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REVIEW

From Trousseau to angiogenesis: the link between the haemostatic system and cancer

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INTRODUCTION

Thrombosis is one of the major complications of cancer. In 10 to 15% of the patients with clinically overt cancer, spontaneous venous thrombosis, thromboembolism after cancer surgery, thromboembolism during chemotherapy and thrombosis of central venous access lines occur as clinical manifestations of thrombosis.[1] The relationship between cancer and thrombosis is also obvious by the clinical observation that thrombosis may be a presenting symptom of cancer. Of all noncancer patients presenting with an idiopathic thromboembolism, 10 to 20% develop cancer in the next three years.[2] It was Armand Trousseau in the 19th century who first noted the 'alteration of the blood' in cancer patients.[3]

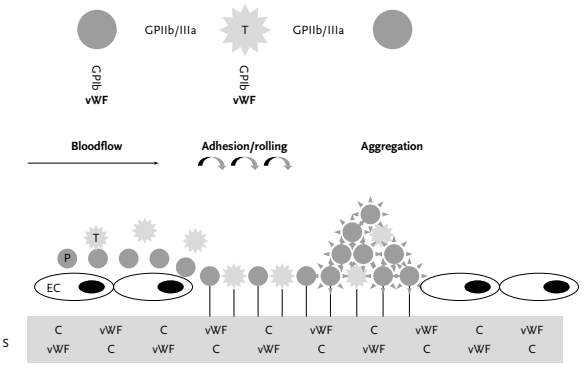
Moreover, there is now considerable evidence that the blood coagulation system is not only involved in cancer-associated thrombosis, but also plays an important role in the biology of malignant tumours. Cancer cells interact with the coagulation system for their growth, for angiogenesis and for the dissemination through the body. Many components of the coagulation system are involved in tumour neovascularisation, and fibrin present in the matrix around tumour cells facilitates tumour cell growth.[4] The interference of tumour cells with the coagulation system leads to an increased activation of several coagulation pathways. And it is this hypercoagulability state that is the major determinant of the increased risk for the above-mentioned thromboembolic complications in cancer patients.

ACTIVATION OF THE PRIMARY HAEMOSTATIC SYSTEM

In normal primary haemostasis a vascular lesion is closed by the formation of a platelet plug. First, the platelets adhere transiently to subendothelial von Willebrand factor (vWF) through the GPIb receptor. This adherence significantly slows the movement of the platelets. Secondly, the slowly moving platelets start to roll across the subendothelium and adhere to vWF and collagen through the GPIb and platelet collagen receptors. Finally, these interactions lead to platelet activation and aggregation through the GPIIb/IIIa receptors on platelets, thereby stably adhering to the damaged vessel wall (*figure 1*). Hence, vWF plays an essential role by promoting the adhesion of platelets to the subendothelium.

In cancer patients both platelets and vWF are believed to be involved in cancer growth and dissemination.[5] It has been shown that platelets release vascular endothelial growth

Figure 1. *Involvement of tumour cells in the primary haemostatic system*



S = subendothelium; EC = endothelial cell; P = platelet; C = collagen; vWF = von Willebrand factor; GP = glycoprotein; T = tumour cell. Circulating platelets and tumour cells adhere to the subendothelial vWF and collagen, leading to rolling, adhesion and aggregation through the GPIb and GPIIb/IIIa receptors on platelets as well as tumour cells.

factor (VEGF), the important regulator of tumour-induced angiogenesis.[5,6] Moreover, VEGF-stimulated endothelial cells promote adhesion and activation of platelets.[7] Previous animal studies show that thrombocytopenia inhibits and platelet transfusion stimulates tumour metastasis in animals.[8] Tumour cell adhesion to platelets might be essential for dissemination. Blocking tumour-binding receptors on platelets inhibits metastasis *in vitro* and *in vivo*.[9] Platelets adhering to tumour cells prolong tumour cell survival in mice by protecting them from lysis by natural killer cells.[10] It is suggested that by binding to activated platelets, tumour cells are able to adhere better to the endothelium (*figure 1*). Moreover, they secrete cytokines increasing the permeability of the vessel wall, thereby enabling dissemination in the surrounding tissue.[9,11]

Elevated vWF levels have been reported in various cancers in humans, including breast cancer and colorectal cancer.[12-15] In the latter it has been shown that vWF levels are associated with tumour stage and metastases.[15] Experimental models *in vitro* and *in vivo* suggest that vWF facilitates binding of platelets to tumour cells thereby hiding the tumour cells from the immune system and enabling the attachment of tumour cells to the endothelium.[9] It has been demonstrated that tumour cells express the GPIb and the GPIIb/IIIa receptor.[16] These receptors can bind the tumour cell to vWF and to platelets (*figure 1*). Patients with disseminated cancer also have a significant increase in unusually large vWF multimers which facilitates further binding to tumour cells. This presence of unusually large vWF multimers is the result of a local acquired deficiency of vWF cleaving protease (ADAMTS 13).[17]

In conclusion, there is cumulating evidence for an important role of platelets and vWF in tumour growth and dissemination.

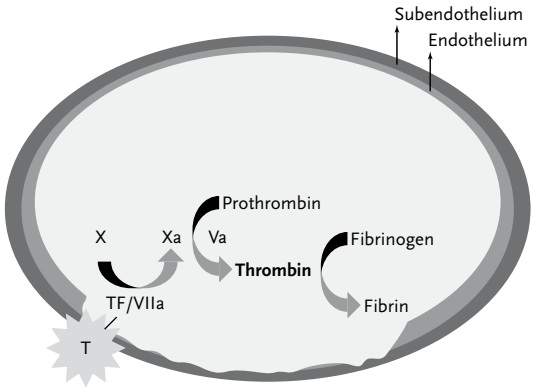
ACTIVATION OF THE SECONDARY HAEMOSTATIC SYSTEM

In normal secondary haemostasis a fibrin clot is formed at the site of a vascular lesion by activation of a coagulation pathway starting with the exposition of subendothelial tissue factor (TF) eventually leading to the conversion of fibrinogen to fibrin. This TF has also been thought to play a pivotal role in cancer-induced hypercoagulability.

TF is the key initiator of the coagulation cascade.[18] In the first or initiation phase TF activates coagulation factor VII to factor VIIa. The formed TF/factor VIIa complex directly activates coagulation factor X to Xa. Together with factor Va, factor Xa is responsible for the conversion of prothrombin to thrombin (i.e. factor II to IIa). Thrombin induces clot formation by inducing the conversion of fibrinogen to fibrin (*figure 2a*).

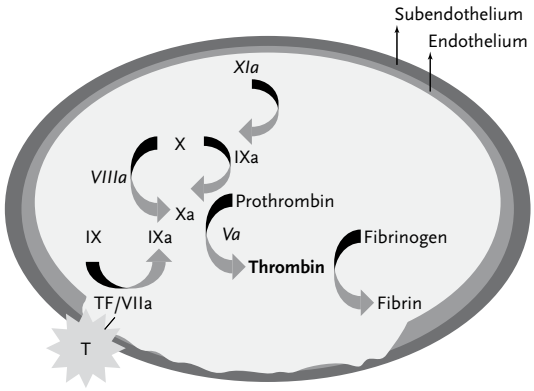
In addition to directly activating factor X, in the next or propagation phase, the TF/factor VIIa complex also indirectly activates factor X to Xa by activating coagulation factor IX to IXa which, together with factor VIIIa, also activates factor X to Xa. Again this leads to the conversion of prothrombin to thrombin and fibrinogen to fibrin (*figure 2b*). Thrombin induces clot formation not only by inducing the conversion of fibrinogen to fibrin but also by directly activating platelets and by stimulating its own formation by activating clotting factors V, VIII and XI (*figure 2b*). Negatively charged phospholipids (e.g. the platelet membrane) and calcium are essential in the whole process of fibrin formation.

Figure 2a. *The initiation phase of the secondary haemostatic system and the activation by tissue factor (TF) on the tumour cell (T)*



The TF/VIIa complex activates factor X to Xa, which, together with factor Va, activates factor II (prothrombin) to factor IIa (thrombin). Finally, thrombin catalyses the conversion of fibrinogen to fibrin.

Figure 2b. *The propagation phase of the secondary haemostatic system and the activation by tissue factor (TF) on the tumour cell (T)*



The TF/VIIa complex also activates factor IX to IXa, which, together with factor VIIIa, activates factor X to Xa. Consequently, this leads to the conversion of prothrombin to thrombin and fibrinogen to fibrin. Thrombin stimulates its own formation by directly activating the clotting factors V, VIII and XI.

TF is normally only localised in extravascular tissues not directly in contact with the blood stream. In case of a vascular lesion the subendothelial TF will be exposed to the blood resulting in platelet activation, fibrin formation and closing of the lesion. In cancer patients, however, TF is expressed aberrantly on endothelial cells, monocytes and, most importantly, on tumour cells themselves (*figures 2a* and *2b*). Moreover, cancer cells may produce a cysteine proteinase, known as cancer procoagulant (CP), which directly activates coagulation factor X to Xa.[19]

Endothelial cells do not normally express TF. TF on endothelial cells is induced by cytokines as TNF-α and IL-1β produced by tumour cells.[20] Moreover, these tumour cytokines induce expression of adhesion molecules on endothelial cells, making them capable of attaching other tumour cells.[21] This accumulation of tumour cells leads to increased cytokine production and thereby increased TF expression on the endothelial cells. It is suggested that this is a major contribution to the cancer-induced hypercoagulability.

Monocytes do not normally express TF. They do express TF when they are activated by stimulating agents such as bacterial endotoxins, inflammatory cytokines and complement factors. TF on monocytes has been demonstrated in cancer patients mainly in *in vitro* studies. Isolated monocytes obtained from cancer patients expressed more tissue factor than monocytes from healthy controls.[22-26] No studies have been performed with direct *in vivo* measuring of the TF expression on monocytes. However, TF expression on monocytes is still thought to have a major role in cancer-induced hypercoagulability.[27]

TF expression on tumour cells has been shown in many cancers, including breast cancer, lung cancer, colorectal cancer and pancreatic cancer. Elevated levels of tissue factor on tumour cells have been correlated with increased angiogenesis, increased vascular density, unfavourable prognosis and advanced disease.[28-31] TF on tumour cells is considered another important factor in the cancer-induced hypercoagulability and plays a pivotal role in angiogenesis. In preclinical studies TF-deficient mice died after ten days of embryonic development because of abnormal formation of yolk-sac vessels, suggesting a role for TF in physiological angiogenesis.[32] The same applies to VEGF-deficient mice, suggesting that TF and VEGF regulate similar functions.[33] Expression of TF in tumours upregulates the expression of VEGF, thereby inducing a switch to a more angiogenic phenotype and inducing sprouting of new blood vessels from pre-existing vessels.[34] Tumour cells overexpressing TF grew more rapidly and formed a larger and more vascularised tumour than TF underexpressing tumour cells.[34]

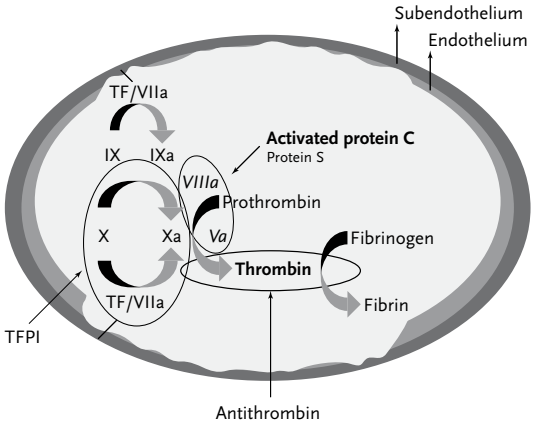
In conclusion, TF plays a central role in the activation of the coagulation system in cancer-related thrombosis and in the enhancement of angiogenesis, tumour growth and tumour metastasis

CHANGES IN THE ANTICOAGULANT SYSTEMS

In normal haemostasis there is a terminating system to prevent ongoing clotting and to confine the fibrin clot to the site of the vascular lesion. Key players in this system are tissue factor pathway inhibitor (TFPI), antithrombin (AT) and activated protein C. TFPI, which is synthesised in the endothelium, is the natural inhibitor of TF. It binds to the TF/factor VIIa complex and binds directly to factor Xa, thereby terminating the initiation phase of the coagulation cascade.[35] Antithrombin is the (slow) inhibitor of coagulation factors IXa, Xa and thrombin, thereby terminating the propagation phase. Its effect can be greatly accelerated by heparins.[36] Activated protein C (aPC), together with its cofactor protein S, inhibits the activity of coagulation factors VIIIa and Va, contributing to the termination of the propagation phase (*figure 3*). Vitamin K-dependent protein C is activated to aPC on the surface of endothelial cells by thrombin bound to the membrane glycoprotein thrombomodulin. The endothelial protein C receptor (EPCR) further stimulates protein C activation.[37]

Decreased activation of the anticoagulant factors TFPI, antithrombin and the proteins of the protein C pathway could lead to activation of haemostasis in cancer patients. Indeed, decreased levels of antithrombin and protein C have been reported.[38] Moreover, there are strong indications that cancer patients without the factor V Leiden mutation have an acquired aPC resistance.[39-41]

Figure 3. *The initiation phase (lower part) and propagation phase (upper part) of the secondary haemostatic system and the termination by tissue factor pathway inhibitor (TFPI), antithrombin and activated protein C in the termination phase*



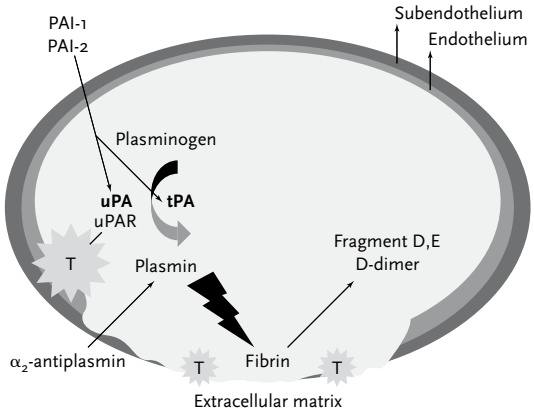
TFPI is the inhibitor of the TF/VIIa complex and factor Xa, antithrombin is the inhibitor of thrombin and activated protein C, together with its cofactor protein S, inhibits the activity of coagulation factors VIIIa and Va.

On the contrary, elevated plasma levels of TFPI have been demonstrated in patients with solid tumours.[42,43] TFPI-1 is the main inhibitor of TF, factor VIIa and factor Xa and directly binds cancer cells to the extracellular matrix, thereby promoting cancer cell migration.[44] TFPI-2 has a low inhibitory activity to TF, factor VIIa and factor Xa, but is a potent inhibitor of plasmin. Plasmin is a protease able to degrade the extracellular matrix directly or indirectly by activating matrix metalloproteinases. These matrix metalloproteinases degrade collagen and other matrix proteins, thereby allowing tumour cells and monocytes to invade the extracellular matrix and the surrounding tissues.[45] TFPI-2 inhibits the plasmin-mediated activation of matrix metalloproteinases involved in tumour progression, invasion, and metastasis.[46] Thus, elevated levels of TFPI-1 stimulate and elevated levels of TFPI-2 inhibit growth and dissemination of cancer cells.
In conclusion, there is cumulating evidence of an important role for the anticoagulant proteins in cancer biology.

ACTIVATION OF THE FIBRINOLYTIC SYSTEMS

The fibrin formed in the initiation and the propagation phase of the secondary haemostatic system is strengthened by thrombin-activated factor XIII, catalysing the formation of cross-links between adjacent fibrin chains to yield the mature clot.[47] The fibrinolytic system is responsible for the lysis of these fibrin clots. In normal fibrinolysis plasminogen is converted to plasmin by activation of tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA). Plasmin cleaves the fibrin network and releases fibrin degradation products fragment D, fragment E and D-dimer. Plasmin is inactivated by α2-antiplasmin with the formation of plasmin-α2-antiplasmin (PAP) complexes. The activity of tPA and uPA is inhibited by specific inhibitors, plasminogen activator inhibitor (PAI) 1 and 2 (*figure 4*).
It has been demonstrated that the fibrinolytic system, in particular the urokinase-type plasminogen activator system, is involved in the process of tumour cell invasion and metastasis. Urokinase-type plasminogen activator binds to the urokinase-type plasminogen activator receptor (uPAR), which is present on tumour cells and monocytes, thus facilitating the conversion of plasminogen to plasmin. Plasmin is a protease not only able to cleave the fibrin network of a clot but, as mentioned before, also able to degrade the extracellular matrix, thereby allowing tumour cells and monocytes to invade the extracellular matrix and the surrounding tissues (*figure 4*).[45]
Elevated tumour levels of uPA, uPAR and PAI-1 are associated with poor prognosis in various malignancies, including cancers of the lung, stomach, colorectum, bladder, ovary and breast.[48] Several studies have been carried out in patients with breast cancer. Breast cancer patients with high tumour levels of uPA had a significantly shorter disease-free and overall survival and the tumour uPA level was a strong prognostic marker in node-positive as well as node-negative breast cancer patients.[49-55] High tumour levels of uPAR were associated with a shorter disease-free and overall survival, particularly in the subgroup of node-positive postmenopausal women with breast cancer.[56,57] Tumour PAI-1 was a strong independent prognostic factor and an important parameter to predict metastatic potential in both node-negative and node-positive breast cancer patients.[53,58,59] On the contrary, elevated tumour PAI-2 levels have been associated with favourable prognosis.[60,61] It has been demonstrated that the plasma levels of soluble uPAR are significantly increased in stage IV breast cancer patients.[62-64]
Elevated D-dimer levels, indicating the degradation of fibrin by the fibrinolytic system, have been described before in breast cancer patients as well as in various other cancers.[65] Recently it has been demonstrated that in breast cancer patients preoperative plasma D-dimer levels correlate with clinical stage and axillary lymph node status.[66] Moreover, in patients with metastatic breast cancer plasma D-dimer levels correlated with tumour volume, progression rate and survival.[67] Plasma D-dimer levels were significantly elevated in breast cancer patients with metastases compared with patients without metastases and were highly significantly correlated with survival.[64]

Figure 4. *Involvement of the fibrinolytic system in the process of tumour cell invasion and metastasis*



In normal fibrinolysis plasminogen is converted to plasmin by activation of tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA), cleaving the fibrin network and releasing fibrin degradation products fragment D, fragment E and D-dimer. Plasmin is inactivated by α-2-antiplasmin; tPA and uPA are inhibited by plasminogen activator inhibitor (PAI) 1 and 2. In cancer patients uPA binds to the urokinase-type plasminogen activator receptor (uPAR) on tumour cells, facilitating the conversion of plasminogen to plasmin, thereby cleaving the fibrin network and degrading the extracellular matrix, allowing tumour cells (T) to invade the extracellular matrix and the surrounding tissues.

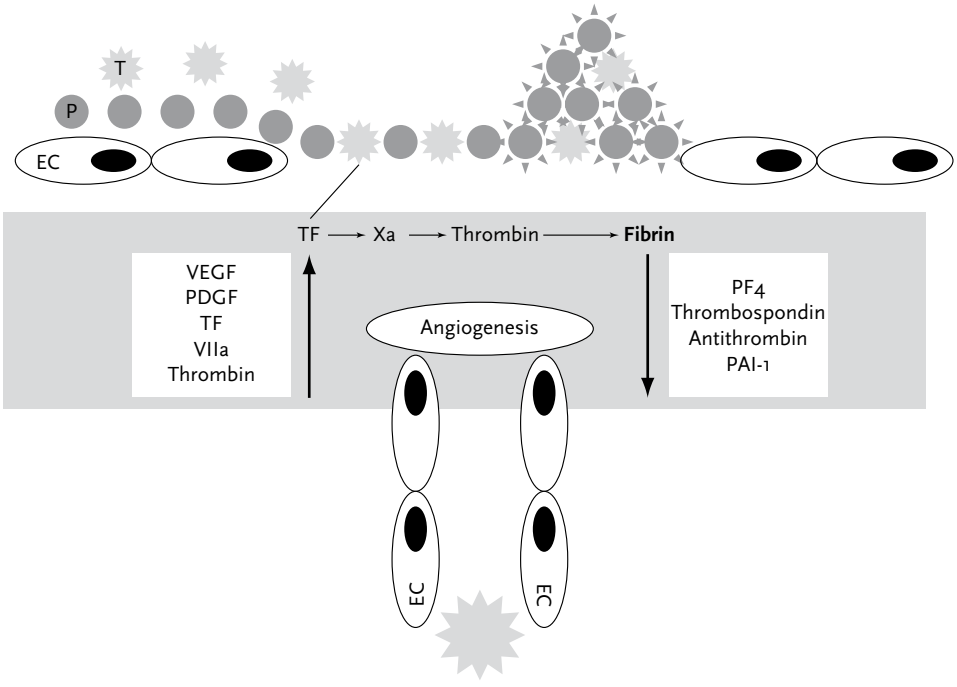
In conclusion, the fibrinolytic system seems to play a significant role in the process of tumour cell invasion and metastasis.

ANGIOGENESIS

Angiogenesis is the development of new blood vessels from the existing vasculature. It occurs in a highly regulated manner in normal physiological processes as wound healing. Angiogenesis is closely related to the haemostatic system in case of vascular damage. Following injury, the haemostatic system regulates platelet adherence and fibrin formation, thereby stopping the bleeding; the angiogenic system regulates the formation of new blood vessels, a vital step in healing the wound. Once clot stabilisation is achieved, angiogenesis is modulated by proteins and peptide fragments generated from the coagulation and fibrinolytic systems.[68-70] The fibrin clot, formed in the haemostatic process, serves as a matrix for migrating endothelial cells. This process is tightly regulated by a local balance of pro- and antiangiogenic factors. Proangiogenic factors stimulate migration, proliferation and differentiation of endothelial cells whereby new vessels are formed. Proangiogenic factors identified in the haemostatic system include platelet release products, such as VEGF and platelet-derived growth factor, and coagulation proteins as thrombin, TF, factor VII and factor XIII. Antiangiogenic factors characterised from the haemostatic system include other platelet release products, as platelet factor 4 and thrombospondin, and coagulation proteins as antithrombin and PAI-1.[68,69] In close cooperation, the haemostatic and angiogenic systems quickly repair the damaged blood vessel.

In 1971 Folkman first described the pivotal role of angiogenesis in tumour growth.[71] For their growth tumours need oxygen and essential nutrients. When tumours are very small, oxygen and nutrients can diffuse into the tumour cells. In order to grow and metastasise a tumour has to develop an adequate vasculature. By activating the haemostatic system, tumour cells induce the production of proangiogenic factors as thrombin, TF and factor VII, thereby creating the environment for the formation of new blood vessels, comparable with physiological angiogenesis. The fibrin locally formed by activation of the haemostatic system by the tumour cells provides the matrix on which the new (often thin-walled, leaky and poorly organised) blood vessels are being formed by stimulation of the proangiogenic factors (*figure* 5). The newly formed blood vessels allow the

Figure 5. *Activating the haemostatic system by tumour cells and platelets leads to the production of proangiogenic factors and antiangiogenic factors thereby creating the environment for the angiogenesis*



VEGF = vascular endothelial growth factor; PDGF = platelet-derived growth factor; TF = tissue factor; VIIa = activated coagulation factor VII; PF4 = platelet factor 4; PAI-1 = plasminogen activator inhibitor 1; EC = endothelial cell; P = platelet; T = tumor cell. The fibrin locally formed by activation of the haemostatic system by the tumour cells provides the matrix in which the new blood vessels are formed.

tumour to grow more rapidly and increase the surface area through which the tumour cells can escape and metastasise.[68]

Because coagulation factors are proteases that have many additional functions in (cancer) cell regulation, the idea that coagulation activation in cancer patients is solely linked to angiogenesis is an optimistic short cut. For example, direct cell signalling by coagulation proteases activating protease-activated receptors (PARs) leads to proliferation and invasiveness of cancer cells. This shows that other mechanisms, many yet to be elucidated, might be more important than regulation of angiogenesis.[72,73]

In conclusion, tumour cells activate the haemostatic system as an essential step in the formation of new blood vessels, in order to grow and eventually metastasise.

ANTICOAGULANTS AND CANCER

Because cancer cells need the coagulation system for their growth, angiogenesis and dissemination through the body, it has been hypothesised that anticoagulants might have an antitumour effect. First coumarin derivates were studied. Although promising results were shown in animal studies, clinical studies in humans are limited and results are conflicting. No differences in survival were observed between warfarin-treated and control groups for advanced non-small-cell lung, colorectal, head and neck and prostate cancer.[74] However, warfarin therapy was associated with a significant prolongation in disease-free and overall survival in patients with small-cell lung cancer.[74] Remarkably, in patients treated with coumarins for six months after a venous thromboembolism significantly fewer (urogenital) cancers were found compared with patients treated for six weeks.[75] However, the incidence of clinically overt cancer was not reduced in patients with idiopathic venous thromboembolism treated with oral anticoagulants for one year compared with three months.[76] More studies have been carried out with low-molecular-weight heparins (LMWH). In studies comparing LMWH and coumarins in the treatment of new patients with a venous thromboembolism three-month mortality data suggested a survival advantage for the patients on LMWH. Subgroup analysis showed that this increased survival was in the cancer patient group.[77,78] Recently it has indeed been shown that LMWH improves survival, although in all studies the effect seems limited to the patient groups with the relatively better prognosis.[79-81] Moreover, adding LMWH to chemotherapy in small-cell lung cancer improved survival compared with chemotherapy alone.[82] It is suggested that apart from the effect of LMWH on coagulation, other mechanisms influenced by heparins are also involved.[83,84] More studies treating cancer patients with LMWH or newer antithombotics, as pentasaccharides and oral thrombin inhibitors, are currently underway.

FUTURE PERSPECTIVES

Several research groups are continuously investigating the molecular pathophysiology of the activation of the coagulation system by tumour cells. At first this was mainly a topic for haematologists. However, since it has been demonstrated that anticoagulant treatment with LMWH might prolong survival in cancer patients, oncologists have been alerted. The survival advantage shown with LMWH in selected patients seems comparable with the survival advantage demonstrated with the very expensive targeted drugs currently used in oncology.[79-82] New studies in various malignancies will follow soon and will attract more attention from oncologists. When the effect of LMWH has been definitely proven in patients with advanced disease, the next step will be to add LMWH to adjuvant treatment in cancer patients. The main goal of adjuvant treatment in, for example, breast or colorectal cancer is to prevent the development of local recurrence and distant metastases. This is currently achieved with standard chemotherapy. Trials in breast cancer have shown that adding targeted therapy with monoclonal antibodies is improving disease-free survival in certain types of breast cancer.[85,86] By adding this targeted immunotherapy to the standard therapy a substantial reduction in recurrences has been achieved. When it has indeed been proven that LMWH prolongs survival in advanced disease, LMWH treatment in the adjuvant setting added to the standard adjuvant treatment with chemotherapy and targeted therapy might give a further reduction in recurrences and distant metastases in breast cancer patients and other cancers. Further understanding of the pathophysiology of the hypercoagulability in cancer will lead to the development of new tools in conquering the cancer.

CONCLUSIONS

Almost one and a half century after Armand Trousseau first noted the hypercoagulability in cancer patients, we are beginning to understand that many proteins of the haemostatic and fibrinolytic system play a pivotal role in tumour biology. By activating the haemostatic and fibrinolytic systems, tumour cells are able to grow, form new blood vessels and metastasise. This activation of the coagulation system leads to an increased risk of thromboembolism in cancer patients. More insight in the underlying mechanisms might lead to the discovery of new agents that interfere with vital processes in tumour behaviour. Armand Trousseau could only dream of these developments.

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