apoptosis [67;68]. Cancer cells enhance P-selectin expression on monocytes, macrophages, endothelial cells and platelets [69]. In addition cancer cells express p-selectin ligands to bind those cells. The latter provides protection against natural killer cells and therefore leads to tumor cell survival and enhanced adhesion to the endothelium [70]. Platelets promote COX-2 synthesis in monocytes and it has been shown that overexpressing of COX-2 leads to inhibited apoptosis, abnormal cell-to-cell interactions and a more invasive phenotype. Furthermore, prostaglandins (COX-2 products and mediators of classic inflammation) might suppress host immunity against tumors [71;72].

More recently, coagulation system activation has been related to hypoxia driven overexpression or mutation of the MET-oncogene (physiological invasive growth). Boccaccio and colleagues induced disseminated intravascular coagulation and the
Trousseau syndrome by targeting the human MET-oncogene in the mouse liver resulting in enhanced PAI-1 and COX-2 expression [73-75]. In glioblastomas it was found that a tumor suppressor gene (PTEN) and hypoxia regulated tissue factor expression and induced intravascular thrombotic occlusion [76].

**Cancer and heparins**

For many decades, experimental and clinical studies have evaluated the effects of anticoagulants on tumor growth with different outcomes. In 1992 Prandoni et al. observed an important reduction in total mortality in the subgroup of VTE patients with cancer at enrolment, who received LMWH. At 3 months, 44% (8 of 18) of the cancer patients died in the standard heparin group versus 7% (1 of 15) in the LMWH group (p=0.021) [77]. These findings of a survival benefit of LMWH in cancer patients were confirmed in a meta-analysis of 9 studies that compared LMWH with standard heparin in the treatment of VTE [78]. These results have led to clinical trials that evaluated the effect of (LMW) heparin on survival in cancer patients without thrombosis.

Figure 4A presents the overall survival results at 6 months after study entry of the five randomized controlled trials that primarily evaluated the effects of (LMW) heparin on cancer progression [79-83]. Overall there is no difference in survival (relative risk 0.91, 95% CI; 0.71-1.16). These studies however differ considerably in study design. In one study UFH was administered, in the others LMWH [79]. In two of the studies only patients with small cell lung cancer were included [79;80], whereas patients with different cancer types were enrolled in the other studies. The duration of heparin therapy differed, from 5 to 52 weeks. In one study a therapeutic dose of LMWH was given [82]. Three studies defined a priori a subgroup consisting of patients with a prognosis of at least 6 months at study entry (also referred to as limited disease) [79;80;82]. Figure 4B shows the survival in this subgroup. Patients treated with (LMW) heparin have a significant survival benefit (relative risk 0.47, 95% CI; 0.30-0.75). Subgroup analysis indicated no benefit for patients with advanced disease (relative risk 0.96, 95% CI; 0.77-1.21). Post hoc analysis of other studies are in agreement with these positive findings in those with limited disease [81;83;84].

These clinical results are supported by proposed mechanisms of heparin on cancer progression, mainly based on in vitro and in vivo studies. The possible mechanisms by which heparins impair cancer growth and progression include decrease of thrombin and fibrin formation, binding to adhesion molecules, anti-heparanase effects and increase of apoptosis.
Fig. 4A. Heparin versus placebo or no anticoagulants in patients with cancer.
Overall survival after 6 months

Fig. 4B. Heparin versus placebo or no anticoagulants in patients with cancer.
Survival after 6 months in patients with limited disease, with a similar definition at study entry

Legend fig. 4: UFH: Unfractioned Heparin, LMWH: Low Molecular Weight Heparin

Heparins have well known anticoagulant effects resulting in inhibition of thrombin and fibrin formation. As discussed above, thrombin as well as fibrin are important for cancer growth and progression. Besides, its coagulant effects heparin is thought to have other properties. This is illustrated by the anti-metastatic effects of non-anticoagulant heparins [85].

The first non-anticoagulant mechanism of heparin is interfering with the interaction between cancer cells and platelets. Specific oligosaccharide structures in heparin can bind to P-selectins exposed on activated platelets [86]; these structures show minimal anticoagulant activity [87]. Ludwig and colleagues tested the impact of UFH, LMWH (nadroparin and enoxaparin), and fondaparinux on P-selectin-dependent tumor interactions in vitro and metastasis formation in vivo. These agents differ widely in their potential to interfere with P-selectin-mediated cell binding. This inhibitory
function strongly correlates to the potency in inhibiting experimental lung metastasis in vivo [88;89]. Selectins are also expressed on leukocytes (L-selectin) and the vascular endothelium (E- and P-selectin). Heparin can inhibit L-selectin binding to its ligands resulting in inhibition of the inflammatory response. Also extravasation of cancer cells is hindered by binding of heparin to selectins. [86;90;91].

The second non-anticoagulant property of heparin is inhibition of cancer cell heparanase [92]. Heparanase is expressed in many human cancers. This enzyme breaks down the polysaccharide barrier facilitating cancer-cell invasion through the vascular basement membrane, also angiogenesis and metastases are promoted [93]. In humans elevated heparanase expression by the tumor has been correlated with a more aggressive behaviour in breast, colon, ovary, pancreas, non-small lung cancer, acute myeloid leukaemia, and myeloma tumours [94-101]. Heparanase also cleaves the growth factor bearing heparan sulphate groups from heparan sulphate proteoglycans localized in the extracellular matrix and cellular membranes [102-104]. Heparins can directly inhibit the effect of growth factors as well. LMWH hinders binding of growth factors to high-affinity receptors to a greater degree than UFH [105].

Heparin, especially LMWH, is thought to increase the apoptosis index in cancer cells. Nasir et al. observed in heparin knockout mice a four fold increase in the apoptosis index with LMWH as compared to controls [106]. Heparin probably enters the cell by endocytosis and induces apoptosis by interfering with transcription factor [107].
REFERENCES


Chapter 2


Cancer and thrombosis: from molecular mechanisms to clinical presentations


