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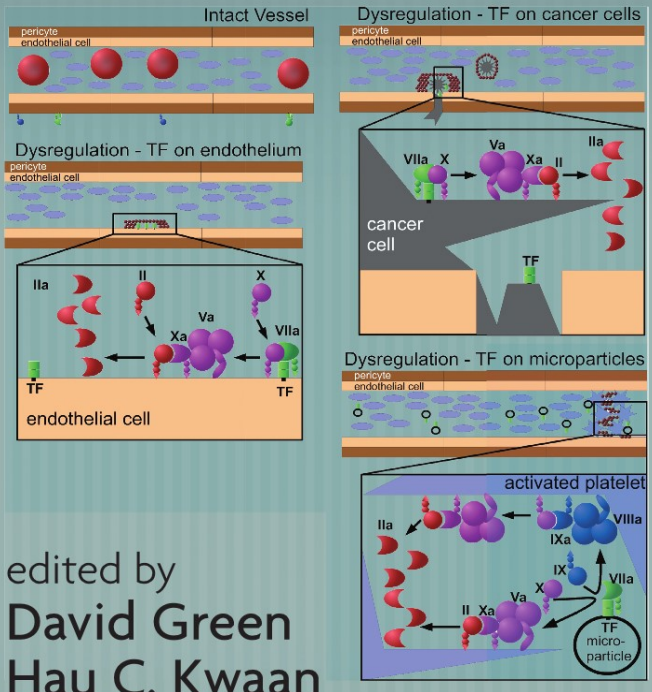
Coagulation in Cancer



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Preface

During the past few years there has been considerable progress in elucidating the effect of cancer on the hemostatic mechanism. In a series of updated reviews, the contributors to this book describe the effects of cancer on coagulation and coagulation on cancer. In the first chapter, Monroe and Hoffman present their current concept of hemostasis, with an emphasis on the cell-based mechanism they recently delineated [1]. The authors describe how this system is perturbed by tumors. Next, Green and Karpatkin describe how platelets and thrombin interact with malignant cells, enhancing tumor cell adhesion and metastasis. In addition to activating platelets, thrombin activates several clotting factors, including factor XIII. Cancer promotes thrombosis by dysregulating tissue factor, cyclooxygenase, and plasminogen activator inhibitor-1, as discussed by Rickles and Falanga. In addition, procoagulants are active in oncogenesis and tumor metastasis. For example, fibrinogen produced by cancer cells promotes the growth of lung and prostate cancer cells through interaction with fibroblast growth factor-2 [2]. Activated FXIII supports the early survival of micrometastases in a mouse model [3]. Components of the plasminogen-plasmin system also play an important role in tumor growth, invasion, and metastasis as described by Kwaan and McMahon. In addition, cancer associated changes in this system increase the risk of bleeding and thrombotic complications. In the final chapter in this section, Sidhu and Soff explain how cancer-induced activation of coagulation promotes angiogenesis which enhances tumor cell proliferation, and how this phenomenon may be manipulated to curb tumor growth.

In the next section, Matzdorff and Green provide an overview of cancer-associated thrombosis, beginning with an historical note on Armand Trousseau, who emphasized the association between malignancy and thrombosis [4]. (Fig. 1-Trousseau). Ashrani and Heit review risk factors for thrombosis in cancer patients, noting that the risk varies by tumor type, stage of disease, and a number of patient-specific factors including inherited thrombophilia. For example, approximately 3% of lung cancer patients will develop venous thromboembolism within 2 years [5], and cancer patients with factor V Leiden have a 12.1-fold increased risk of thrombosis as compared to those without this mutation (5.1-fold) [6]. Specific thrombotic disorders associated with malignancy are tumor-associated microangiopathy, disseminated

Fig. 1 Armand Trousseau, 1801–1867

intravascular coagulation (DIC), and migratory thrombophlebitis. Zakarija discusses thrombotic microangiopathies, their clinical manifestations and management. Saba, Morelli, and Saba review DIC. They note that the interplay of many mediators from the circulation, the cancer cells, and the host cells may be responsible for a particular thrombotic manifestation. The authors emphasize that control of the tumor is of primary importance, but there are other approaches that may be helpful in limiting the coagulopathy. In the next chapter, Tefferi reports that major thromboses at the time of diagnosis are found in 9.7–29.4% of patients with essential thrombocythemia, and in 34–38.6% of patients with polycythemia vera. He discusses the current management and risk stratification in these disorders as well as in primary myelofibrosis.

Iatrogenic thromboses in cancer patients may be due to chemotherapy and intravenous catheters. These risks are intensified by patient characteristics such as the site of the cancer, marked obesity (body mass index $>35\,kg/m^2$), anemia, thrombocytosis, or leukocytosis [7]. Ashrani and Rajkumar review the chemotherapeutic agents most commonly associated with thrombosis, and outline measures to prevent thrombotic complications when using these drugs. The topic of catheter-related thromboses is addressed by Freytes, who notes that there has been a recent decline in the frequency of this complication. Anticoagulant prophylaxis is probably not warranted for most patients needing central venous catheters, but when catheter-related thrombosis does occur, the use of antithrombotic agents is warranted. Allen and Bhat address thrombotic problems in children with cancer, and describe the diagnosis and management options specific to this population. Next, Lee discusses the management of venous thrombosis in cancer patients. The choice of anticoagulant, and the dose and duration of therapy, are described.

The treatment of the cancer patient presents certain challenges not present in patients without malignancies. These include the risk of bleeding due to chemotherapy-induced thrombocytopenia, recurrent and refractory venous thromboembolism, and concerns about maintaining the quality of life of patients with advanced disease. In the final chapter of this section, there is an examination of the effects of anticoagulants on cancer. The pioneering work of Zacharski [8, 9] suggested that the administration of warfarin to patients with small cell carcinoma of the lung resulted in a longer time to disease progression ($p = 0.016$) and improved overall survival ($p = 0.018$). We reported lower mortality in cancer patients treated with low molecular weight heparin as compared with standard heparin, and this was not due to a difference in deaths from thrombosis or bleeding [10]. Pineo and Hull review the literature regarding the beneficial effects of heparins on cancer survival and the effects of these anticoagulants on experimental models of tumor growth and metastasis. They illustrate the many pathways in hemostasis and angiogenesis that may be influenced by heparins and related compounds.

The final section of the book discusses bleeding problems in cancer, beginning with a review of cancer-associated thrombocytopenia by Eklund. A diagnostic algorithm for assessing thrombocytopenia in the cancer patient is presented, and the use of platelet transfusions for patients with decreased platelet production, as proposed by the American Society of Clinical Oncology [11], is discussed. Next, Zangari, Elice, Tricot, and Fink describe bleeding disorders associated with dysproteinemias. Bleeding is most frequent in patients with amyloidosis or Waldenstrom's macroglobulinemia, but hemorrhage due to many other types of circulating clotting inhibitors has been described. In particular, acquired von Willebrand's disease and specific inhibitors of factor VIII may be associated with recurrent and severe bleeding [12, 13]. The treatment of bleeding problems in cancer patients is addressed by Pereira. The use of topical agents, laser photocoagulation, and palliative embolization, as well as systemic therapy, is described, and the chapter concludes with a discussion of blood component usage.

This book is aimed at informing professionals working in the field of cancer about the pathophysiologic mechanisms of cancer-related thrombosis and bleeding. It will provide assistance in recognizing the various bleeding and clotting disorders associated with cancer. Further, it includes current recommendations for the management of hemorrhage, and prevention and treatment of thrombosis in the patient with malignancy. The editors anticipate that it will be a useful addition to the literature on cancer and coagulation.

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Part I
Alterations in Hemostasis Due to Cancer

Chapter 1
Dysregulation of Hemostasis by Cancer

Dougald M. Monroe and Maureane Hoffman

Dysregulation of the coagulation system in cancer patients can lead to both thrombosis and bleeding. An examination of the normal hemostatic processes suggests possible mechanisms by which a breakdown in the physiologic mechanisms might lead to the dysregulations observed in cancer.

1.1 Model of Coagulation

In many conceptual models of coagulation, the maintenance of hemostasis has been attributed to a strict segregation of the initiating protein (thromboplastin or tissue factor) to the outside of the blood vessel and the procoagulant proteins on the inside of the blood vessels. In these models coagulation occurs because a break in the blood vessel allows contact between plasma proteins and tissue factor. Current data support a more complex picture and suggest that interactions between the coagulation proteins is not the dominant control mechanism in coagulation; rather the dominant control mechanism is regulation by various cell types.

It is clear that intact healthy blood vessels do not provide an absolute barrier to the passage of plasma proteins [1]. Studies on the half-life of coagulation proteins suggest that they circulate through the extravascular space [2]. This process serves to concentrate at least two coagulation proteins around blood vessels where there are specific receptors. Factor IX has a specific, tight (nM) binding site for collagen IV and appears to localize around blood vessels in the same sites as collagen IV [3]. Tissue factor is present to varying degrees in a number of tissues [4]; in addition, tissue factor is found on the pericytes that surround larger vessels [5]. Recent data suggest that this tissue factor has factor VII(a) bound to it even in the absence of any detectable vascular injury [6]. These factor VIIa/tissue factor complexes have been implicated in the low level activation of coagulation proteins referred to as idling [7].

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Procoagulant factors are activated at low levels during the process of idling, so that, even in the absence of injury, activated coagulation factors are present in the tissues surrounding the vasculature. This process accounts in part for what has been called the "hemostatic envelope" that surrounds vessels [4]. However, coagulation only proceeds to fibrin formation following a break in vessel wall when platelets and the larger coagulation factors such as factor VIII/von Willebrand factor, factor V, and fibrinogen come in contact with the extravascular space. The idling process allows for rapid progression of coagulation after an injury.

Coagulation leading to hemostasis can be thought of as occurring in three overlapping phases: initiation, amplification, and propagation. The process of hemostasis is limited to an area of injury through the active participation of undamaged endothelium outside the area of injury.

1.1.1 Initiation

In the initiation phase (Fig. 1.1), a disruption in the vasculature allows platelets to flow across collagen in the extravasculature. Platelets adhere through interactions between collagen and a number of platelet receptors, including glycoprotein VI and the glycoprotein Ib/IX/V complex (mediated by von WIllebrand factor) [8]. These platelets become partially activated and can degranulate. This degranulation releases partially active factor V [9]. Bleeding also brings plasma factors to the area of injury. Additional activated factor IX and factor X, beyond that formed during the idling process, can be rapidly produced by the preformed factor VIIa/tissue factor complexes surrounding the blood vessels [10]. Factor Xa can complex with the factor Va deposited by the activated platelets and convert some prothrombin to thrombin. This thrombin generation can promote local fibrinogen conversion to fibrin resulting in deposition of fibrin at the margins of the wound area [11, 12].

1.1.2 Amplification

The major role of thrombin formed in the initiation phase is to act in an amplification phase (Fig. 1.1) that activates platelets throughout the wound bed [11]. Thrombin formed in the initiation phase can localize to platelets through a specific binding site on glycoprotein Ib [13]. There thrombin activates platelets through cleavage of the Protease Activated Receptors (PAR1 and PAR4). It has been established that activation of platelets with different agonists can lead to different levels of platelet activity [14]. These different levels of platelet activity are due in part to enhanced binding of coagulation factors on platelets stimulated with different agonists, especially combined simulation with thrombin and collagen [15, 16]. Thus platelets at the margins of the wound are highly activated relative to platelets aggregated onto each other or onto a fibrin matrix [17].

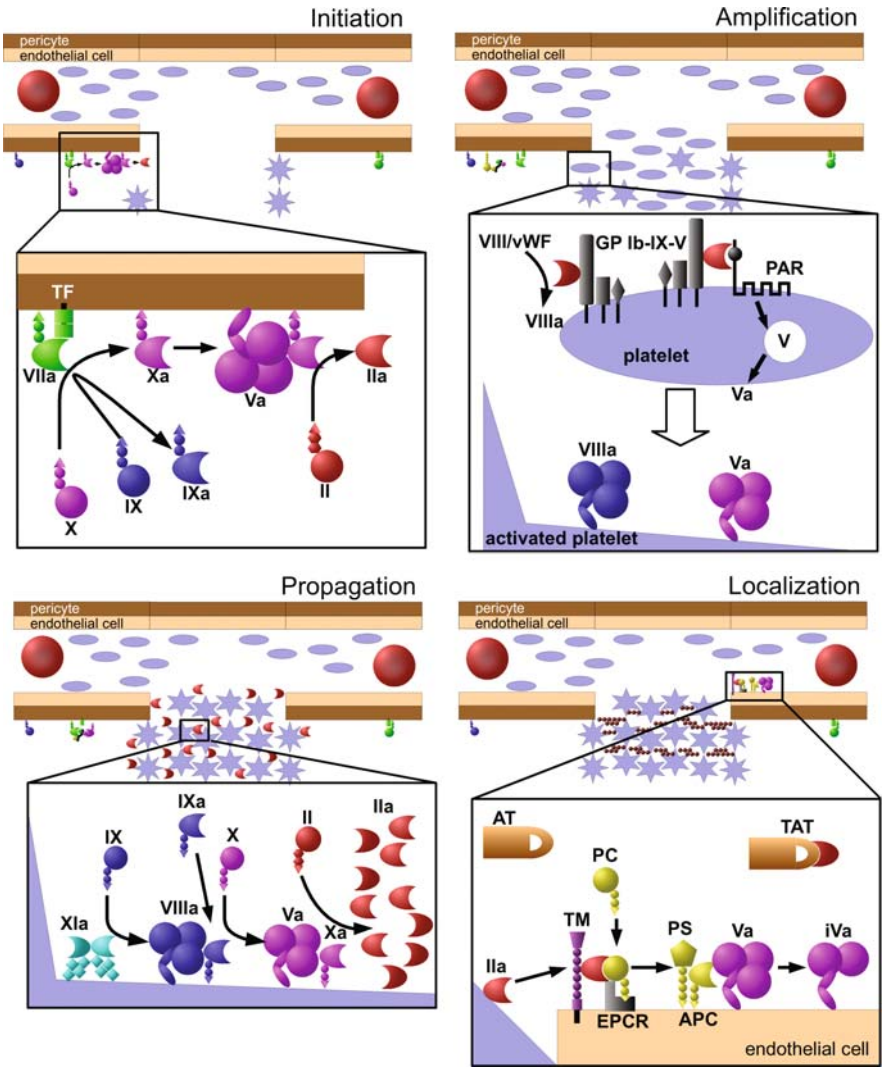

Fig. 1.1 Hemostasis can be visualized as three overlapping phases: *Initiation*, *Amplification*, and *Propagation*. Active processes *Localize* coagulation to the area of injury. *Initiation:* Platelets adhere and degranulate, releasing factor Va. Factor VIIa/tissue factor (TF) activates factors IX and X. Factor Xa complexes with factor Va to convert some prothrombin (II) to thrombin (IIa). *Amplification:* Thrombin formed in the initiation phase binds to the glycoprotein (GP) Ib-IX-V complex on platelets and activates platelets through a signaling process initiated by cleavage of PAR1. Platelet activation causes platelet degranulation releasing factor V. Factor VIII is cleaved from Von Willebrand factor (VWF) and released onto the platelet surface as factor VIIIa. *Propagation:* Factor IXa combines with factor VIIIa to activate factor X. This platelet surface factor Xa combines with factor Va to provide for a strong burst of thrombin. This thrombin can participate in a positive feedback loop by promoting activation of factor XI on the platelet surface leading to additional factor IXa. *Localization:* Thrombin released from activated platelets is swept downstream and inhibited

Platelet activation results in degranulation that releases partially active factor V from platelet alpha granules [9]. Von Willebrand factor, which carries factor VIII, is also bound to platelet glycoprotein Ib so it is likely that factor VIII and thrombin are thereby brought into proximity. Thrombin cleaves factor VIII, releasing it onto the platelet surface [18, 19]. At the end of the amplification phase the activated platelet surface, with both activated factor V and factor VIII, is primed for rapid thrombin generation.

1.1.3 Propagation

In the propagation phase (Fig. 1.1), factor IXa, activated by factor VIIa/tissue factor in the initiation phase, associates with the platelet surface and combines with factor VIIIa to convert factor X to factor Xa on the platelet surface. This platelet surface generation of factor Xa combines with factor Va to provide a strong burst of thrombin generation. This thrombin can participate in a positive feedback loop by promoting activation of factor XI on the platelet surface, leading to additional factor IXa and more factor Xa and thrombin [20]. A strong burst of thrombin during the propagation phase seems to be a key component in forming a stable fibrin structure leading to a durable platelet clot [21, 22].

Factor Xa generated by factor VIIa/tissue factor during the propagation phase is not equivalent in function to factor Xa generated on activated platelets. Factor Xa bound to factor Va and in the presence of prothrombin is resistant to inhibition [23]. However, FXa that dissociates from the cell membrane is rapidly inactivated by the inhibitors antithrombin and tissue factor pathway inhibitor. Thus factor Xa is probably confined to acting on the surface where it is generated. While factor Xa generated by factor VIIa/tissue factor can promote some thrombin generation, this thrombin generation by itself does not appear sufficient to stabilize the entire wound area. When the propagation phase is defective due to a lack of platelet surface thrombin generation (as in hemophilia), the platelet plugs are not stabilized by a strong fibrin mesh [11] and thus the patient is prone to delayed bleeding [12].

1.1.4 Localization

The platelet thrombin generation process appears to be self-limiting; tissue factor initiated thrombin generation in whole blood does not result in complete conversion of prothrombin to thrombin [24]. Blood samples from different

Fig. 1.1 (continued) by antithrombin (AT). Thrombin that moves to endothelial cells binds thrombomodulin (TM); this IIa/TM complex cleaves protein C to activated protein C (APC). Cleavage is accelerated when protein C is bound to the endothelial cell protein C receptor (EPCR). APC cleaves and inactivates factor Va (iVa)

individuals show very reproducible limitations on their prothrombin conversion [24]. However, it is not clear that the coagulation complexes on platelets are actively shut down. Recent data suggest that active complexes might persist on platelets for an extended time [25]. These preformed complexes could quickly generate thrombin if damage to the plug brings in a fresh supply of procoagulant proteins.

Even if coagulation is not actively shut down, it does appear to be limited by a number of forces. One force limiting coagulation is flow. When blood vessels are broken, the initial platelet plug creates an area of stasis where coagulation proceeds [21]. However, at the interface between the platelet plug and intact vessels there is still an area of flow. Activated factors released from the margins of the wound or from activated platelets are swept downstream where they can be acted on by inhibitors.

Intact endothelial cells have active mechanisms to prevent platelets from adhering. Among these mechanisms are production of prostaglandins and ectonucleosidase that inhibit platelet activation [26]. Endothelial cells also have active mechanisms to shut down thrombin generation (Fig. 1.1). Thrombin that becomes associated with the endothelial surface does not participate in a positive feedback loop as seen on platelets; rather, thrombin on the endothelial surface participates in a negative feedback loop [27]. Thrombin that reaches endothelial cells binds tightly (nM) to thrombomodulin [28]. This thrombin/thrombomodulin complex converts protein C to activated protein C. Activated protein C inactivates factor V and factor VIII, resulting in the shutdown of any thrombin generating mechanism on the healthy endothelium. Thrombin/thrombomodulin activation of protein C is significantly accelerated by platelet factor 4 [29] meaning that activated platelets tend to promote this negative feedback loop on endothelial cells near a site of injury. Thus platelet activation, thrombin generation, and fibrin formation are localized to a site of injury.

1.2 Dysregulation of Coagulation in Cancer

The literature suggests that it is hard to generalize about coagulation effects across all cancers, as cancers of different origins may have very different characteristics. Nonetheless, cancer patients are at a clear risk of thrombosis ([30] and Chap. 7) and idiopathic thrombosis may be a marker for underlying, undetected cancer. For example, one large study showed a clear association between idiopathic venous thrombosis and development of cancer, an association that extended for 10 years or more after the thromboembolic event [31].

While it is very clear that cancer patients have an increased risk of thrombosis, the mechanism(s) that promote(s) this enhanced thrombosis risk are not clear and several different mechanisms have been proposed.

1.2.1 Altered Tissue Factor in Cancer

There are a number of studies that examine the role of tissue factor in alterations of hemostasis in cancer. As is known from in vitro assays, even relatively small amounts of tissue factor can promote rapid clotting in a test tube. Also, injection of sufficiently large amounts of tissue factor into animals can induce fibrinogen depletion and fibrin deposition, sometimes resulting in thrombosis [32]. So a number of studies have hypothesized that tissue factor expression might contribute to thrombosis in cancer patients.

1.2.2 Tissue Factor Expression on Tumor Cells

The earliest concept was that tumor cells themselves were the focus for thrombosis. During hematogenous spread, tumor cells transport through the blood stream and adhere at sites distant from the primary tumor. Since many tumor cells express TF and other procoagulants [33] it was reasonable to postulate that they directly activate coagulation (Fig. 1.2). During metastasis it is possible to observe a fibrin mesh work around some tumor cells and it has been suggested that this fibrin mesh plays a role in shielding the tumor from attack by the inflammatory system [34]. Also, microthrombi consisting of fibrin encapsulated metastatic cells can lodge in different tissues, providing a possible mechanism for transport of metastatic cells through the vasculature.

1.2.3 Tissue Factor on Endothelium

Upregulation of tissue factor on endothelium of vessels lining tumors has been observed in several types of cancer [35, 36]; fibrin deposition can be observed in proximity to this upregulated tissue factor [37]. Healthy endothelium does not typically express any significant amount of tissue factor [4, 5], so upregulation of tissue factor coupled with the increased vascular permeability that is associated with cancer could provide a pathway to thrombin generation on the endothelium.

Tumor cells and tumor-derived endothelial-like cells can also participate in formation of microcirculatory channels through which blood can flow. In one viral induced model of hepatic carcinoma, cells lining microcirculatory channels expressed markers of both endothelial cells and hepatic cells; also, these cells expressed tissue factor [36].

1.2.4 Tissue Factor Microparticles

While it is attractive to construct a model of coagulation in which the initiator, tissue factor, is completely separated from plasma components, such a model does not fit with a growing body of data suggesting that there are low but measurable levels of tissue factor in the circulation of even healthy individuals

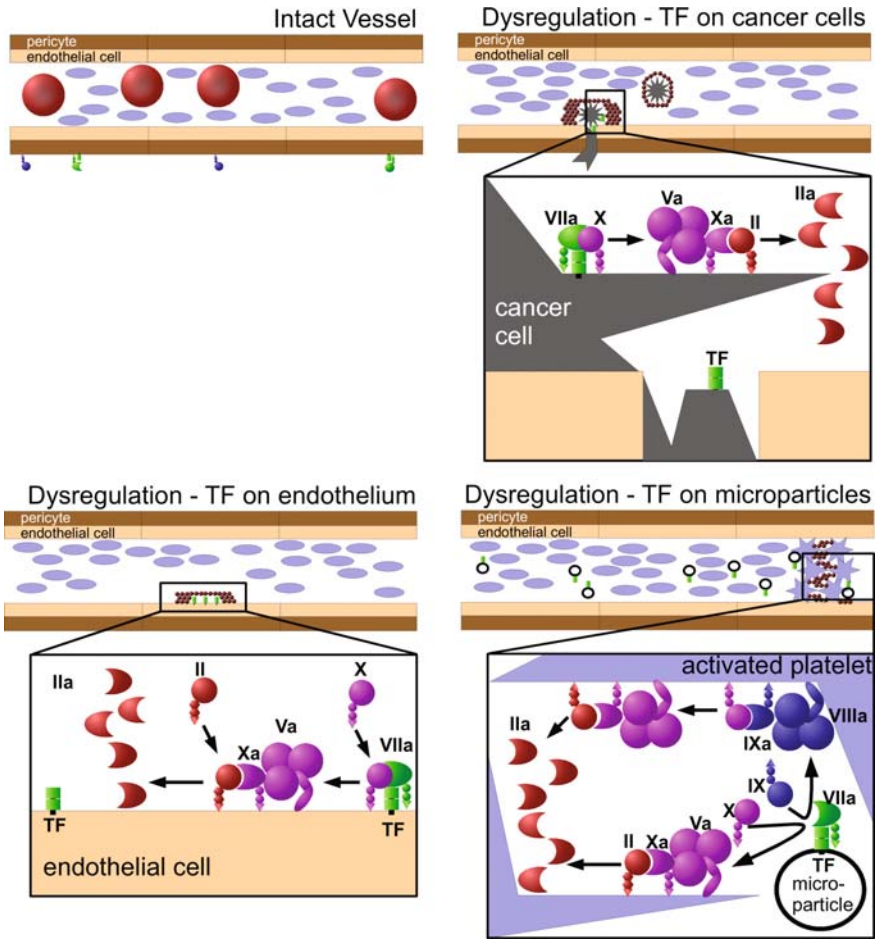

Fig. 1.2 Healthy endothelium has little procoagulant function and has active mechanisms to prevent platelet adhesion. *Tissue factor on cancer cells:* Tumor cells can be a focus for thrombosis. Tumor cells can invade the blood stream at a number of sites including junctions between endothelial cells. Tumor cells that express TF could directly activate factor X and promote factor Xa interaction with factor Va leading to thrombin (IIa) generation. This could result in formation of microthrombi consisting of fibrin encapsulated metastatic cells that could travel through the vasculature. *Tissue Factor on Endothelium:* Upregulation of tissue factor on endothelium of vessels associated with tumors could lead to VIIa/TF activation of factor X. This factor Xa, in complex with factor Va, could promote conversion of prothrombin (II) to thrombin. When thrombomodulin is downregulated, the normal negative feedback mechanisms would be overcome (compare to Fig. 1.1*, Localization*). *Tissue Factor on Microparticles:* Circulating microparticles with tissue factor (TF) have been observed in cancer patients. Association of these TF microparticles with activated platelets could promote thrombosis by giving increased activation of both factors IX and X (compare to Fig. 1.1*, Propagation*). Factor Xa with platelet released factor Va would supplement the existing propagation mechanism and enhance thrombin generation. Also, factor IXa with factor VIIIa would activate additional factor Xa, leading to even more thrombin generation. Since microparticles could potentially travel throughout the vasculature, thrombosis could occur anywhere, even at sites distant to the tumor

(see [38] for a review). This tissue factor can be found either associated with microparticles [39] or as an alternatively spliced form that has no membrane association [40]; however, it is unclear whether the alternatively spliced form can promote factor Xa and thrombin generation [41]. In animal models of thrombosis induced by stasis, microparticles containing tissue factor could enhance thrombus formation in a dose dependent fashion [42]. Another animal study has suggested that tissue factor in microparticles contributes to thrombus formation [43], but yet another study using the same mice and techniques but different injury suggested that tissue factor microparticles did not contribute significantly to thrombus formation [44].

Circulating tissue factor on either a cell or a microvesicle could bind factor VII, leading to factor VII activation. There appear to be mechanisms for regulation of tissue factor so it is not inevitable that tissue factor exposure will lead to factor Xa generation [45]; however, there is a clear possibility that circulating tissue factor can promote factor Xa generation. If these microparticles also express procoagulant lipids, this factor Xa generation can lead to thrombin production and disseminated intravascular coagulation [46]. In addition to overt DIC, a significant proportion of cancer patients experience a low grade compensated state of DIC (for a review see [47]).

There are data showing that thrombi formed under flow accumulate significant amounts of tissue factor [48]. This tissue factor accumulation is very different to than which is seen in hemostatic wounds where there is no detectable tissue factor except at the periphery of the wounds [49]. It is very reasonable to speculate, although the data are not yet conclusive, that tissue factor that becomes associated with activated platelets could contribute to the propagation phase by enhancing thrombin generation (Fig. 1.2). If so, then enhanced levels of tissue factor containing microparticles might contribute to thrombosis by disrupting the normal platelet surface regulatory mechanisms that limit thrombin generation. This thrombosis would not be limited to the site of the cancer or tumor, but could occur anywhere in the vasculature where platelet aggregation and activation is occurring.

Circulating microparticles with tissue factor levels above those found in healthy patients have been observed in cancer patients (for example see [50]). While cultured cancer cell lines can shed tissue factor positive procoagulant microparticles, it is not clear that in vivo such microparticles reach the circulation in large amounts. One careful study showed that while patients with colorectal cancer did not have an increase in the total number of microparticles, tissue factor expressing microparticles doubled. However, only 12% of this increase was from cells of unknown origin with the remainder of the increase being associated with microparticles having markers of a hematopoietic origin [51]. Another study showed that only 3% of the tissue factor microparticles had markers corresponding to the cancer cell type [52]. This suggests that the tissue factor increase may be secondary to an inflammatory response to cancer ([53] and Chap. 3). If true, these circulating tissue factor microparticles are an attractive mechanism to account for the thrombosis risk associated with cancer.

1.2.5 Inflammatory Cells and Endothelium

Some data suggest that inflammatory cells can become associated with and activated on vessels within tumors [37, 54]. These activated inflammatory cells, especially monocytes, can express high levels of tissue factor [46]. If these data are generalizable, it suggests that tissue factor might be available as an initiator on undamaged blood channels (Fig. 1.2). Coupled with low flow, this could lead to generation of sufficient thrombin and fibrin to produce occlusive thrombi [37].

1.3 Other Dysregulations of Coagulation

In addition to tissue factor mediated mechanisms of thrombosis, there are other potential disruptions of the coagulation process.

1.3.1 Platelet Activation on Endothelium

Whereas healthy blood vessels have mechanisms to prevent platelet adherence and activation, in cancer the endothelium can be disturbed or, as described above, microcirculatory channels can be lined with peusdoendothelium. In vivo models suggest that tumor cell activation of endothelial cells can promote platelet adhesion [55]. This suggests that a platelet/fibrin mass might be able to overcome localizing mechanisms in the cancer setting.

1.3.2 Downregulation of Thrombomodulin on Endothelium

In addition to upregulation of tissue factor, some data suggest that, in selected cancers, endothelial cell thrombomodulin can be downregulated (see review in [56]). Where thrombomodulin was downregulated, the prognosis was poorer with an enhanced thrombosis risk. Overall, these alterations in endothelium in cancer can result in a thrombotic phenotype due to changes in initiation, propagation, and a loss of localization mechanisms.

1.4 Summary

While there is no doubt that cancer patients are at increased risk of thrombosis and hemorrhage, the data to date do not justify suggesting one dominant mechanism to account for this observed dysregulation of coagulation. The mechanisms discussed here have focused on unusual expression of tissue factor because that constitutes the largest body of literature. However, that does not mean that strong data exist correlating thrombosis with tissue factor expression in cancer patients. A number of these studies have extrapolated from

biochemical or in vitro tissue culture work. In patients, even studies that concluded that thrombosis was correlated with some particular disruption of coagulation had a number of patients that did not show thrombosis, even in the presence of high levels of the stimulus. So, despite the large number of existing studies, the correlation between any particular mechanism and bleeding or thrombosis is relatively poor.

Establishing the factors that contribute to thrombosis and bleeding in cancer patients remains an important goal of ongoing work and the current knowledge is discussed in detail in subsequent chapters of this book. Identification of a dominant underlying mechanism, if such a dominant mechanism exists, would allow for targeted therapeutics to address the dysregulation of coagulation seen in cancer patients.

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Chapter 2
Effect of Cancer on Platelets

David L. Green and Simon Karpatkin

2.1 Introduction

Platelets play an essential role in hemostasis, the arrest of bleeding. In response to vascular injury, platelets become adherent and undergo activation and aggregation. The formation of platelet plug occurs simultaneously with activation of coagulation leading to fibrin formation. Platelet function also contributes to pathologic thrombus formation leading to vascular occlusion often in the context of underlying vascular disease, which is a leading cause of morbidity and mortality. Other less well appreciated functions of platelets include a vascular maintenance function, regulation of angiogenesis, as well as putative roles in inflammation and immunity. Platelets are now linked to diverse physiologic and pathologic processes including wound healing and tissue regeneration, response to microbial infection, inflammatory diseases, atherogenesis, tumorigenesis and metastasis. Platelets contain many biologically active mediators which are released upon activation which include growth factors, coagulation factors, adhesive ligands, proteases, heparanase, cytokines, chemokines and vasoactive lipids.

Platelets as components of the tumor microenvironment become activated and release growth factors, chemokines, matrix metalloproteinases and inflammatory mediators, and thereby recruit inflammatory cells, and stimulate the production and remodeling of extracellular matrix and tumor angiogenesis. Platelets may enhance metastatic efficiency by a number of mechanisms including stabilization of tumor emboli, promoting tumor cell adhesion, and by protection from host anti-tumor defenses. In this chapter, we review the role of platelets and thrombin in metastasis and tumor cell adhesion, and we discuss what has been learned from genetic mouse models and the role of platelets in angiogenesis. We briefly review clinical

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trial data with aspirin in cancer and cancer prevention and speculate on the effect of thrombin on tumor dormancy.

2.2 Thrombosis and Cancer

The association of thrombosis with cancer is well recognized [1–4] and thrombosis is an important cause of mortality in cancer patients [5]. That there may be causal connections between cancer and thrombosis [6] is suggested by an observed increased risk of cancer in individuals who present with idiopathic venous thromboembolism (VTE), the majority of which presents clinically within 6 months of the thrombotic event [7, 8]. The prognosis of cancer patients with VTE at diagnosis or within 1 year of diagnosis is significantly worse than those patients without VTE and does not appear to be accounted for by excess mortality attributable directly to VTE [8, 9] which could be explained by the association of hypercoagulability with more aggressive malignancies which is supported by epidemiologic data [10].

2.3 Laboratory Findings in Cancer Hypercoagulation

Markers of activation of coagulation are commonly detected in cancer patients [10]. Hemostatic derangements include thrombocytosis [11], hyperfibrinogenemia, elevated fibrin degradation products [12]. Chronic disseminated intravascular coagulation (DIC), which in some cases precedes the cancer diagnosis itself, is a feature of the Trousseau syndrome [13]. Thrombocytosis is commonly observed in cancer patients and is noted to be an adverse prognostic feature in diverse malignancies such as renal, prostatic, cervical, endometrial, ovarian, gastric, lung cancers and mesothelioma. In pancreatic cancer a low platelet count is an adverse feature. Increased platelet turnover and reduced platelet survival have been reported [14]. Thrombocytopenia may result from DIC or alternatively may be immune-mediated. Fibrinogen turnover is increased [15] as are coagulation activation markers fibrinopeptide-A [16], derived by thrombin cleavage of fibrinogen A chain, and prothrombin activation fragment F1 + 2. Proposed mechanisms of hypercoagulation in malignancy include the constitutive expression of tissue factor (TF) on most tumor cells, expression of cancer procoagulant activity and direct activation of factor X. Platelet activation, as well as inhibition of physiologic anticoagulant pathways including tissue factor pathway inhibitor (TFPI), protein C, and antithrombin [17] have all been proposed to contribute to the hypercoagulability of malignancy.

2.4 Role of Platelets in Metastasis

Invasion and metastasis are hallmarks of malignancy [18] and it is metastasis that affects cancer mortality [19]. Cells are shed from the primary tumor and released into lymphatics or the blood stream (intravasation) to spawn new colonies. In the case of blood-borne metastasis, tumor cells lodge in the vasculature and extravasate into tissues and co-opt normal stromal elements in the process. Tumor cells proliferate and induce an angiogenic response which supports additional tumor growth.

Accumulated evidence points to a role for platelets in promoting blood-borne tumor metastasis, which appears to play a critical role particularly during the early stages of tumor intravasation. Classic studies by Gasic in 1968 [20] and confirmed in subsequent studies [21] demonstrated the importance of platelets and platelet-tumor emboli in experimental tail vein metastasis. Depletion of platelets by administration of antiplatelet antibody greatly reduces the efficiency of experimental pulmonary metastasis observed after tail-vein injection of tumor cells. In experimental metastasis, shortly after injection, tumor cells become enmeshed within a platelet-rich thrombus [22, 23] that may serve to promote tethering and adhesion to vascular endothelium [24], perhaps providing protection from shear forces. Tumor cell lines aggregate platelets in vitro [25, 26]. Some tumor cell lines when injected into animals will result in transient thrombocytopenia [25]. In some studies the platelet aggregating activity correlates with metastatic potential [25, 26]. A number of tumor cell lines exhibit a platelet requirement for metastasis [25–27]. After intravenous injection, tumor cells arrest in arterioles and are rapidly enmeshed in a platelet thrombus [28]. Subsequent steps include penetration of endothelial cell tight junctions, contact with subendothelial matrix, tumor-thrombus remodeling, intravascular tumor cell penetration and invasion through the subendothelial matrix.

The interaction of CT26 colon carcinoma cells with platelets is mediated by Necl-5, an immunoglobulin-like receptor originally identified as the poliovirus receptor [29]. It colocalizes with $\alpha v\beta 3$ in the leading edge of migrating cells and binds to CD226, a counter-receptor on platelet. CD226 also mediates interaction of thrombin-stimulated platelets with vascular endothelium [30] and may provide a mechanism to bridge tumor cells to the vessel wall.

Metastatic efficiency is extremely low, as the vast majority (>98%) of tumor cells when injected intravenously into mice are eliminated within 24 h [31]. Tumor microemboli may be stabilized by platelets and leukocytes and are thereby protected from otherwise rapid elimination. Other plausible mechanisms wherein platelets contribute to metastatic efficiency include stabilizing adhesion to the vascular endothelium and promoting subsequent extravasation. The release of various adhesive ligands, platelet-derived growth factors, chemokines, and coagulation factors all potentially contribute to this process. Thrombin generation and fibrin deposition results in the formation of a provisional matrix. In addition, platelets may provide protection from host

anti-tumor response by binding to tumor cells and inhibiting their killing by natural killer cells possibly by providing a physical barrier to NK cell targets [32, 33].

2.5 Tumor Cell Adhesion

The requirement of platelets for experimental metastasis has led to numerous studies evaluating antiplatelet agents as potential inhibitors of cancer growth and metastasis. A few studies report antitumor activity in carcinogen-induced and transplantable tumor models treated with inhibitors of platelet function. However, results are mixed with negative studies also reported for aggregation, cyclooxygenase and phosphodiesterase inhibitors as well as for prostacyclin [34–36] with respect to treatment of established tumors. These negative studies of antiplatelet agents may not be surprising when considering that the platelet requirement is manifest during the earliest stages of the metastatic process [21], namely tumor cell adhesion. More recent studies using specific αIIβ3 inhibitors have shown anti-metastatic activity in some tumor models, confirming earlier observations [37, 36] and the platelet targeting approach merits additional study

Tumor cell adhesion to platelets and vascular endothelium is complex with participation by integrins, adhesive ligands and cell adhesion molecules (Fig. 2.1). Under static conditions, CT26, B16a and T241 tumor cells adhere to platelet αIIβ3 via fibronectin and von Willebrand factor [36]. Antibody to von Willebrand factor (VWF) and to platelet αIIβ3 inhibits experimental pulmonary metastasis with these tumor cell lines [36]. Surprisingly, given these results, mice genetically deficient in VWF demonstrate increased susceptibility to tumor metastasis [38]. Restoration of VWF lowers the metastatic efficiency in vivo and leads to tumor cell apoptosis in vitro [39]. More recent studies have demonstrated that combined blockade of integrins αIIβ3 and αvβ3 in preclinical models results in antiangiogenic, antitumor and antimetastatic effects [37]. β3 Integrin null mice are protected from B16-F10 melanoma cell osteolytic bone metastases [40]. A specific inhibitor of activated αIIβ3 and platelet aggregation inhibits B16 visceral metastases in an arterial-mediated metastasis model [40].

Soluble fibrin monomer enhances platelet-tumor cell adhesion [41]. Human melanoma cells adhere to collagen I matrix under flow conditions mediated by specific interactions with platelets [42]. The interaction of tumor cells with the matrix is dependent on platelet activation and thrombus formation and is dependent on ß3 integrin function in platelets and melanoma cells [42].

In a study with human colon cancer cell lines LS174HT and COLO205 under dynamic flow conditions, adhesion to immobilized platelets is mediated by initial tethering via platelet P-selectin followed by stable adhesion via the αIIbß3 [43].

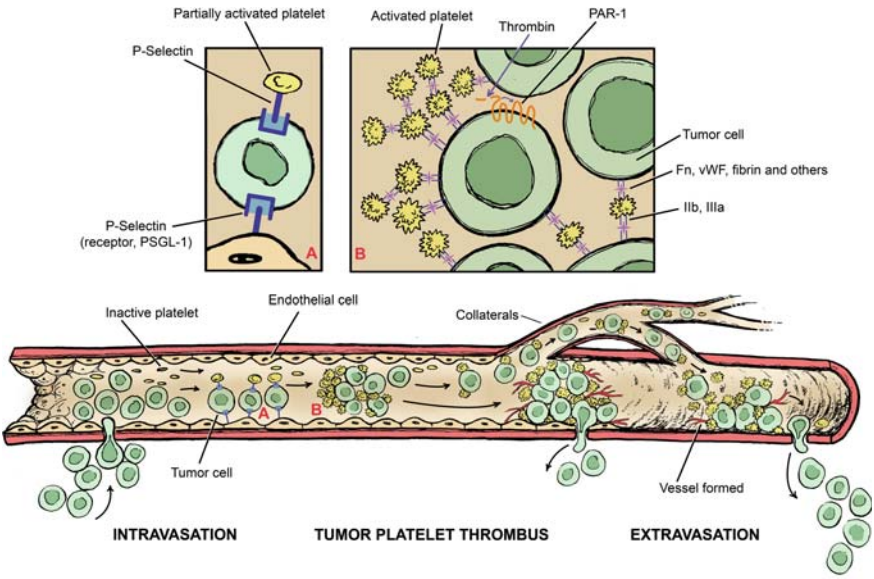

Fig. 2.1 The putative of role of platelets and thrombin generation in the processes of tumor cell intravasation, platelet-tumor thrombus formation, tumor-platelet embolization, leading to colonization and angiogenesis. Thrombin stimulates increased shedding of tumor cells and leads to a more aggressive phenotype. Platelet P-selectin binds to P-selectin glycoprotein ligand (PSLG) and mediates initial tumor cell tethering to the vessel wall. Thrombin promotes bridging of activated platelets and tumor cells via interactions of platelet integrin IIb–IIIa and tumor cell integrins with VWF, fibronectin (Fn) and other adhesive RGDS-containing ligands. Platelet-tumor aggregates stabilize tumor cells in the circulation. Distal embolization of platelet-tumor aggregates may lead to ischemia and endothelial cell injury. Adhesion to the subendothelial matrix permits tumor cell extravasation followed by eventual colony formation. (Reproduced with permission from Elsevier, Cancer Cell 2006; 10:357)

P-selectin has also been implicated in tumor progression. Mice deficient in P-selectin have attenuation of experimental tumor growth and metastasis [44]. Tumor cell selectin ligands bind to P-selectin on platelets. Heparin inhibits metastasis of human carcinoma by a P-selectin-dependent mechanism [45], as well as by its anti-thrombin effect. Although thrombin has long been implicated in tumor progression (see next section), this result suggests that an alternative mechanism for the antineoplastic activity of heparin is its anti-adhesive function, distinct from the inhibition of thrombin. Leukocyte L-selectin mediates metastasis and L- and P-selectin are synergistic with respect to promoting tumor metastasis [45].

The complexity of tumor cell adhesion is underscored by the involvement of numerous other adhesive ligands such as laminin [46], vitronectin [47], type IV collagen [48] and thrombospondin [49] and the numerous integrin receptor combinatorials such as $\alpha 3\beta 1$, $\alpha 5\beta 1$, and $\alpha v\beta 3$ [47, 50] which bind to extracellular matrix components and mediate adhesion, platelet-tumor interaction and metastasis and trigger signal transduction events.

2.6 Role of Thrombin

In addition to its well known actions on the coagulation cascade, thrombin is a potent growth factor for mesenchymal cells [51–53], a proangiogenic factor [54] that stimulates endothelial cell mitogenesis and migration. The cellular effects of thrombin are mediated by the G-protein-coupled seven transmembrane-spanning protease-activated receptors (PARs)-1, -3 and -4. The unique mode of PAR-1 receptor activation results from cleavage of its N-terminal end which exposes a tethered ligand capable of binding to the second extracellular loop of the receptor. Thrombin stimulates platelet-tumor adhesion in vitro and enhances experimental pulmonary metastasis in vivo [55]. Its action on platelets includes activation of GPIIb-IIIa and enhancement of surface deposition of von Willebrand factor and fibronectin, which may bridge tumor cells to platelets and result in their tethering to the vessel wall of the microvasculature. Thrombin also exerts effects on tumor cells which are stimulated to bind to platelets [55] and cultured endothelial cells [50]. Similar findings were noted under flow conditions for thrombin-stimulated human melanoma 397 cells in which adhesion was found to be P-selectin and GPIIb-IIIa dependent [56].

PAR-1 expression is detectable in many tumor cell lines [57] and in metastatic breast cancer [58] contributes to thrombin-stimulated tumor cell motility [59]. Overexpression of PAR-1 in B16 melanoma cells increases experimental pulmonary metastasis fivefold [60]. Thrombin-stimulation of tumor cells results in changes in gene expression that promote oncogenesis. In murine tumor cell lines, B16F10 and UMCL, thrombin upregulates GRO-α [61] and Twist [62] gene expression. Gro-α is required for thrombin-induced angiogenesis [61]. Twist also enhances tumor growth and angiogenesis [62]. Twist is a transcription factor that regulates embryonic morphogenesis and plays an essential role in murine breast tumor metastasis [63] by increasing cell motility and causing loss of E-cadherin-mediated cell-cell adhesion [63]. Cathepsin D is elevated and secreted by many cancers, particularly breast, and is associated with poor prognosis. It has recently been shown to increase angiogenesis by activating matrix metalloproteinase-9 and to enhance cancer growth [64].

Experimental tail vein metastasis models have obvious limitations of tumor load and thrombin concentration at tumor host interface. The role of endogenous thrombin was evaluated in spontaneously metastasizing murine breast tumor 4T1. Specific inhibition of thrombin by hirudin, a direct acting thrombin inhibitor that binds with high affinity, inhibited tumor growth, reduced circulating tumor cells, lowered metastatic potential and prolonged survival of tumor bearing mice [65].

Local thrombin generation within the tumor microenvironment may result in more aggressive tumor biology. Indeed, tumor cells express tissue factor and provide an efficient surface in conjunction with activated platelets for thrombin generation. Tissue factor expression promotes hematogenous metastasis of melanoma [66] and generation of thrombin. Tissue factor and VIIa may signal

through the thrombin receptor [67]. Surgical tumor specimens have demonstrable surface thrombin activity as detected by hirudin binding [68].

In summary, thrombin generation may reprogram cancer cell gene expression to a more malignant phenotype, which can set up a positive feedback. Thrombin stimulates adhesion of tumor cells to endothelium and activates a proangiogenic switch by releasing vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and angiopoietin (ANG)-1 from activated platelets and upregulation of VEGF, ANG-2, Gro-α, Twist and cathepsin D by tumors.

2.7 Genetic Models

Genetic approaches using knock out mice have confirmed the importance of platelets in experimental hematogenous metastasis [27, 69]. Lung metastasis is markedly impeded in nuclear factor-erythroid 2 (NF-E2) (−/−) mice that have few circulating platelets [27]. Similar findings were reported for fibrinogen (−/−) mice and in PAR-4 (−/−) mice, which have platelets that fail to respond to thrombin [27]. Gαq (−/−) mice are deficient in platelet signaling and unresponsive to agonists in vitro [70] and show protection from experimental and spontaneous metastasis [33]. Interestingly, neither fibrinogen [69] nor Gαq [33] depletion had an observable impact on growth of primary subcutaneous tumors. The additional enhanced protection from metastasis observed with hirudin treatment of PAR-4 (−/−) [27] and fibrinogen (−/−) [69] mice indicates that thrombin promotes metastasis by a mechanism that is partially independent of platelet activation and fibrin deposition.

2.8 Platelets as Regulators of Angiogenesis

Classical studies showed that platelets contribute to vascular integrity [71] and release factors that nurture the vascular endothelium. Infusion of platelet-rich plasma supports organ preservation and vascular integrity [72]. Conversely, experimental thrombocytopenia leads to thinning and fenestrations of endothelial cells [73], an effect that is attenuated by administration of corticosteroids [74]. Platelets promote survival and proliferation of endothelial cells in vitro [75] by release of fibroblast growth factor and vascular endothelial growth factors. Additionally, platelets are a source of other angiogenic growth factors such as angiopoietin-1 and platelet-derived growth factor. Platelets stimulate endothelial cell sprouting and tube formation in matrigel [76]. Platelets are a major storage reservoir for growth factors and their release can be regulated in an activation-dependent manner, allowing for an on-demand delivery system in the vasculature. Other growth factors found within platelets include hepatocyte growth factor (HGF), epidermal growth factor (EGF), insulin-like growth factor (IGF)-1, -2, platelet-derived endothelial cell growth factor (PD-ECGF),

and TGF-β [77]. Platelets also contain inhibitors of angiogenesis such as angiostatin, thrombospondin-1, plasminogen activator inhibitor-1, platelet factor-4 and endostatin. In addition, platelets are a source of bioactive lipids such as sphingosine 1-phosphate (S1P) and lysophasphatidic acid (LPA). Endothelial differentiation gene (Edg)-1 is a G protein-coupled receptor for S1P and is required for vascular maturation [78]. Edg-1 knockout mice exhibit lethal embryonic hemorrhage and are deficient in vascular smooth muscle cells and pericytes [78], mesenchymal-like perivascular cells which closely associate with endothelial cells from capillaries and post-capillary venules. S1P mediates vascular maturation by modulating N-cadherin function [79]. LPA receptors are detected in human primary breast tumors [80]. Platelet-derived LPA promotes breast cancer cell line MDA-BO2 osteolytic bone metastases [80].

Regulation of the balance of platelet-derived angiogenic vs anti-angiogenic factors is presently unknown, although in the aggregate the platelet releasate is proangiogenic [81, 82]. Of interest in this regard is that stimulation of platelets by selective PAR-1 agonist exerts a proangiogenic effect by inducing VEGF release while inhibiting endostatin, while selective agonist PAR-4 stimulation has the reverse effect, potentially allowing for counter-regulation of angiogenesis via these two receptors [83]. PAR-1 and PAR-4 are expressed on human platelets and are activated by thrombin. The recent finding of the organization of pro- and anti-angiogenic proteins into separate α-granules establishes a mechanism for selective platelet release [84]. Interestingly, platelets of tumor bearing mice sequester angiogenic regulators which may serve as biomarker of early tumor growth [85].

Platelets circulate in proximity to vascular endothelium in flowing blood but do not attach to the endothelium under normal conditions. Thromboresistance of pristine endothelium is maintained by heparin-like mucopolysaccharides, prostacyclin synthesis and CD39 (ecto-ADPase). Platelets, similar to leukocytes, roll on stimulated venular endothelial surface in a manner that is dependent upon endothelial P-selectin expression [86]. Platelets express P-selectin glycoprotein ligand 1 (PSGL-1) and mediate platelet-endothelial interactions in vivo [87]. Hematopoietic cytokines promote revascularization and angiogenesis by the recruitment of bone marrow derived hemangiocytes that is induced by the release of platelet SDF-1 [88]. Thus platelets may be important regulators of physiologic and tumor angiogenesis [89]. Platelets are a major transporter of VEGF [90] which can enhance vascular permeability and stimulate angiogenesis.

2.9 Clinical Trials

The anticancer activity of aspirin has been established in numerous epidemiologic studies particularly with respect to chemoprevention in colorectal cancer [91–93]. A 40–50% reduction in fatal colon cancer was reported in aspirin users

[91]. Case control studies also support a favorable effect of aspirin in the prevention of cancers of the esophagus, breast, ovary and lung [93]. The association is most robust for prolonged and regular usage of aspirin. To our knowledge, aspirin has not shown meaningful anti-tumor activity in clinical trials in cancer patients. No effect of the addition of aspirin to chemotherapy was seen in a randomized controlled study of 303 patients with SCLC [94].

2.10 Does Thrombin Influence Tumor Dormancy?

Autopsy studies have revealed microscopic or in situ cancers as a common finding without apparent clinical disease. Examples of such reported series include prostate, thyroid, and breast cancers. Shulman and Lindmarker [10] treated patients with deep vein thrombosis for either 6 weeks or 6 months of oral anticoagulant and found fewer cancers in the 6 month treated group. Cancer was diagnosed in 66 of 419 patients vs 45 of 435 patients in the 6-month group (odds ratio 1.6, 95% CI 1.1-24, $P = 0.02$), although there was no difference in overall cancer mortality. The difference in cancer incidence became apparent 2 years after treatment, implying an effect on early cancer. Most of the difference in cancer incidence was accounted for by fewer urogenital cancers diagnosed in the extended treatment group. It is conceivable that the inhibition of thrombin delays or prevents the onset of clinically evident cancer. In the Second Northwick Park Heart Study, 3052 middle-aged men were evaluated for hypercoagulability yearly for 4 years and monitored for morbidity and mortality with average follow-up of 11 years [95]. The intent of the study was to examine association of hypercoagulability with the subsequent development of coronary heart disease. While there was no associated increased risk of heart disease, cancer mortality was increased in the group with elevated activation markers of coagulation 11.3 vs 5.1 per thousand person-years ($P = 0.01$). Persistent activation was defined by 2-yearly consecutive measurements of fibrinopeptide A and prothrombin activation fragments $1 + 2$ with values exceeding the upper quartiles of the population (approximately 5% of the population). The excess mortality was mainly due to increased incidence of cancers of the digestive tract (relative risk 3.26, $P<0.001$). The median interval between detection of activation and diagnosis of malignancy was 4.8 years.

These observations provide further validation of the link between thrombin activation and malignancy. Activation of a procoagulant axis may convert an otherwise dormant tumor to a more biologically aggressive phenotype. The mechanism of persistent thrombin activation remains unclear but it may be contributed by host factors such as age or genetic risk. These findings provide a rationale for future clinical investigation of anticoagulants in cancer prevention and treatment.

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Chapter 3
Activation of Clotting Factors in Cancer

Frederick R. Rickles and Anna Falanga

3.1 Introduction

Evidence for "hypercoagulability" is commonly found in patients with cancer and increases the risk of thromboembolism (TE) [1]. While the pathophysiology of TE in cancer is complex, it can be viewed classically as related to abnormalities of Virchow's triad: stasis of the blood; vascular injury; hypercoagulability (or, as described by Virchow himself, as "abnormalities of the fixed elements of the blood") [2]. Epidemiologic, laboratory, pathologic and clinical evidence supports this important association. However, association is clearly not the same as causation and, until recently, TE was thought largely to be an *epiphenomenon* in cancer – a secondary manifestation of the inflammatory response to tumor growth and/or to the therapy (e.g. chemotherapy, surgery, radiation therapy).

Recent evidence from several laboratories [3–5], however, has linked malignant transformation, tumor angiogenesis and metastasis, to thrombus formation, mediated perhaps by signaling cascades that can be triggered in a clotting-dependent and/or clotting-independent manner. Tissue factor (TF), the ubiquitous activator of blood clotting, has been shown, for example, to induce synthesis of vascular endothelial growth factor (VEGF) in human tumor cells independent of its ability to activate factor Xa-catalyzed conversion of prothrombin [6]. The TF-VIIa complex and factor Xa are among known activators of G-protein-coupled protease-activated receptor-2 (PAR-2) in tumor cells, while the TF-VIIa-Xa complex and thrombin efficiently activate PAR-1. Both PAR-1 and PAR-2 have been implicated in signaling pathways leading to angiogenesis and metastasis [7–9]. The precise role of the cytoplasmic domain of TF, which has been targeted as the likely signaling region of the molecule, remains controversial [6, 8]. Nevertheless, multiple cell-signaling cascades are triggered during the

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generation of these clotting intermediates that are believed to influence tumor cell migration, adhesion, cell-cell interaction, tumor cell diapedesis and replication, as well as new vessel growth.

Strong epidemiologic evidence has supported the negative impact of activation of blood clotting in cancer patients [10–13]. Investigators are now exploring with renewed interest the potential that inhibitors of blood clotting proteins (with and without anticoagulant effect) can impact on cancer survival. The results of recently published randomized, controlled trials of various forms of the anticoagulant, low-molecular-weight heparin (discussed in depth elsewhere in this volume) have documented increased survival in patients with advanced cancer [14–18]. While the mechanism(s) for the salutary effects of anticoagulants on cancer survival has (ve) yet to be elucidated, some evidence suggests that various heparin fractions, in addition to their anticoagulant properties, can interfere with a variety of important tumor functions, including, for example: (1) tumor angiogenesis; (2) heparanase-mediated extravasation of blood-borne tumor cells; and, (3) interactions between carcinoma mucins and selectins.

Targeting blood clotting reactions in cancer may provide a unique approach to treatment. New agents are being developed that can activate clotting selectively only in tumor vessels, thus producing localized infarction and reduction in tumor size [19–22]. Other approaches target tumor cell and tumor-associated endothelial cell TF to deliver immuno-toxins or selective inhibitors of tumor growth with specificity to spare the surrounding normal tissue [23, 24]. Both selective activation and inhibition of clotting may prove to be useful adjuncts to traditional therapeutic approaches in cancer patients.

3.2 Epidemiology

As noted, cancer significantly increases the risk for TE and this risk is greatest within the first 3–6 months following the diagnosis of cancer [1]. Among all patients in whom the diagnosis of venous TE (VTE) is established, cancer is responsible for approximately 20% of the attributable risk [25] and patients with cancer are at nearly three times the risk for recurrence of VTE as are matched control subjects after a first episode [26]. Although the absolute risk of VTE in cancer patients remains relatively small (e.g. 0.6% in one large population-based study) [11], cancer patients undergoing surgery have at least a twofold increase risk of postoperative thrombosis compared to non-cancer controls undergoing the same procedures [27], and even without the added stress of surgery, the adjusted odds ratio for VTE is nearly 30 in patients with some tumor types [1]. Finally, the risk of death due to cancer appears to increase significantly if the diagnosis of VTE is established simultaneously or within the first year after the cancer diagnosis is established [10–13], supporting long-standing experimental data that suggest that thrombus formation provides some biological advantage to the growth and dissemination of cancer [2, 28].

3.3 Mechanisms of Cancer-Induced Thrombosis

Stasis, vascular injury and hypercoagulability, the three components of Virchow's Triad [29], each play a role in the pathogenesis of thrombus formation in cancer patients, examples of which include: *stasis*: bed rest; extrinsic compression of blood vessels by tumor; *vascular injury*: direct invasion of vessels by tumor; prolonged use of central venous catheters; endothelial damage secondary to chemotherapy; *hypercoagulability*: release of tumor-associated PCAs and cytokines; impaired endothelial cell defense mechanisms and reduction of naturally occurring inhibitors (e.g. antithrombin, protein C or protein S deficiency; activated protein C resistance); increased adhesive interactions between tumor cells, vascular endothelial cells, platelets and host monocyte/macrophages, which is further enhanced in mucin-secreting tumors (e.g. selectin-mediated). These and other probable mechanisms for thrombosis in cancer patients have been examined in detail elsewhere [2, 28, 30]. Suffice it to say that the pathogenesis of thrombosis in cancer is complex and most likely involves multiple mechanisms that may differ dramatically from patient to patient. However, until recently, it has been presumed that all of these mechanisms are secondary and have no primary role in the molecular events leading to the development of cancer.

3.4 Molecular Pathogenesis of Thrombosis in Cancer – Direct Link to Oncogenesis

Boccaccio and her colleagues developed a model for human liver carcinoma by targeting activated human *MET* oncogene to mouse liver with a lentiviral vector and liver-specific promoter [3]. The animals slowly developed progressive hepatocarcinogenesis, which was preceded and accompanied by a thrombohemorrhagic syndrome ultimately indistinguishable from Trousseau's Syndrome with disseminated intravascular coagulation (DIC). Venous thrombosis in the tail vein of the mouse occurred early and was followed by a progressive coagulopathy and fatal internal hemorrhage. The syndrome was characterized in the animals by elevated blood levels of fibrin D-dimer, a prolonged prothrombin time and a marked reduction of the platelet count (i.e. DIC). Genome-wide expression profiling of hepatocytes expressing the *MET* oncogene demonstrated impressive upregulation of both the plasminogen activator inhibitor 1 (PAI-1) and cyclooxygenase-2 (COX-2) genes with a two- to threefold increase in circulating protein levels. Inhibitors of either PAI-1 (XR5118) or COX-2 (Rofecoxib®) prevented both laboratory and clinical evidence of DIC in the mice.

Based on their observations in this mouse model, the investigators postulated a five-step process (Fig. 3.1), whereby *MET* induction by hypoxia results in increased expression of the tyrosine kinase receptor for hepatocyte growth factor/scatter factor (step 1), activation of prothrombotic, hemostasis genes

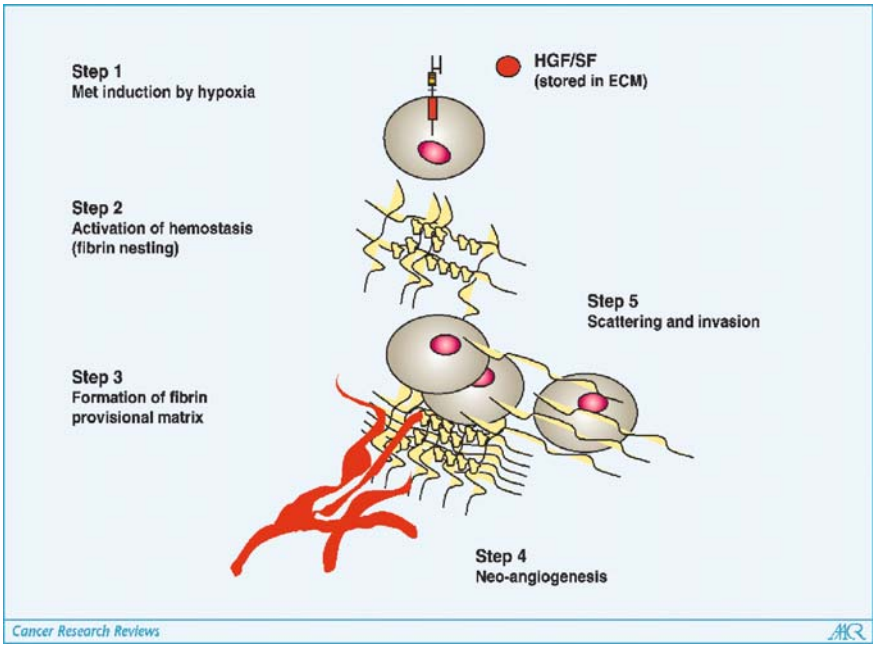

Fig. 3.1 How activation of the coagulation cascade may drive invasive growth. Pericellular activation of the coagulation cascade is a critical early step for invasive growth. 1. In the avascular focus of transformation, hypoxia induces transcription of the MET oncogene, elevating the cell surface level of the MET tyrosine kinase receptor. Availability of hepatocyte growth factor/scatter factor (HGF/SF) in the extracellular matrix (ECM) may influence MET activation, or the ligand requirement may be bypassed as a result of MET overexpression. 2. MET signaling induces transcription of a number of hemostasis genes, most prominently the plasminogen activator inhibitor type 1 (PAI-1) and cyclooxygenase type 2 (COX-2) genes (see text). 3. Induction of hemostasis leads to fibrin polymerization around the cell, forming a provisional extracellular matrix that provides a scaffold that promotes angiogenesis (4) and the outward migration of cancer cells. (Reproduced from *Cancer Research* 2005 (reference [31]) with permission of the publishers)

(e.g. COX-2 and PAI-1) by *MET* signaling (step 2) with fibrin nesting of the tumor cells, formation of a fibrin provisional matrix for the tumor (step 3), induction of neo-angiogenesis by the fibrin matrix and other coagulation proteases (step 4) and, finally, "scattering" of tumor cells with invasion (step 5) [31]. Thus, for the first time, experimental evidence has been generated linking directly activation of hemostasis with oncogenesis.

Inactivation of the tumor suppressor gene *Pten*, together with hypoxia, which leads to Akt activation and upregulation of the *Ras*/*MEK*/*ERK* signaling cascade, has recently been shown to induce TF gene expression in human astrocytoma cell lines [4], an in vitro model for malignant transformation. Cells transformed with Akt showed the greatest incremental increase in hypoxia-induced TF expression and secretion, exhibiting procoagulant activity,

the latter of which was factor VII-dependent and inhibited by anti-TF antibodies. These findings were partially reversible by induction of *PTEN*. *PTEN* is inactivated by mutation, promoter methylation, or by other mechanisms in upwards of 80% of human glioblastomas and TF is expressed in >90% of such tumors. In low grade astrocytomas, however, only 10% of tumors express TF and evidence for *PTEN* inactivation is rarely found. The histopathologic hallmark of high grade astrocytomas (glioblastoma multiforme) is so-called pseudopalisading necrosis, thought to represent a "wave of tumor cells actively migrating away from a central hypoxic zone that is created following vascular compromise and associated with intravascular thrombosis." Thus, the finding by Rong et al. that pseudopalisading cells produced increased TF in seven human glioblastoma specimens further supports the molecular relationship between malignant transformation and clotting activation [4].

Yu and colleagues [5], using the well-established, step-wise model of human colorectal cancer (CRC), in which activation of mutant K-*ras* and subsequent inactivation/loss of *p53* "drive many interrelated aspects of the malignant phenotype…..", demonstrated that "TF is required for full expression of the K-*ras*-dependent tumorigenic and angiogenic phenotype of CRC cells in vivo, but not for cellular transformation in vitro." Conversely, activation of K-*ras* and loss of *p53* were both necessary to achieve full expression of TF both on the cell membrane and on the surface of microvesicles shed into the circulation of the mouse. With each subsequent mutation, increasing TF levels were documented in the respective cell lines in vitro and in vivo in the more aggressive tumors produced in SCID mice, which were shown to have acquired a new K-*ras* mutation. Finally, the investigators showed that TF gene-silencing with small interfering RNA (siRNA) markedly inhibited in vivo tumor growth and angiogenesis.

Thus, three different tumor model systems have now provided complimentary evidence that oncogene activation and/or tumor suppressor gene inactivation upregulates blood clotting in vivo (by increasing TF, PAI-1 and COX-2), strongly implicating clotting pathways in the basic biology of cancer. Furthermore, this data implies that targeting clotting intermediates might prove to be a rational strategy for both reducing the thrombotic risk in cancer and, perhaps, impairing tumor growth [31].

3.5 Targeting Tissue Factor and Other Coagulation Intermediates in Cancer Therapy

Two recent therapeutic approaches, one applied to experimental tumor models and one applied to patients with advanced malignancy, support the rationale for further testing of "anticoagulant" and/or anti-tissue factor strategies for treating cancer.

The first approach either utilizes a modified TF construct to induce local tumor infarction [22] or down-regulates the expression of TF in tumor cells and tumor vascular endothelium [24]. Both methods exploit the increased expression of TF by tumors and tumor-associated endothelial cells. Various modifications of this therapeutic strategy also take advantage of high affinity binding of factor VIIa to TF, which allows targeting of the compounds to the malignant cells and the abnormal, angiogenic endothelium [32–34]. Along with other endothelial-specific targets, investigators have succeeded in reducing the size of experimental tumors by inducing local thrombosis and subsequent tumor infarction [19–23] (Table 3.1). The delivery of a "toxic" construct that binds to TF in the neoangiogenic vasculature of the tumor activates local blood coagulation, producing an obstructive thrombus and tumor infarction. Reported most recently by El-Sheikh and colleagues [22], the investigators linked a soluble, truncated form of TF (tTF) to the heparin-binding domain of VEGF, the latter of which targets at least three receptors hyperexpressed on tumor endothelium – the VEGF receptor, VEGF neuropilin 1, or coreceptors and chondroitin 6 sulfate proteoglycan. The investigators were successful in inducing selective tumor necrosis quite rapidly (5 min) after infusion of the construct, together with recombinant factor VIIa (rVIIa), into mice harboring the

Table 3.1 Targeting tissue factor in tumor models of cancer chemoimmunotherapy

Construct	Target	Presumed mechanism	Evidence for efficacy (Reference)
Truncated TF (tTF)[a] + bifunctional antibody[b]	I-A[d]	Conversion of tTF to TF on tumor endothelium with infarction induction	Neuroblastoma regression in vivo [19]
VIIai[c] + IgG Fc	TF	Destruction of endothelium of tumor	Melanoma and prostate cancer regression in vivo [20,23]
tTF + antibody to ED-B domain of fibronectin (FN)	FN	Conversion of tTF to TF on tumor endothelium	Complete regression of #3 different syngeneic tumors (~30% of mice) [21]
tTF + HBDt domain[d] of VEGF (+/− VIIa)	VEGFR-2[d], Npn-1[e], C6S of PG[f]	Conversion of tTF to TF	Selective mouse tumor necrosis (colon cancer) [22]
Curcumin analog (+/− rVIIai[c])	TF; VEGF[d]	Inhibition of tumor angiogenesis	Human breast tumor regression in nude mice [24]

[a] tTF = soluble, hypofunctional, extracellular domain of tissue factor;
[b] Bifunctional antibody to TF and to MHC class II antigen I-A[d]
[c] VIIai = inactivated recombinant factor VIIa
[d] VEGF = vascular endothelial growth factor and VEGFR-2 receptor
[e] Npn-1 = neuropilin-1; [f]C6S = chondroitin 6 sulfate proteoglycan

CT26 colon cancer [22], apparently sparing non-tumor vasculature and avoiding systemic activation of coagulation.

Utilizing a small, molecular weight inhibitor of TF and VEGF gene expression, which was *not* designed to activate TF in the tumor endothelium, Adams and colleagues demonstrated regression of human breast tumors in nude mice. They explored the properties of a variety of synthetic analogs of curcumin; the lead compound reduced expression of TF and VEGF in MDA-MB-231 human breast cancer cells and in other human tumor cell lines, as well as in endothelial cells [24]. More recent data demonstrated the feasibility of linking the lead compound in that series to inactivated rVIIa in order to target the tumor endothelium and tumor cells; upwards of 60% uptake of the labeled complex by the malignant cells was achieved by the investigators, supporting the rationale of the targeting strategy [24a]. A somewhat similar anti-TF approach, using small molecular weight inhibitors of the NF-κB and EGR-1 oxidative stress pathways in cancer cells and angiogenic endothelium, has achieved potentially important reduction of TF PCA expression by tumor cells in vitro and both regression of growth and angiogenesis of established human tumors in xenogeneic models [35, 36]. One or more of these targeting strategies may prove useful in cancer chemotherapy. Of interest, the anti-angiogenic strategy, using a factor VIIa cassette for targeting TF in the neoangiogenic endothelium, is currently being tested in Phase II studies of patients with "wet" macular degeneration [37] and is contemplated for prostate cancer.

The direct anticoagulant strategy of cancer treatment focuses typically on LMWH compounds or other newer anticoagulants and will be reviewed in more detail elsewhere in this volume. However, it seems clear that additional well-designed studies are needed to determine whether patients with cancer will have a survival advantage by utilizing LMWH (or other anticoagulants) before this approach to cancer therapy can be widely adopted. Regardless, the preliminary results of the these studies of patients with poor prognosis cancers have stimulated renewed interest in the anticoagulant approach, particularly since the increased survival observed was not due to prevention of fatal pulmonary emboli or other thrombotic complications. Instead, patients lived longer with their tumors and ultimately died of cancer, lending support to the concept that some anticoagulants may alter tumor biology.

Less certain is the mechanism(s) by which anticoagulants might effect this change in tumor behavior. As indicated earlier, possible mechanisms include (but are not limited to): inhibition of angiogenesis; inhibition of tumor proteases (e.g. heparanase); inhibition of selectins [38–47]. It is very likely that these and other mechanisms are impacted by LMWH and are overlapping – e.g. inhibition of thrombin generation and thrombin action, for example, likely reduces its effects on angiogenesis as well as effects of thrombin on cell growth genes, selectin expression, etc.

3.6 Summary

The risk for thromboembolism (TE) in patients with cancer is well known and is related at least in part to the production of a hypercoagulable state mediated by the procoagulant properties of tumor cells and tumor-associated host cells. The principal tumor procoagulant activity (PCA) is tissue factor (TF). Tumor cell expression of TF appears to correlate best with the hypercoagulable state characteristic of patients with cancer. While the increased risk of TE has been considered by many to be an *epiphenomenon* – not causally related to the transforming malignant events – recent advances in the understanding of the pathogenesis of cancer have suggested a direct relationship between clotting activation and oncogenesis. These new observations, made possible by the development of key molecular probes and tumor models in experimental animals, now provide the missing "link" that explains better the close relationship between thrombosis, aggressive behavior of tumors, angiogenesis and metastasis. The regulation of expression of TF has been demonstrated to be controlled at the molecular level by several oncogenes, as also appears to be true for the enzyme, cyclooxygenase-2 (COX-2), an important regulator of platelet function and for plasminogen activator inhibitor type 1 (PAI-1), an inhibitor of fibrinolysis. Engagement of protease activated receptors (PARs) by the TF-VIIa complex, factor Xa and/or thrombin, have now been shown to be important for tumor growth, angiogenesis and metastasis. Targeting blood clotting reactions in cancer, therefore, may provide a unique approach to cancer treatment.

In summary, blood clotting reactions, particularly in response to TF induction, are intimately involved in the biology of cancer, rendering the cancer patient susceptible to thrombosis. In addition, these same clotting reactions appear capable of stimulating tumor growth genes, tumor cell diapedesis, cell adhesion to the endothelium, angiogenesis, and a host of other processes critical to both primary tumor growth and metastasis. Rational strategies for the down-regulation of these coagulation pathways hold the promise of a duality of beneficial effect – i.e. reduction in thrombosis potential and tumor proliferation in patients with cancer.

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Chapter 4
The Role of Plasminogen-Plasmin System in Cancer

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Abstract Components of the plasminogen-plasmin system participate in a wide variety of physiologic and pathologic processes, including tumor growth, invasion and metastasis, through their effect on angiogenesis and cell migration. These components are found in most tumors and their expression not only signifies their function but also carries a prognostic value. Their expression is in turn modulated by cytokines and growth factors, many of which are up-regulated in cancer. Though both tPA and uPA are expressed in tumor cells, uPA with its receptor (uPAR) is mostly involved in cellular functions, while tPA with its receptor Annexin II on endothelial surface, regulates intravascular fibrin deposition. Among the inhibitors of fibrinolysis, PAI-1 is a major player in the pathogenesis of many vascular diseases as well as in cancer. Therapeutic interventions, either using plasminogen activators or experimental inhibitor agents against PAI-1, have shown encouraging results in experimental tumors but not been verified clinically.

Keywords Cancer · PAI-1 · Plasminogen – plasmin system · Thrombosis · uPA · uPA receptor

4.1 Introduction

The plasminogen-plasmin system is involved in not only the regulation of hemostatic balance but also a wide range of biologic processes. These include embryogenesis, development, wound healing, cell proliferation and migration. As such, the system plays an important functional role in both physiologic and pathologic conditions. When this system was first discovered, it was named the fibrinolytic system. However, with the realization that fibrin is not the only

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substrate for the active enzyme plasmin, the term plasminogen-plasmin system is more appropriate and will be used here. This chapter will review our current understanding of how components of this system affect tumor growth and metastasis.

4.2 Historical Background

The first association between the pathology of cancer and fibrin must be credited to Billroth [1], who first described the presence of tumor cells within a thrombus. Later, Iwasaki found that tumor cells within the vascular channels of recanalizing thrombi were viable [2]. The significance of fibrin within tumors was again noted by O'Meara and Jackson [3], who postulated that. if unresolved, the fibrin would induce further tumor growth and vascular proliferation. Subsequent observations by others led to experimental and clinical trials with anticoagulants [4], defibrinating agents [5], and thrombolytic agents [6]. Though the results were controversial, experimental tumor metastases were found to be enhanced by increasing the fibrin content in tumors through the use of antifibrinolytic agents, or by induction of hyperfibrinogenemia [7]. In the ensuing years, the role of the plasminogen-plasmin system gained attention not only in the pathogenesis of bleeding and thrombotic complications in the cancer patient, but also in tumor growth and metastasis [8].

4.3 Plasminogen-Plasmin System

The precursor of the active protease plasmin is plasminogen [9–17]. This is a single-chain glycoprotein of 92 kDa synthesized mainly in the liver. It is present in plasma and extracellular fluids at a concentration of 1–2 μM, with a biologic half-life of 2.2 days. In the native form, the amino-terminal is occupied by glutamic acid. On activation by a plasminogen activator or by plasmin, proteolytic cleavage at Lys-77 and Lys-78 results in the formation of Lys-plasminogen (Fig. 4.1). Further cleavage of the Arg-560 and Val-561 peptide bond results in the formation of a two-chain plasmin held together by two disulfide bonds.

Its structure contains five triple-looped structures with three disulfide bonds known as kringles. These kringles are involved in binding of plasminogen to cell surfaces and to fibrin. Of interest is that the structure of a potent anti-angiogenic protein, angiostatin, is identical to the first four of the five kringles.

The lysine at the amino-terminal of plasminogen functions as the binding domain for many proteins, including fibrin, α2-antiplasmin, thrombospondin and the plasminogen receptor annexin II. It also enables plasminogen to bind to specific lysine binding sites on many cell surfaces. Furthermore, plasminogen

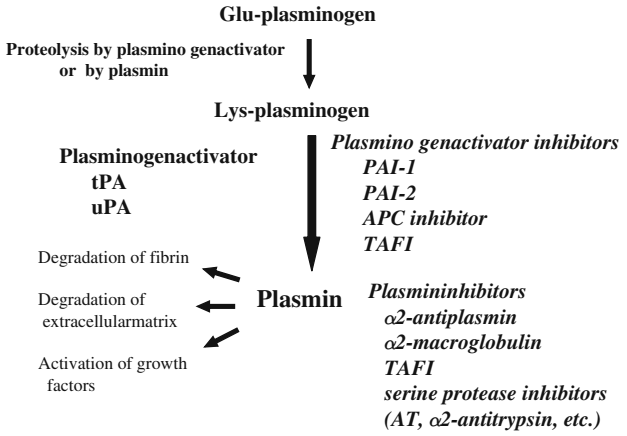

Fig. 4.1 The plasminogen-plasmin system

activation is blocked by lysine binding to ε-aminocarboxylic acids (epsilon amino-caproic acid, tranexamic acid). These are clinically useful antifibrinolytic agents.

Plasminogen has no proteolytic activity prior to conversion to plasmin. On the other hand, plasmin is a serine protease with broad substrate specificity, which includes fibrin, fibrinogen and extracellular matrix (ECM) proteins such as laminin and fibronectin, either directly or indirectly through the activation of latent metalloproteinases [8, 17, 18]. Thus, it is an intermediary protease with a wide range of functions in health, such as tissue remodeling and wound healing, and involvement in pathologic processes including tumor growth and metastasis. Other substrates of importance include the pro-forms of growth factors, which can be cleaved and activated by plasmin. Plasmin can also proteolyse specific cleavage sites on plasminogen to generate angiostatin.

4.3.1 Plasminogen Activators

Though there are many proteases derived from bacteria, fungus, insects and other animals that can proteolyse plasminogen, only two activators are present in man.

The first one is tissue-type plasminogen activator (tPA) [19–21]. It is a 70-kDa glycoprotein which, under physiologic conditions, is synthesized mainly by endothelial cells. It is responsible for maintaining vascular patency in response to intravascular fibrin formation. However, it is also produced by neurons, keratinocytes, melanocytes and various tumor cells. Observations in transgenic mice with tPA null$^{-/-}$ have now shown that, in addition to maintaining vascular patency, tPA participates also in neuronal development and neurologic functions [22].

The resting plasma level of tPA is low, around 5 ng/mL, but large amounts can be released from endothelial cells under a variety of circumstances. It is then quickly bound to the circulating inhibitor PAI-1. A lesser amount is also bound to α_2-macroglobulin. It is then rapidly removed from the circulation by the liver with a plasma half-life of around 5 min.

The source of the circulating tPA is believed to be the vascular endothelium. Earlier observations from our laboratory indicated that fibrinolytic activity can be released from the vascular wall by ischemia [23, 24], serotonin [25] and other vasoactive stimuli [23]. This fibrinolytic activity was later shown to be derived mostly from tPA and, to a lesser extent, from uPA. However, recently, there is evidence that tPA present in the neuronal terminals in the autonomic nervous system can also be released through the vascular wall [26]. This new finding explains our earlier observation that stimulation of a vessel wall can release fibrinolytic activity from a vessel located distally to the site of stimulation, indicating that the stimuli is transmitted via perivascular sympathetic nerves [23].

The "finger" domain present at the amino-terminal enables tPA to have a high affinity for fibrin, thus making tPA a more efficient thrombolytic agent than uPA. In addition to its high affinity for fibrin, tPA also binds to extracellular matrix (ECM) proteins, including laminin and fibronectin, and the mannose-6-phosphate/insulin-like growth factor. Recently, a cellular surface receptor for both tPA and plasminogen, termed annexin II, was found in endothelial cells, macrophages and certain tumor cells [27, 28]. The close proximity of these two ligands on this dual receptor enhances plasminogen activation. The high expression of annexin II in acute promyelocytic leukemia and other malignant conditions may explain the high bleeding risk in these disorders [29].

The second plasminogen activator in man is urokinase-type plasminogen activator (uPA). This fibrinolytic enzyme was first discovered in urine, hence its name urokinase. It originates from kidney cells [30, 31]. When first released, it is in a single-chain form, pro-urokinase, which is then rapidly converted by plasmin or kallikrein to the two-chain form connected by a disulfide bond. The single-chain glycoprotein has a molecular mass of 53 kDa. Both forms are fibrinolytic and are used as thrombolytic agents, but the single-chained uPA has a higher affinity for fibrin. The tertiary structure of uPA is composed of the amino-terminal fragment (ATF) that contains a growth factor domain, as well as a kringle domain. Both the single-chain and the two-chain uPA binds to the uPA receptor (uPAR) via the ATF, forming a uPA-uPAR complex [32]. The complex form facilitates plasminogen activation by uPA.

In addition to being a potent activator of plasminogen, uPA also directly activates procollagenase. This allows it to exert a regulatory effect on cell migration as well as tumor growth and metastasis.

uPA is secreted by many other cell types including endothelial cells and tumor cells in addition to kidney cells. The role of tumor-derived uPA in tumor growth and metastasis has been the subject of many studies and will be reviewed in a later section of this chapter.

uPAR is anchored to the cell surface by its glycosyl-phosphatidylinositol (GPI) domain at the C-terminal, and released by phosphatilyl-inositol specific phospholipase C [33]. On the cell surface, uPAR has a high affinity for uPA, and can also bind uPA which had been inactivated by PAI-1, forming a uPA-uPAR-PAI complex. This complex is rapidly internalized [34], a process facilitated by several members of the low density lipoprotein receptor (LDLR) family [35]. Following internalization, the uPA-PAI-1 portion of the complex is degraded while uPAR is recycled and emerges at a different site on the cell surface [34]. This process is believed to be directional and important in cell migration.

4.3.2 Inhibitors of the Plasminogen-Plasmin System

Inhibitors of plasmin are α2-antiplasmin (α2-AP), and α2-macroglobulin. α2-AP belongs to the family of serpins (serine protease inhibitors) [36]. Plasmin generated in circulating blood binds to a lysine binding site on α2-AP and is rapidly inhibited. The level of the plasmin-α2-AP complex in blood is often used as an indicator of the intensity of fibrinolytic activity. α2-AP also cross-links with the α-chain of fibrin, preventing the latter's proteolysis by plasmin. When excess circulating plasmin in blood has saturated all the available α2-AP, a slower acting inhibitor α2-macroglobulin acts as a second line of defense. In addition, there are other serine protease inhibitors including antithrombin, α1-antitrypsin, α1-antichymotrypsin, inter-α-trypsin inhibitor and C-1 inactivator. The recently discovered thrombin activatable fibrinolytic inhibitor (TAFI) inhibits plasmin as well as both plasminogen activators, tPA and uPA.

4.4 Plasminogen Activator Inhibitors (PAIs)

4.4.1 Plasminogen Activator Inhibitor Type 1 (PAI-1)

PAI-1 is a member of the serine protease inhibitor (SERPIN) family (Table 4.1). It is perhaps the most important component of the plasminogen-plasmin system in the regulation of many physiologic processes and in the pathogenesis of many disorders including cancer [37–40]. PAI-1 is synthesized by the endothelial cells, liver, adipose tissues, vascular smooth muscle cells, and a large number of tumor cells. In addition to being a potent inhibitor of both tPA and uPA, PAI-1 inhibits plasmin directly.

PAI-1 is stored in the α-granules of platelets. Most of the PAI-1 in platelets is in a latent form, but when the active portion is released into a thrombus or into the ECM, it can exert their effects on fibrinolysis as well as ECM functions.

The plasma level of PAI-1 in resting healthy individuals is around 1 nM, an amount which is two to three times more than needed to inhibit the circulating plasminogen activators. As discussed below, multiple stimuli, including

Table 4.1 Characteristics of plasminogen activator inhibitor type 1

Mechanism of action
Inhibits plasminogen activators
Inhibits plasmin directly
Binds to and activates vitronectin
Binds to glycosaminoglycans
Binds to LDL-R
Inhibition of apoptosis
Factors regulating PAI-1
Cytokines: TNFα, IL-2, IL-6
Growth factors: TGFβ1, EGF, FGF
Hormones: Insulin, glucocorticoids
Angiotensin II, IV
Hypoxia
Reactive oxygen species
Pathogenic role in
Thrombotic disorders
Arterial and venous thrombosis
Acute myocardial infarction
Atherosclerosis
Obesity
Insulin resistance syndrome
Polycystic ovarian syndrome
Pulmonary fibrosis
Tumor growth and metastasis

inflammatory and tumor derived cytokines, can further increase this level. The plasma level of PAI-1 is elevated in obesity, metabolic syndrome, type II diabetes, inflammatory states, and cancer [37]. PAI-1 levels are often used as a prognostic marker for thromboembolic complications in patients with cancer, type II diabetes and veno-occlusive disease in the post-bone marrow transplantation setting. PAI-1 is present in many tumor cell types and in the stromal fibroblasts of the tumor microenvironment as well as in tumor associated endothelial cells. In these settings, it may modulate tumor growth, invasion and angiogenesis.

PAI-1 is the principal inhibitor of both uPA and tPA. In addition, it has a high affinity for ECM proteins, especially vitronectin. The interactions between vitronectin, uPA, uPAR and PAI-1 modulate multiple functions of uPA. The binding of PAI-1 to vitronectin stabilizes the adhesion of cells to the ECM. Both of these processes are essential for cell migration. PAI-1 bound to the uPA-uPAR complex is internalized by endocytosis. While PAI-1 and uPA are degraded intracellularly, uPAR is secreted by the cell and recycled. This process facilitates the propelling action observed in cell migration and tissue remodelling. In addition, PAI-1 binds to glycoaminoglycans, and to low density lipoprotein receptors (LDL-R) [38]. LDL-R facilitates the internalization of the PAI-1-uPA-uPAR complex.

PAI-1 added to cell cultures inhibits apoptosis of both normal vascular smooth muscle cells and tumor cells, and may thus contribute to tumor proliferation and to angiogenesis [39, 40].

4.4.2 Regulation of PAI-1

As the PAI-1 gene is expressed in almost every cell type in the body, transcription of this gene is regulated by numerous signals generated by cellular responses to various stimuli [41, 42] (Table 4.1). These responses include inflammatory cytokines such as TNFα and IL-1, growth factors, including TGFβ1, EGF, and FGF, hormones such as insulin and glucocorticoids, angiotensin II and angiotensin IV [43] and hypoxia-inducing factor and reactive oxygen species. Through these pathways, PAI-1 is up-regulated in obesity and metabolic syndrome, type II diabetes, hypertension and many types of cancer. These observations led to the concept that PAI-1 not only plays a major role in thrombogenesis by inhibiting fibrinolysis, but is also involved in the pathogenesis of many other disorders by its modulation of cellular interactions.

Several anti-PAI-1 agents are being developed for possible therapeutic use in cancer and other disorders affected by PAI-1 [3, 37].

4.4.3 Plasminogen Activator Inhibitor Type 2 (PAI-2)

PAI-2 is synthesized by the placenta, monocytes [44, 45], eosinophils and keratinocytes as well as by ovarian tumors and myeloid leukemic cells. PAI-2, as in the case of PAI-1, is an inhibitor of both uPA and tPA [46]. Its expression by the placenta is believed to contribute to the increased prothrombotic risk in late pregnancy. Also, like PAI-1, it binds the uPA-uPAR complex on the cell surface and is then internalized. However, unlike PAI-1, most PAI-2 exists within the cell in the cytosol and only a small fraction is secreted. The plasma level is barely detectable. While the secreted portion takes part with PAI-1 in the inhibition of the plasminogen activators, the main functions of PAI-2 within the cell are not clear. One known intracellular function is the protection of cells against TNFα-mediated apoptosis [47]. It is notable that the PAI-2 gene is located in chromosome 8, less than 300 mbp from the apoptosis-inhibiting BCL-2 gene. In follicular lymphoma with the t(14;18) translocation, BCL-2 is over-expressed, resulting in inhibited apoptosis of the lymphoma cells [48] . However, it is not known whether PAI-2 has a similar effect on lymphoma.

There are a number of observations of a tumorogenic effect of PAI-2. Studies with oligonucleotide microarrays indicate there is at least a 12-fold increase in PAI-2 genes in ovarian serous papillary carcinoma compared to normal ovarian tissues [49]. The level of PAI-2 has been correlated with a poor prognosis for ovarian, and colorectal carcinomas, while low expression in epithelial

carcinoma such as head and neck squamous cell carcinoma signifies invasion [50]. In contrast, a high PAI-2 level indicates a favorable prognosis in breast carcinoma. Recently, high expression of PAI-2 was found in the gastric mucosa of patients with *Helocobacter pylori* infection, suggesting another possible link between the proliferative and apoptotic inhibitory actions of PAI-2 and the ultimate gastric cancer formation [51].

4.4.4 Thrombin-Activatable Fibrinolytic Inhibitor (TAFI)

Thrombin-activatable fibrinolytic inhibitor, also known as procarboxypeptidase U, is a 55-kDa carboxypeptidase synthesized in the liver that has been shown to play an important role in fibrinolysis. It is initially produced as a proenzyme and is converted to an active, zinc ion dependent carboxypeptidase B-like enzyme (TAFIa) through cleavage at the Arg92-Ala93 bond. This process can be mediated by trypsin or plasmin. However, these activators are much less efficient than thrombin. Thrombin conversion of TAFI to TAFIa is accelerated 1250-fold in the presence of thrombomodulin [52], and the activation is calcium-ion dependent. TAFIa production occurs in two peaks, with the first peak occurring shortly after initiation of clot formation and the second stimulated by thrombin generation. The half-life of the enzyme is approximately 10 min and therefore the first increase in concentration is transient. The second peak is prompted by plasmin formation and unlike the first, has little effect on fibrinolysis [53].

TAFIa is able to attenuate fibrinolysis by preventing plasmin formation through removal of carboxyterminal arginine and lysine residues from fibrin and fibrin cleavage products. This blocks the binding of tPA and plasminogen, thereby interfering with the positive feedback of plasmin formation [54]. Removal of the arginine and lysine residues from fibrin also removes the inhibitory effect fibrin has on antiplasmin. Fibrinolysis is then prevented by the greater resulting influence of antiplasmin on free plasmin [55]. Plasmin may also be directly inhibited by TAFI, to impair fibrinolysis further. The increased amounts of TAFIa generated by activation of the intrinsic system continue to propagate the downregulation of fibrinolysis. There have been no reported inhibitors of TAFIa to date, and the activity appears to decay spontaneously.

Due to its effect on fibrinolysis, levels of TAFIa may correspond to an increased risk of thrombosis or bleeding. Increased levels have been shown to be a weak risk factor in incident (twofold increased risk) [56] and recurrent (twofold increased risk) [57] venous thromboembolism. Concurrent elevations in factor VIII may enhance this thrombogenic risk. On the other hand, low TAFI activity is seen in patients with acute promyelocytic leukemia, and this finding may in part explain the hyperfibrinolysis and propensity for hemorrhage seen in this disease [58]. TAFI levels have also been investigated in a number of other disease states where perturbations of clotting may play a

central role. Examples include demonstration of increased TAFI levels in ischemic stroke, in patients with angina, and in men requiring coronary artery bypass grafting [59–61].

It is unclear what role, if any, TAFI has in malignant disease states. A mouse model failed to show any affect of TAFI deficiency on growth or metastasis of different tumor cell types [62]. Malignancy may increase TAFI expression through a cytokine-mediated process. Theoretically, increased levels of TAFI may promote growth and spread of tumor cells through intra-tumoral fibrin deposition, and may accentuate the several prothrombotic features of various malignant states. Clinical data in humans supporting these possibilities is lacking.

4.5 Role of the Plasminogen – Plasmin System in Tumor Growth and Metastasis

The relationship between the plasminogen-plasmin system and tumor biology is complex [8, 42, 63, 64] (Fig. 4.2). It involves several important steps as shown in Fig. 4.2. The major ones are cell proliferation, apoptosis, cell migration and invasion and angiogenesis. In all these steps, one or more of the components of the plasminogen-plasmin system participate in the process.

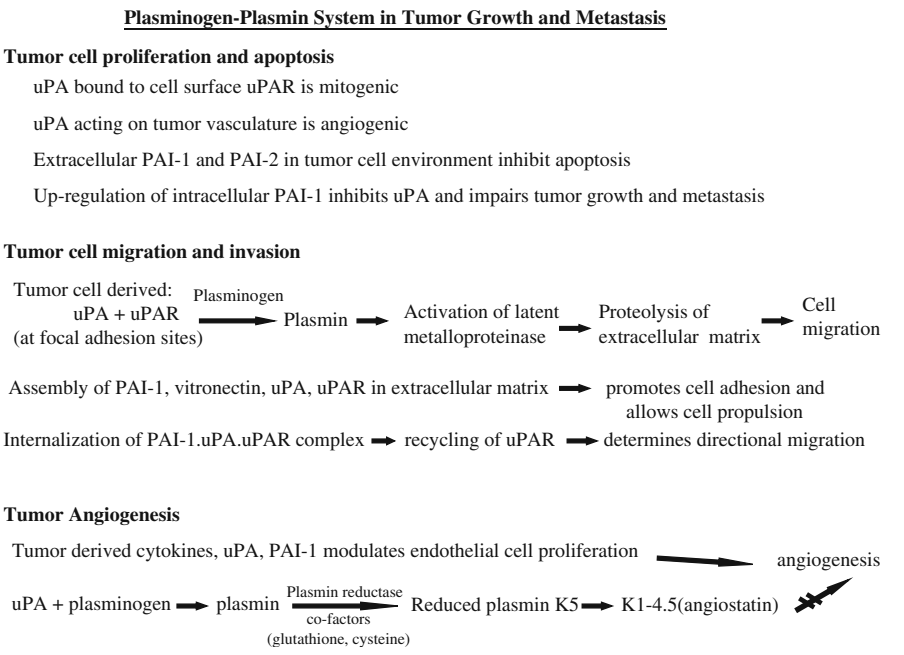

Fig. 4.2 Plasminogen-plasmin system in tumor growth and metastasis

4.5.1 Tumor Cell Proliferation and Apoptosis

uPA bound to uPAR is mitogenic. On the surface of tumor cells, initiation of intracellular signaling follows the assembly of the amino-terminal fragment of uPA (containing an epidermal growth factor-like domain), and uPAR, along with an ECM protein (such as vitronectin or fibronectin) and the epidermal growth factor receptor, leads to growth stimulation [65, 66]. The same events occur with stromal cells, vascular smooth cells and endothelial cells in the tumor microenvironment. These characteristics of uPA form the basis for a high uPA expression in tumors signifying a poor prognosis. On the other hand, we had shown previously that, in vitro and in animals, high expression of PAI-1, by inhibiting uPA, impairs tumor growth, angiogenesis and metastasis [67]. When PAI-1 was transfected into an aggressive human prostate cancer cell, PC-3, tumors with transfected cells that over-expressed PAI-1 had a slower growth rate in vitro. Tumors in athymic mice given PC-3 clones with high PAI-1 expression were smaller, less metastatic and contained less vasculature. This anti-tumorogenic effect is attributed to the inhibitory action of uPA on tumor invasion, and on uPA activation of plasmin. However, in a different setting, PAI-1 has an opposite effect by being inhibitory to apoptosis. When the stable form of PAI-1 is added to tumor cell cultures, or endothelial cell culture, both spontaneous and induced apoptosis in tumor cells are inhibited [39]. In this respect, PAI-1 enhances tumor growth. PAI-2 added to tumor cell cultures also has an anti-apoptotic action. As tumor growth involves both cell proliferation and apoptosis, PAI-1 may favor tumor growth by inhibiting apoptosis. In certain tumors, a high PAI-1 content signifies a poor prognosis. These observations of stimulated and inhibited tumor growth were confirmed by others [38]. Which of these opposing effects of PAI-1 are acting on the tumor must be dependent on a number of factors, many of which are still not fully understood. PAI-1 injected into animals or added to tumor cell cultures likely inhibits uPA on the tumor cell surface, where the assembly of PAI-1 and the uPA-uPAR complex are internalized, leading to the degradation of uPA. Also, it is likely that the effect may be dose-dependent. One determinant may also be the tumor type. For example, in carcinoma of the breast, the known tumorogenic effect of estradiol may in part be mediated through the down-regulation of tPA, uPA, uPAR and PAI-1 [68].

The activation of pro-forms of growth factors is another way in which plasmin, tPA or uPA can participate in tumorogenesis. One example is that plasmin can activate latent β-FGF. Plasmin has also been shown to activate the propeptide of VEGF-C and VEGF-D, both angiogenic and lymphangiogenic factors [69]. Of interest, tPA can directly activate the latent form of PDGF-CC by proteolytic removal of the CUB domains [70].

4.5.2 Tumor Cell Migration and Invasion

The regulation of cell migration by uPA, uPAR and PAI-1 in cell migration is important in wound healing as well as in cancer cell invasion, and metastasis. uPA bound to uPAR is present at focal adhesion sites on the cell surface [71, 72]. Locally generated plasmin from the activation of plasminogen at these sites in turn activates latent metalloproteinases and latent growth factors [5]. It is believed that the resulting proteolysis of the extracellular matrix (ECM) frees the cell from its adhesion site allowing cell migration. Since uPA bound to uPAR is a much more potent activator of plasminogen, the location of uPAR determines the direction of the cell migration. There is also another major cellular process, involving vitronectin in the ECM, uPA-uPAR complex, PAI-1 and the recycling of uPAR [34]. Both monomeric and dimeric forms of uPAR are present on the cell surface [33, 73]. Vitronectin in the ECM binds to uPAR, preferentially the dimeric form [74], and affects the distribution of uPAR on the cell surface. The complex of uPA, uPAR and PAI-l binds to vitronectin on the cell surface, resulting in changes in the cell shape and cell adhesiveness to the ECM. With the endocytosis of the complex assembly, de-adhesion occurs. Following the recycling of uPAR, readhesion takes place [33]. These steps regulate cell migration and propulsion.

4.5.3 Tumor Angiogenesis

The plasminogen-plasmin system is involved in tumor angiogenesis in two aspects [75–79]. First, along with tumor derived cytokines and VEGF, uPA and PAI-1 modulate endothelial cell proliferation. Second, plasmin and plasminogen activators proteolyse plasminogen and, acting with several cofactors, release one or more of the kringle structures. These kringles possess inhibitor effects on tumor angiogenesis, best exemplified by angiostatin. Plasmin, derived from uPA activation of plasminogen, can be reduced by plasmin reductase (phosoglycerate kinase) in the presence of co-factors, including glutathione and cysteine. The disulfide bonds between kringles are further proteolysed to form kringle peptides with potent inhibitory activity against the proliferation of microvascular endothelial cells. Despite encouraging results of tumor reduction in animals, these results were not reproduced in early human clinical trials [79].

4.6 Role of the Plasminogen-Plasmin System in Thrombotic Complications in Cancer

Though procoagulants and their activation in cancer play a major role in thrombogenesis, changes in fibrinolytic components are also important contributory factors. Evidence for this is provided by in vitro studies as well as

observations of inhibited fibrinolysis in vivo. The inhibited fibrinolysis is primarily due to increased in PAI-1 activity in the plasma of cancer patients. Both uPA and tPA are regulated by PAI-1. While uPA acts on cell proliferation and migration and thus plays an important role in cancer progress, tPA, on the other hand, is the body's defense against extension of intravascular fibrin thrombi, and thus a high PAI-1 level is thrombogenic.

4.7 The Plasminogen-Plasmin System in Acute Leukemia

Both thrombotic and bleeding complications are linked to perturbation of the plasminogen-plasmin system in acute leukemia [80, 81]. In acute promyelocytic leukemia (FAB: M-3), annexin II, a dual receptor for tPA and for plasminogen [27, 28], is highly expressed in the leukemic cells [29, 82]. Its level is also increased in a small number of patients with AML (FAB: M4-5) or with ALL. Annexin II is a cell membrane surface protein found in endothelial cells, macrophages and several malignant cell lines. It is a protein with a molecular weight of 40 kDa. In addition to binding tPA, it is a co-receptor for plasminogen, with tPA binding at the amino-terminal of the core 1 domain, while plasminogen binding occurs at the lysine binding site in the core 4 domain. The close proximity of the two ligands on the cell surface facilitates their interaction in plasmin generation, with in vitro enhancement of 60-fold. Annexin II on endothelial cell surface has the highest expression on the microvascular endothelial cells in the brain [83]. In acute promyelocytic leukemia (APL), this location of a higher Annexin II expression is of clinical significance. APL cells in the bone marrow and peripheral blood as well as the APL cell line NB4, express both uPA and tPA [84]. After treatment with *trans*-retinoic acid, the uPA expression is further dysregulated in NB4 cells for 24 h before returning to normal levels as these cells undergo differentiation [85]. Thus, an increased expression of uPA by APL cells, in conjunction with the expression of tPA as well as the presence of annexin II contributes to the excessive fibrinolysis [29]. In addition, TAFI level is low in APL and thus there is less inhibitory control over fibrinolysis [59]. Larger amounts of plasmin were also found to be generated in vitro by the brain endothelial cells [84]. This may provide an explanation for the relatively higher incidence of intracranial hemorrhage in APL. In a recent analysis of the early deaths seen in patients with APL, hemorrhagic complications were found to be the major cause of early deaths, accounting for over 50% of patients with or without ATRA therapy [86–90]. Intracranial bleeding accounts for most of the fatal hemorrhages, with other sites including diffuse pulmonary alveolar and gastrointestinal hemorrhage. In addition to plasmin induced fibrinolysis, elastase and chymotrypsin released by leukemic blasts may also contribute to the impaired hemostasis by proteolysis of von Willebrand factor [91].

4.8 Clinical Observations of the Plasminogen-Plasmin System in Cancer

As discussed, the plasminogen-plasmin system has potential biological effects extending beyond thrombosis and hemorrhage. Tumor growth and metastasis may be promoted by uPA-mediated enhancement of cell proliferation, adhesion, migration, and degradation of the extracellular matrix. Alterations of this system in cancer could lead to a variety of altered clinical outcomes, which has been demonstrated in a number of cancer types.

The majority of the clinical literature evaluating upregulation of the plasminogen-plasmin system has focused on prognosis through the enhancement of tumor growth and progression. As mentioned in detail previously, this may come about through inhibition of apoptosis and through increased release of TGFβ, FGF2, ILGF-1, and hepatocyte growth factor. Degradation of the extracellular matrix and promotion of cellular adhesion may assist in the development of metastatic disease. These features of the plasminogen-plasmin system have been correlated with its over-expression, leading to adverse outcomes in a number of malignancies (Table 4.2). High uPA and/or PAI-1 levels have been shown to be adverse prognostic markers in breast, colorectal, esophageal, gastric, ovarian, prostate, renal, and endometrial cancers. These findings may prove to be a vital addition to previously known prognostic markers and potentially assist in individualizing cancer treatments.

Table 4.2 Plasminogen-plasmin system: clinical and laboratory features in malignancy

Malignant site	Reference	Laboratory and clinical findings
Breast	Look et al. [92]	Pooled analysis (8,377 patients). uPA and PAI-1 prognostic independent of patient age, tumor size, grade, or hormone receptor status, and nodal status. uPA and PAI-1 levels more predictive of RFS and OS in lymph node negative disease than ER status and tumor size. May aid in determining need for additional therapy in some women
	Janicke et al. [97]	Longer DFS with low PAI-1 & uPA in node negative disease compared with high PAI-1/uPA
		uPA and PAI-1 more informative than size, hormonal status, age, locoregional treatment. High uPA and PAI-1 in node negative disease benefit most from adjuvant chemotherapy
	Foekens et al. [98]	High PAI-2 associated with favorable RFS and OS in tumors with high uPA. No PAI-2 prognostic association in overall population
	Sternlicht et al. [99]	Shorter OS with high tumor PAI-1 mRNA levels. PAI-1 levels influenced by connective tissue growth factor. No prognostic association with PAI-1 gene promoter polymorphism

Table 4.2 (continued)

Malignant site	Reference	Laboratory and clinical findings
	Grondahl-Hansen et al. [100]	High uPAR levels associated with shorter OS, but no affect on RFS Shorter OS and RFS with elevated PAI-1 levels
	Duffy et al. [101]	uPA is a stronger prognostic indicator for RFS than lymph node status Lymph node status is a stronger predictor for OS than uPA. uPA also predictive of OS and DFI in node negative disease whereas tumor size, ER status were not
	Demirkan et al. [102]	Anthracycline based chemotherapy did not alter TAFI or PAI-1 levels
Gastric	Wojtukiewicz et al. [103]	Tumor tissue staining: fibrinogen seen throughout. Fibrin/D-dimer at tumor margin; No TFPI present; high molecular weight urokinase and plasminogen not detected; weak tPA staining on tumor cells; strong PAI-1 expression. Conclude that TFPI, fibrinolysis does not balance tumor coagulation
	Heiss et al. [104]	High uPA associated with more aggressive disease, and prognostic in lymph node positive, T1/T2 disease. Not predictive in lymph node negative disease
	Nekarda et al. [105]	Decreased OS with elevated uPA or PAI-1 from completely resected tumors. Only PAI-1 was a significant prognostic marker in multivariate analysis
	Cho et al. [106]	uPA and PAI-1 higher in cancer than normal gastric tissue, with higher levels corresponding to decreased RFS. Only uPA was a significant prognostic marker in multivariate analysis
	Beyer et al. [107]	High uPAR staining correlated with *H. pylori* infection. High tumor PAI-1 expression is an independent predictor of poor prognosis
	Luebke et al. [108]	Unable to find any correlation between uPA or PAI-1 with tumor size, grade, nodal status, or metastatic disease on prospective evaluation. No survival association was found with uPA or PAI-1
Colorectal	Skelly et al. [109]	uPA levels highest in carcinoma vs adenomatous polyp or normal mucosa. High uPA associated with lower OS. Prognostic effect is independent of tumor stage. May indicate more aggressive disease
	Mulcahy et al. [110]	High grade uPA staining in Duke's B colon ca is associated with worse prognosis compared with low grade staining (8 year survival 81 vs 43%)
	Yang et al. [111]	Higher tumor expression of uPA and uPAR are independently predictive of distant metastatic disease, cancer specific survival, and overall survival
	Ganesh et al. [112]	High uPAR levels associated with decreased OS; independent of age, stage, tumor grade
	Stephens et al. [113]	Pre-operative plasma soluble uPAR levels independently predicted survival in colorectal cancer. High soluble uPAR levels associated with increased risk of mortality

Table 4.2 (continued)

Malignant site	Reference	Laboratory and clinical findings
	Kockar et al. [114]	Heightened fibrinolysis (elevated D-dimer, global fibrinolytic activity) in metastatic vs local disease
Glioma/ glioblastoma	Sciacca et al. [115]	Increased tPA and PAI-1 levels in high grade glioma vs neurologic and healthy controls. No difference in factor V Leiden or Prothrombin gene mutations. May indicate significant role for PAI-1 in prothrombotic state
	Landau et al. [116]	uPA and PAI-1 are elevated in gliobastome multiforme but not normal brain or benign tumors. May facilitate infiltration of tumor cells into normal brain parenchyma
	Hsu et al. [117]	Stronger cytoplasmic uPA staining associated with higher grade glioma and shorter OS. On multivariate analysis: high uPA staining, tumor grade, and age>50 are associated with shorter survival
Liver	Kwaan et al. [118]	Increased markers of plasma fibrinolysis found in cirrhosis but not hepatocellular carcinoma
	De Petro et al. [119]	uPA mRNA found in hepatocellular carcinoma, but not in normal hepatocytes. Higher levels of uPAR and tPA in malignant vs non-malignant cells. Higher uPA correlated with shortened OS in male patients
Lung	Hataji et al. [96]	Highest TAFI levels in small cell vs adeno- or squamous carcinoma. TAFI levels higher in chemotherapy responders vs nonresponders
	Pavey et al. [120]	Elevated plasma D-dimer, fibrinogen, and PAI-1 antigen associated with increased risk of death in non-small cell lung carcinoma
Prostate	Miyake et al. [121], and Hienert et al. [122]	uPA and uPAR levels higher in advanced disease with metastatic bone disease vs organ confined disease
	Shariat et al. [123]	Higher uPA and uPAR plasma levels found in patients with prostate cancer than those without
		uPA and uPAR levels correlated with extent of disease at diagnosis and higher levels pre-prostatectomy predicted for prostate specific antigen doubling times, failure to respond to salvage radiation therapy, and development of metastatic disease
Esophageal	Torzewski et al. [124] and Nekarda et al. [125]	High uPA expression is an independent predictor of disease outcome in both squamous and adenocarcinoma. Independent of tumor grade, stage, lymph node status, lymphatic/vessel invasion
Laryngeal	Wojtukiewicz et al. [126]	Plasminogen, tPA located on tumor cells. Low molecular weight uPA located on undifferentiated cells. Weak/variable PAI-1,2, and 3 expression found. Trace uPA receptor staining
Ovarian	Kuhn et al. [127]	Increased uPA and PAI-1 were predictive of decreased OS in univariate, but not multivariate, analysis
	Konecny et al. [128]	uPA associated with malignant progression and independently associated with shortened OS. PAI-1 not prognostic in multivariate analysis

Table 4.2 (continued)

Malignant site	Reference	Laboratory and clinical findings
Melanoma	Kwaan et al. [129]	tPA and inhibitors of fibrinolysis (e.g., α2 PI) expressed in tumor cells, but only tPA found in melanoma cell lines and xenografts. Increased fibrinolytic inhibitors may contribute to hypercoagulability
Endometrial	Tecimer et al. [130]	Higher uPA and PAI-1 levels are associated with more advanced stage of disease and disease recurrence. Shorter disease-free and overall survival found with elevated PAI-1 levels
		uPAR levels had no prognostic value
Cervical	Kobayashi et al. [131]	Higher rates of lymph node involvement with strong uPA and PAI-1 staining in primary malignant tissue. Decreased OS and RFS with strong PAI-1 and uPA staining of malignant cells
Renal cell	Hofmann et al. [132]	Strong tumor tissue staining for uPA, uPA-R, or PAI-1 correlates with developing relapsed and/or metastatic disease
	Ohba et al. [133]	Higher uPA, uPAR, PAI-1 and PAI-2 expression correlated with higher tumor grade and metastatic disease. uPA, uPAR, and PAI-1 expression also correlated negatively with survival, although PAI-2 did not

RFS: relapsed-free survival; OS: overall survival; ER: estrogen receptor; DFI: disease-free interval; TFPI: tissue factor pathway inhibitor

The prognostic significance of the plasminogen-plasmin system has best been demonstrated in breast cancer. Levels of uPA and PAI-1 were more predictive of both disease-free and overall survival than ER status and tumor size in a pooled analysis of over 8,000 patients with breast cancer [92]. Increased uPA and PAI-1 were associated with a worse prognosis. Application of this finding to clinical practice was demonstrated in a prospective study of 761 patients [93]. Based on the hypothesis that poor outcome is associated with high PAI-1 and uPA, patients with lymph node negative disease, but with high PAI-1 and uPA, were given adjuvant cyclophosphamide-methotrexate-5-fluorouracil (CMF) chemotherapy. Another large trial of over 3,000 women showed increased levels of PAI-1 and uPA correlated with greater benefit from adjuvant chemotherapy vs those with lower levels [94]. Similar findings are present with regard to adjuvant hormonal treatment, with uPA and PAI-1 negative breast tumors responding better to intervention with tamoxifen than those with high expression, independent of ER/PR status [95].

Application of the prognostic information given by the plasminogen-plasmin system to clinical care has also been extended to other malignant diseases. For example, it has already been shown that higher TAFI levels in lung cancer directly correlate with a more favorable response to chemotherapy [96]. Such observations allow better individualization of cancer care, with administration of more aggressive treatment to those patients who are likely

to benefit most. In addition, those who are unlikely to benefit could be spared the toxicities associated with many therapeutic interventions. Additional data is needed in this area to justify its wider application.

Available evidence indicates a pathophysiologic role of the plasminogen-plasmin system in the prothrombotic nature of malignant disease, with high tumor expression of PAI-1 and resultant inhibition of fibrinolysis potentially exacerbating the hypercoagulability associated with malignancy. To date, clinical studies demonstrating this correlation have largely been lacking. Whether components of this system can be used successfully in the treatment or prevention of thrombosis in cancer remains to be established. It is certainly plausible that alterations in the expression of the various components of the uPA system may predict risk for thrombosis in the same way as with disease outcome. This type of information may prove useful in targeting those at highest risk, with greater surveillance and possibly prophylactic treatment for those patients whose thrombotic potential is greatest.

4.9 Conclusion

Though fibrin was found in cancer tissues as early as the late nineteenth century, the active investigation of the role of the plasminogen-plasmin system in cancer has accelerated only in the past two decades. This has greatly increased our understanding of how the components of this system, especially uPA, uPAR and PAI-1, affect tumor growth, invasion and angiogenesis. Undoubtedly these findings have contributed to the elucidation of the pathogenesis of many forms of malignant disorders. However, little progress has been made in translating the findings from in vitro studies and animal experiments into innovative therapeutic approaches. In experimental tumors in animals, perturbation of uPA and of PAI-1 has been found to impair tumor growth and metastasis, while only a few anecdotal results have been reported in humans. As the pathogenesis of cancer is complex, one would expect that the influence of uPA and PAI-1 is only one part of this process. New agents are being designed to interdict these effects, especially those of PAI-1. Whether they will be effective remains to be determined by clinical trials. Questions to be addressed in future clinical trials will concern the effect of anti-PAI-1 or anti-uPA agents by themselves, or whether effectiveness will require a combination with cyto-reductive measures including chemotherapy or radiation, and in addition, the combination with anti-angiogenic agents or with hormonal therapy wherever applicable.

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Chapter 5
The Coagulation System and Angiogenesis

Gurinder Sidhu and Gerald A. Soff

5.1 Introduction

In 1865, Armand Trousseau recognized the relationship between an activated coagulation system and malignancy [1]. He suggested that patients presenting with idiopathic venous thrombosis might be harboring an occult cancer. Numerous recent studies have also demonstrated an increased odds ratio of patients with idiopathic venous thromboembolic disease having an occult malignancy (i.e. [2]). Venous thromboembolic disease (VTE) is the second most common cause of death in cancer patients, after deaths from the underlying malignancy [3]. The prevalence of VTE depends on tumor type and treatment received. Recent studies note that activation of the coagulation system, in addition to predisposing to VTE, may also contribute directly to the growth of primary and metastatic cancers, in large part by promoting angiogenesis. In this chapter, we will review the role of platelets and the coagulation system in promoting tumor-associated angiogenesis. We will also discuss the effects of anticoagulation on angiogenesis and the effects of anti-angiogenesis therapy on the coagulation system and risk of thrombosis.

5.2 Platelets and Angiogenesis

Reactive thrombocytosis is common in cancer patients, and is observed in 30–60% of cancer patients [4, 5]. Increased granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), thrombopoietin (TPO), and interleukin-1 (IL-1), have been associated with tumor-associated thrombocytosis [6]. In addition to thrombocytosis, elevated levels of β-thromboglobulin and P-selectin are found

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in prostate, breast, lung, colon, and gastric cancers, suggesting that platelets are activated in cancer [7–11] and are relevant to cancer biology. Recent studies have supported the hypothesis that platelets contribute to angiogenesis and therefore, to cancer growth and metastasis.

Gasic and colleagues were the first to show the contribution of platelets to hematogenous metastasis [12]. They induced thrombocytopenia in mice by administration of neuraminidase or antiplatelet serum, followed by tail vein injection of TA_3 ascites tumor cells. They observed a dose-dependent reduction in the formation of pulmonary metastases, corresponding to the induction of thrombocytopenia. Since those early observations, other studies also have supported a role of platelets in tumor growth and invasion, and at least part of the process is related to the ability of platelets to enhance and regulate angiogenesis.

It is well-established that platelets contribute to the earliest stage of the coagulation by adhering to the subendothelial matrix of damaged or altered blood vessels. In addition, platelets can express and deliver molecules involved in angiogenesis [13–15]. Platelets can stimulate endothelial cells in culture and can promote the assembly of capillary-like structures in vitro, a surrogate for in vivo angiogenesis activity [16]. Other recent studies have also shown decreased angiogenesis by induction of thrombocytopenia and administration of anti-platelet agents [17]. Platelet alpha-granules contain both promoters and inhibitors of angiogenesis. Platelets may influence angiogenesis by releasing promoters of angiogenesis, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGFR), platelet derived growth factor (PDGF) and matrix metalloproteinases (MMPs) [13, 18–22]. Thrombin treatment of platelet-rich plasma from healthy individuals exhibited higher VEGF levels than platelet-free plasma [23]. Platelet alpha-granules also possess inhibitors of angiogenesis, including endostatin, platelet factor-4, thrombospondin-1, alpha-2-macroglobulin, plasminogen activator inhibitor-1, and angiostatin [24, 25].

A recent study by Italiano and colleagues suggest that pro- and anti-angiogenic factors exist in different alpha-granule populations [26]. Using double immunofluorescence and immuno-electron microscopy, they found that the majority of the alpha-granules stained for either VEGF or endostatin, but there was little evidence for co-localization [26]. Further, the angiogenesis inhibitor, thrombospondin-1, and the angiogenesis promoter, bFGF, also segregated into separate, distinct granules. von Willebrand factor (vWF) co-localized with endostatin while fibrinogen co-localized with VEGF. The packaging of VEGF and endostatin into separate alpha-granules suggested that distinct granule subpopulations could undergo selective release. This was supported by the observed selective release of endostatin-containing alpha-granules, but not VEGF containing granules, by protease-activated receptor (PAR)4 activation. In contrast, PAR1 activation resulted in release of VEGF containing granules, but not endostatin containing granules. This interesting study suggests that selective activation of specific PARs results in selective release of promoters or inhibitors of

angiogenesis related factors, and may regulate the angiogenic potential of the adherent platelets [26].

Following platelet adhesion and activation in the tumor microvasculature, released VEGF stimulates endothelial cells. VEGF stimulation of endothelial cells is followed by adhesion of additional, non-activated platelets [27]. The adhesion function of platelets, as mediated by the vWF receptor GPIb-α, significantly contributes to the process [15]. In an in vitro model of angiogenesis, inactivated human platelets are potent promoters of tube formation by endothelial cells on Matrigel. Tube formation was accelerated in the presence of platelets, suggesting that platelets affect both the magnitude and the kinetics of this process [16]. Kisucka and colleagues used the cornea neovascularization assay and the Matrigel plug angiogenesis model to evaluate the regulatory effect of platelets in an in-vitro model of angiogenesis. The number of growing vessels in thrombocytopenic mice was lower in the cornea assay, and they showed significantly increased appearance of hemorrhage compared with control mice. The thrombocytopenic mice also showed more protein leakage and developed hematomas using the Matrigel model. These data suggest that platelets not only stimulate angiogenic vessel growth, but also play a critical role in vessel wall stability by preventing hemorrhage from the angiogenic vessels.

Platelet derived microparticles (PMPs) have also been shown to have proangiogenic properties [28]. Addition of in vitro generated PMPs to isolated rat aortic ring models was associated with a concentration-dependant proangiogenic effect. This effect can be reduced by inhibition of VEGF, bFGF, and PDGF, suggesting these three factors contribute to the PMP-associated proangiogenic effect. PMP exerted this effect via PI 3-kinase, Src kinase, and ERK. The pro-angiogenic effect of PMP was also demonstrated in vivo. In a subcutaneous pocket model, agarose beads containing PMP (100 μg/mL) were as effective or more effective in inducing angiogenesis than beads with a combination of pure VEGF/bFGF (50 ng/mL each) [28]. PMPs were also shown to be angiogenic in a mouse model of myocardial ischemia. Following coronary artery ligation and myocardial ischemia in mice, addition of PMPs resulted in an almost threefold increase in coronary revascularization, again confirming the pro-angiogenic activity of PMPs [28].

5.3 Tissue Factor and Angiogenesis

Tissue factor (TF) is 47-kDa transmembrane protein that initiates the coagulation cascade [29, 30]. Mature TF is composed of 263 amino acids: a 219 amino acid extracellular domain, a 23 amino acid transmembrane domain and a 21-residue intra-cytoplasmic domain [29, 30]. Following vessel injury or endothelial cell damage, circulating factor VII binds to TF and is converted to the active serine protease, factor VIIa. The TF:FVIIa complex can activate factors IX and X and hence initiates the coagulation cascade. Factor X is activated by

TF:FVIIa complex and forms part of the prothrombinase complex that converts prothrombin to thrombin. Thrombin has multiple procoagulant activities, including cleavage of fibrinogen to fibrin monomers, activation of factors V, VIII, XI, and XIII, and activation of platelets [31]. Inhibition of TF mediated activation of coagulation is regulated by tissue factor pathway inhibitor (TFPI) that forms a quaternary complex with TF, FVIIa and FXa [32]. The inhibition of TF function by TFPI can be increased if it is bound to thrombospondin, an angiogenesis inhibitor [33].

In addition to its procoagulant properties, TF is known to have an important role in angiogenesis, as first demonstrated by the embryological lethality of TF knock-out mice [34]. These mice die by day 10.5 during embryonic development, in part due to disorganization of the yolk sac vasculature [34]. Similar pathological findings are associated with lethality in VEGF-deficient embryos [35], suggesting regulation of similar functions by TF and VEGF. Indeed, TF and VEGF have been found to be co-localized on malignant cells from human lung and breast cancer samples [36]. The increased expression of TF in tumors results in an angiogenic phenotype, by up regulation of the pro-angiogenic factor VEGF and down regulation of anti-angiogenic factor thrombospondin [37].

Under physiologic conditions, activation of the phosphatidylinositol 3-kinase-AKT signaling pathway causes suppression of TF production in normal endothelial cells. Within the hypoxic environment of tumors, however, decreased PI3-K activity along with increased p38 and MAPK activity, induces up-regulation of TF expression in tumor-related endothelial cells [38]. The aberrant increased expression of TF is known to occur in a variety of tumor types including breast cancer [39], leukemia [40], glioma [41], non-small cell lung cancer [42] and colon cancer [43–45]. Increased levels of TF have been associated with increased angiogenesis, advanced stages of disease, and poorer outcome. Nakasaki and colleagues analyzed the expression of TF and VEGF, and microvessel density in 100 colon cancer specimens and found the expression of TF correlated with the Duke's stage [43]. Examination of non-small cell lung cancer specimens has also revealed a correlation between TF expression, microvessel density, and VEGF expression [46].

5.4 Thrombin

Thrombin is one of the key serine proteases of the coagulation cascade. The inactive zymogen prothrombin is proteolytically activated to thrombin by the prothrombinase complex (FXa, FVa, calcium ions) that assembles on the phospholipid membranes of platelets, monocytes, activated endothelial cells, or tumor cell surfaces [31, 47]. The best characterized function of thrombin is to cleave fibrinogen to fibrin monomers, which polymerize to form the basic

structure of the fibrin clot [31]. Cancer-associated fibrin matrix has been shown to be pro-angiogenic, and is discussed below. Thrombin is also one of the most potent platelet agonists. It induces shape change in platelets and the release of the platelet activators ADP, serotonin and thromboxane A2 [48]. Thrombin also activates the integrin GpIIb/IIIa, which mediates platelet aggregation, and induces alpha-granule release [48]. Thus, the generation of thrombin contributes to angiogenesis by serving as a platelet activator as well as by contributing to the fibrin polymer [21, 49]. Thrombin also regulates cellular behavior by activating G-protein coupled PARs that activate a network of signaling cascades [48]. Thrombin generated cleavage of the N-terminal domain of the PARs exposes a neo-N terminus that functions as a tethered ligand. This tethered ligand binds to the second transmembrane domain of the seven transmembrane G-protein coupled receptor. Activation of PAR causes a conformational change which results in the exchange of bound GDP for GTP on the associated G proteins. Thrombin activation of PARs leads to the up-regulation of many angiogenesis related genes including VEGF, VEGFRs, TF, bFGF, and MMP-2. These genes can promote to a number of pleotropic responses such as endothelial cell shape change, increased vascular permeability, increased cell proliferation, and increased proteolysis, that all contribute to increased tumor angiogenesis.

5.5 Fibrin

Fibrinogen and fibrin, the major components of the clot, also have important roles in angiogenesis. Tumor-associated blood vessels are typically leaky and hence fibrinogen and other clotting molecules extravasate from the defective vessels forming fibrin deposits around the tumor. These deposits provide a pro-angiogenic matrix that facilitates endothelial cell adhesion, migration, proliferation, and differentiation into tubules [50]. Fibrin contains multifunctional domains that act as bridging molecules between cell-matrix interactions during physiological and pathological processes [51]. Circulating cellular components specifically bind to fibrin matrices via integrin and non-integrin-mediated cell surface receptors and affect cell behavior. Integrin binding occurs specifically thorough Arg-Gly-Asp (RGD) sequences located at Aα95–98 (RGDF) and Aα572–575 (RGDS) of the human fibrinogen molecule [52].

Formation of the fibrin matrix allows the malignant cells to adhere to and invade the tissues in metastatic sites and protects the cancer cells from immune surveillance [53–55]. Fibrin has been reported to induce expression of the pro-angiogenic cytokine, interleukin 8, by endothelial cells [56]. The fibrin matrix also supports the migration of tumor cells and provides a scaffold for the formation of new blood vessels [57].

5.6 Plasmin/Angiostatin

The key component of the fibrinolytic system is the zymogen plasminogen, which is activated by a plasminogen activator to the serine protease plasmin. Plasmin's best characterized function is to degrade fibrin. There are also specific protease inhibitors that regulate plasmin generation and function, including plasminogen activator inhibitors (PAI-1 and PAI-2), and alpha-2-antiplasmin, and alpha-2-macroglobulin [58, 59]. Plasmin generation has been shown to occur on the surface of a number of cancer lines [58, 59]. Plasmin generation has a complex and biphasic effect on angiogenesis. Plasmin may promote angiogenesis by degradation of the extracellular matrix and basement membrane of the surrounding blood vessels, thus facilitating endothelial cell and cancer cell migration and invasion [60]. Plasminogen conversion to plasmin is localized to the surface of cancer cells by globular β-actin, which binds the fifth kringle domain of plasminogen and plasmin [58, 59]. The urokinase receptor (uPAR), also found on the surface of the cancer cells, binds urokinase (uPA) and the bound uPA converts plasminogen to plasmin [58, 59]. Plasmin remains bound to the surface globular β-actin and locally promotes angiogenesis. However, the surface globular β-actin serves as a co-factor for plasmin autoproteolysis, resulting in an intra-kringle 5 cleavage and the generation of the natural angiostatin isoform, angiostatin4.5 (AS4.5) [58, 59, 61–63]. AS4.5 is a potent angiogenesis inhibitor and consists of the first four kringles of plasminogen and 85% of kringle 5, and induces endothelial cell apoptosis, and inhibits angiogenesis in vitro and in vivo [64, 65]. Because the cleavage of plasmin to AS4.5 is within kringle 5, the domain that keeps plasminogen/plasmin anchored to the surface by globular β-actin, AS4.5 is released from the surface and conveys a systemic antiangiogenic effect. AS4.5 has been shown to inhibit tumor growth in mouse models [61, 66, 67]. AS4.5 has also been induced in human cancer patients, with promising anti-tumor effects [68]. Thus, the balance between plasmin and AS4.5, two plasminogen-derived proteins, influences the relative pro- and anti-angiogenic nature of a particular tumor.

5.7 Anticoagulation and Angiogenesis

If the coagulation process is important for angiogenesis and neoplastic growth, it would be expected that drugs that alter the coagulation system can impact tumor progression. As noted above, persistent activation of the coagulation system commonly accompanies advanced malignancies [69]. Vitamin K antagonism has been shown to decrease the number of metastases in animal experiments [70, 71]. Colucci and colleagues showed that induction of vitamin K deficiency had the same anti-metastatic effect on Lewis lung carcinoma cells as warfarin [72]. Vitamin K agonists (VKAs) appear to exert their anticancer effect through their anticoagulant effect [70]. Animal models have shown

positive effects with thrombolytic agents. Brown and colleagues showed that when streptokinase was administered 30 min after inoculating rats with mammary carcinoma cells, there was a decrease in the number of metastases as compared to controls [73].

While anticoagulants have shown significant antiangiogenesis activity in mouse models of cancer [74–77], the human experience has been less convincing and remains under study. Two trials using urokinase in addition to doxorubicin for intravesical treatment of superficial bladder cancer showed no improvement in response rates, rate of recurrence, or overall survival [78, 79]. Postoperative infusion of urokinase had no effect on 4-year survival [80]. There has been no rigorous study showing a statistically significant benefit of thrombolytic agents on human cancer.

A meta-analysis of cancer patients with venous thromboembolism who were initially treated with low molecular weight heparin (LMWH) vs unfractionated heparin (UFH) showed that patients treated with LMWH had a significantly better survival (see Chap. 15). This led to interest in the effect of anticoagulants on cancer progression [81]. In one study, cancer patients without evidence of venous thrombosis undergoing breast or pelvic cancer surgery, received prophylactic doses of UFH or LMWH perioperatively [82]. Improved survival with LMWH was observed as far out as day 650. Subgroup analysis showed survival advantage with LMWH to be only in ovarian and uterine carcinoma, and not in breast cancer [82]. It is not known whether the improved survival was due to differences in the anticoagulant properties of the two agents (UFH and LMWH), or some other property.

One of the largest trials in the use of LMWH in cancer patients is the CLOT study, which randomized 676 cancer patients with VTE to receive acute treatment with the LMWH dalteparin for 5–7 days, followed by either dalteparin or an oral vitamin K agonist [83] (see Chap. 14). There was a statistically significant decrease in the number of recurrent VTE events with dalteparin, without a significant increase in bleeding. However, there was no difference in survival at 6 month follow up, with 90% of deaths in both groups due to progressive cancer [83]. In the FAMOUS study, 385 cancer patients without venous thrombosis were randomized to receive LMWH or placebo for 1 year, designed to detect an anti-cancer effect of the LMWH [84]. The 1-, 2-, and 3-year survival of the LMWH treated group showed a trend to improved survival compared with the placebo group, but the results did not reach statistical significance [84]. A subgroup analysis of better prognosis patients in the FAMOUS study did suggest improved survival with the LMWH, but since this was not defined *a priori*, further study is warranted.

In another study of patients with advanced malignancy and absence of VTE, 302 patients were enrolled and then randomized to receive a six week course of LWMH or placebo. There was noted to be a modest but statistically significant improvement in survival at 6 months and 1 year [85]. Altinbas and colleagues randomized 84 patients with small cell lung cancer to undergo either chemotherapy alone or chemotherapy and LMWH [86]. There was noted to be a

statistically significant improvement in the response rate, progression free survival, and overall survival with addition of LMWH to chemotherapy. LMWH have a clear role in management of thrombotic complications with cancer, but the anti-neoplastic effects though encouraging, remain to be further explored.

5.8 Antiangiogenic Therapy and Coagulation

Antiangiogenic therapy is one of the most promising new classes of cancer therapy. However, these agents appear to convey an increased risk of thrombosis. While the mechanism by which angiogenesis inhibitors predispose to thrombosis is not fully understood, they do target the endothelial cells in tumor-associated vessels. Therefore, successful antiangiogenic therapy is associated with diminution of the endothelial cell lining of the blood vessels, exposing the blood to a relatively thrombogenic surface. Thus some increase in the rate of thrombosis may be expected with angiogenesis inhibition.

Thalidomide, one of the earliest antiangiogenic agents successfully used in human cancer patients, significantly increases the risk of both venous and arterial thrombosis [87, 88]. The anti-VEGF antibody bevacizumab also increases the risk of thrombosis. In the pivotal phase III trial of bevacizumab in metastatic colorectal cancer, Hurwitz and colleagues reported improved survival with addition of bevacizumab to chemotherapy, but this was also associated with an increased incidence of thrombosis [89]. They observed a 19.4% incidence of any thrombotic event in the bevacizumab treated arm, compared with a 16.2% incidence in the placebo arm. The rates of major bleeding were 3.1% in the bevacizumab arm and 2.5% in the placebo arm [89]. Patients with metastatic colon cancer developing thromboembolism during treatment with bevacizumab appear to tolerate anticoagulation without a higher risk of serious bleeding [89].

A phase III trial (ECOG 3200) comparing FOLFOX (oxaliplatin, fluorouracil, and leucovorin) chemotherapy with FOLFOX and bevacizumab found no increased incidence of arterial thrombotic events in the combination arm [90]. However, a statistically significant increase in the incidence of bleeding was noted in the bevacizumab arm [90]. A pooled analysis of five randomized controlled trials in metastatic colon cancer [89, 91, 92], breast cancer [93] and lung cancer [94] found a modest increase in the risk of arterial thromboembolic events among patients treated with bevacizumab [95]. The risk appeared to be increased more in patients who had a history of an arterial thromboembolic event and were aged 65 years or more [95].

Kuenen and colleagues investigated the effects of SU5416, a potent small molecule inhibitor of vascular endothelial growth factor (VEGF) receptor-1 and -2, on endothelial cell function and coagulation [96]. They noted a significant increase in the levels of soluble E-selectin, which reflects activation of

endothelial cells (ECs), and the levels of vWF and soluble-TF, which reflect activation of these cells and others that circulate. Interestingly, they noted that levels of soluble-E-selectin and soluble-TF were significantly higher at baseline and increased to a significantly greater extent in patients experiencing a thromboembolic event compared with control patients, suggesting a subpopulation of patients have a predisposition to cancer-associated thrombosis.

5.9 Conclusions

Since the insightful observations of Trousseau almost 150 years ago, our understanding of cancer biology has made great strides. Still, a number of key aspects of Trousseau's understanding remain current to this day. Cancer activates coagulation, and this activation of the coagulation system contributes to the cancer growth, at least in large part by promoting angiogenesis. This observation has led to an association of the cancer-promoted thrombotic risk with the new and promising field of angiogenesis. Safer and more effective treatment modalities may result.

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Part II
Thrombotic Disorders Associated with Cancer

Chapter 6
Overview of Cancer and Thrombosis

Axel C. Matzdorff and David Green

6.1 Introduction

Historically, the French physician and scientist Armand Trousseau (1801–1867) has been honored as the first scientist to report the association between malignant disorders and thromboembolism [1]. In 1861, in his book *Clinique medicale de l'Hôtel-Dieu de Paris,* he postulated that most patients with cachexia and thrombosis have undiagnosed cancer. A few years after his observation Trousseau noted thrombophlebitis of his left upper arm and a few months later he succumbed to gastric cancer.

While Trousseau's original description was about venous thrombosis in patients with visceral cancer, today we have broadened the term "Trousseau's syndrome" to apply to any type of venous thromboembolism occurring in patients with solid tumors or hematologic malignancies. The eponym has also been ascribed to chronic disseminated intravascular coagulopathy, microangiopathy, verrucous endocarditis, and arterial emboli in cancer patients. It might be of interest that Trousseau concedes in his original treatise that it was actually his student J. Werner who first noticed this clinical association; in fact, 20 years earlier Rudolf Virchow in his seminal work on thrombosis and emboli had described several patients with cancer and thromboembolism [2]. This historical perspective highlights our daily clinical experience that cancer patients have an increased risk for thromboembolism. As will be discussed below and in subsequent chapters, tumors are thrombogenic and cancer patients have more time to develop thromboses because modern therapy has prolonged the life of the cancer patient. However, many of these treatments are themselves thrombogenic; for example, post-operative thromboembolism is a major risk after tumor excision or debulking. Also, some of the chemotherapeutic agents such as thalidomide/dexamethasone, tamoxifen, and inhibitors of vascular

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endothelial growth factor (VEGF) are thrombogenic. Preventing and treating coagulation abnormalities in cancer patients has become a major health issue for oncologists.

6.2 Epidemiology

Venous thromboembolism is common in patients with malignant disorders [3]. Of all cancer patients, 10–15% develop symptomatic thromboembolism during their disease, but this is only the tip of the iceberg. The incidence at autopsy approaches 50%. This means that many thromboses have been asymptomatic or their clinical signs were obscured by symptoms from the underlying cancer. The highest incidence of thromboembolism has traditionally been attributed to cancer of the lungs, pancreas, stomach, colon and ovaries [4, 5]. Recently CNS tumors were added to this list [6].

While we have learned that the presence of cancer should prompt greater alertness for signs of thrombosis, it is less clear whether the occurrence of spontaneous and idiopathic thrombosis justifies an extensive search for underlying yet undiagnosed cancer. Studies show that 10–15% of patients with idiopathic thromboembolism will develop cancer within the subsequent 2 years, particularly patients over the age of 40 [5, 7]. Some clinicians therefore advocate extensive tumor screening including computerized tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning [8, 9]. Whether this will eventually translate into improved survival or be cost-effective is less clear.

6.3 Pathophysiology

Many years ago, A. T. Billroth noted that cancers are often covered with fibrous material [10], suggesting that they release procoagulants. Furthermore, coagulation activation and tumor-progression are closely linked. Multiple and interacting pathophysiologic mechanisms are responsible for this association.

Colon, breast, lung and renal cancers secrete Cancer Procoagulant A (CPA) [11], a serine protease that activates factor X. Blast cells from acute promyelocytic leukemia (AM_3L) also express CPA on their surface as well as annexin 2 that enhances fibrinolysis [12].

Tissue factor (TF) is another factor secreted by tumor cells. TF activates factors VII and X which eventually leads to thrombin generation and the formation of fibrin [13]. Thrombin acts as a growth factor for cancer cells and is a survival factor as well, increasing the resistance of tumors to chemotherapy agents. Fibrin enhances the adhesion of circulating tumor cells to the vessel wall where they are joined by endothelial progenitor cells, proliferate, and form

macrometastasis [14]. In addition to its effects on coagulation, TF triggers release of VEGF, which is another important growth factor for tumors.

Carcinoma mucins are glycosylated molecules that bind to selectins. When secreted in large quantities they enter the blood stream and bind to L-selectin on leucocytes and P-selectin on platelets and endothelial cells leading to the formation of platelet-rich thrombi [15]. These interactions can be prevented by heparin derivatives, which might account for some of their anti-neoplastic activity.

Both the coagulation and the fibrinolytic system may be activated by tumors. Some secrete pro- and antifibrinolytic factors [urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA), plasminogen activator inhibitor-1,2 (PAI-1, 2] to support local tumor invasion and proliferation [16]. It is an established phenomenon that breast and prostate cancers release plasminogen activator, sometimes so much that systemic hyperfibrinolysis develops [17–19].

In addition to direct coagulation activation, there are indirect, immune-mediated pathways. Cancer cells interact with monocytes and lymphocytes leading to the release of cytokines (TNFα, IL-1). Cytokines induce TF expression on monocytes and endothelial cells and the release of PAI-1. They also down regulate thrombomodulin, a potent inhibitor of thrombogenesis. The net effect is that the vessel wall becomes more "sticky". Cytokines increase plasma C4-binding protein which binds free protein S and reduces protein S activity. Without its cofactor, free protein S, the protein C anticoagulant pathway is impaired (Fig. 6.1).

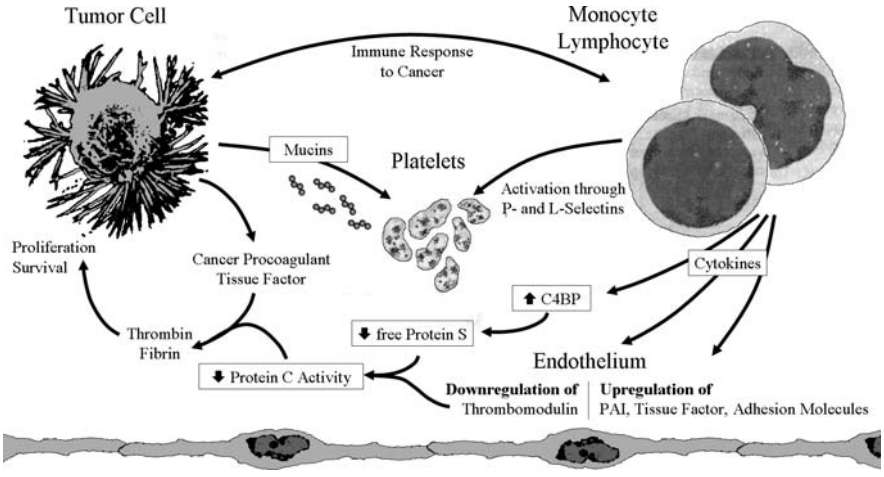

Fig. 6.1 Cancer cells secrete mucins, cancer procoagulant A and tissue factor. This leads to the activation of platelets and coagulation factors. Thrombin and fibrin act as survival and growth factors for cancer cells. Inflammatory cytokines released from immune cells render the endothelium more thrombophilic. Downregulation of free protein S and thrombomodulin reduces protein C activity. All these mechanisms can occur at the same time and trigger venous thromboembolism

Cancer chemotherapy and several of the newer anti-tumor agents carry a substantial thrombotic risk themselves. It has long been known that asparaginase decreases coagulation factors (AT III, protein C, fibrinogen) and disturbs the balance between pro- and anticoagulant factors. Tamoxifen increases the thrombotic risk 1.5- to 2-fold; considering the large number of women taking tamoxifen, this small increase has considerable relevance for clinical practice. The newer aromatase inhibitors have a much smaller risk which is an important argument in favor of the use of these agents [20].

Several of the older chemotherapy drugs and many of the new anti-tumor agents increase the risk of thrombosis. Thromboembolism may develop in 5–25% of myeloma-patients receiving thalidomide in the absence of effective thromboprophylaxis [21, 22]. To prevent this complication, low-molecular-weight heparin (LMWH) may be more efficacious than aspirin [23]. The new anti-VEGF-antibody bevacizumab (Avastin^{TM}), which is currently used for the treatment of some patients with colon, lung, breast and renal cancer, may increase thrombotic risk. The reason for this is not clear but alterations in the endothelium have been suspected.

6.4 Symptoms and Diagnosis

Thromboembolism should be suspected in cancer patients with even minor or only a single symptom. Not only are the cancer cells thrombogenic but the dehydration and reduced mobility associated with the disease enhance the risk. Wells et al. [24] have developed a widely used scoring system to assist in the diagnosis of venous thromboembolism; several of the criteria are routinely observed in cancer patients, which places them in the highest risk category. Any additional symptom such as new chest or leg pain, shortness of breath, or unilateral leg edema, justifies a thorough clinical workup that includes imaging.

However, many cancer patients with thromboembolism may be asymptomatic or the symptoms due to the cancer may obscure the signs of thrombosis. In fact, up to 7% have undiagnosed venous thrombosis or pulmonary embolism [25]. Attending physicians need to be acutely aware of the high likelihood of an occult thrombosis in hospitalized cancer patients, and should inquire about symptoms and check for signs of venous thromboembolism on a daily basis.

Combining low clinical probability and negative D-dimer has proven to be safe and reliable in ruling out DVT in the general population. This is also true for cancer patients with a low clinical probability score [26]. As has been outlined above, most cancer patients fall into the intermediate or high probability group. Therefore, ancillary tests such as the D-dimer are less helpful.

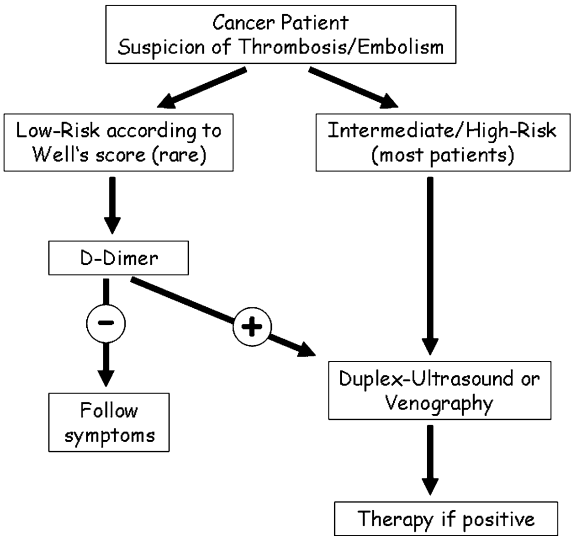

Fig. 6.2 Most cancer patients with symptoms suggestive of thrombosis require duplex-ultrasound or venography. Few patients have a low clinical risk index according to the Well's score [24] and only in these patients it is safe to rule out thromboembolism when D-dimer testing is normal

Symptomatic patients, even those with a normal D-dimer, should have imaging studies to exclude the diagnosis of venous thrombosis (Fig. 6.2).

6.5 Thrombophilia Screening in Cancer

Patients with cancer undergo a variety of procedures that may increase their risk of thrombosis. These include exposure to hormonal and other therapies, extensive surgical procedures, and placement of indwelling catheters. Prior to initiating these interventions, one may consider testing the patient for the presence of thrombophilic mutations, such as factor V Leiden and prothrombin gene mutation. Thrombophilic mutations increase the risk of thrombosis in tumor patients [27, 28, 29]. However, screening for these mutations in all cancer patients is unlikely to be cost effective and should probably be reserved for those patients who would not otherwise receive thromboprophylaxis. Thus, thrombophilia screening would not be indicated in patients about to receive thalidomide or undergoing major surgery, since thromboprophylaxis is already indicated for such patients. On the other hand, thrombophilia significantly increases the risk of central-line thrombosis [30, 31], and thromboprophylaxis is not routinely indicated in this situation. If thrombophilia is detected in a cancer patient one may consider avoiding central lines and switch to oral therapies or, if this is not an option, discuss low-dose heparin prophylaxis in the absence of clinical data for this approach [32]. Type, duration and intensity of anticoagulant therapy in patients with central lines will be discussed further below.

6.6 Prophylaxis of Venous Thromboembolism in Cancer Patients

Patients with malignancy belong to a high risk group for perioperative venous thromboembolic complications [33]. LMWHs have been shown to be safe and effective for prophylaxis in cancer patients [34]. There are some important differences from non-cancer patients:

- It is not sufficient to give heparin until discharge from hospital or ambulation. In patients with abdominal or pelvic cancer surgery prophylaxis should be extended over 4 weeks [35].
- Cancer patients belong to the highest-risk category for venous thromboembolism. Dosing of the LMWH should be higher than in standard surgery patients (>3,400 U daily), similar to the doses used in high-risk orthopedic surgery [36].

As noted previously, cancer inpatients are at a heightened risk for venous thromboembolism. They are usually immobile – otherwise they would be treated as outpatients – or they may be receiving hormonal and chemotherapies which increase the risk of thromboembolism even further [37]. Therefore thromboprophylaxis is recommended for all hospitalized cancer patients in the absence of bleeding or other contraindications [22].

6.7 Central Venous Catheters and Thromboembolism

The incidence of catheter related thromboses in cancer patients ranges from 0.3% to 30%. This large variation is due to patient-related factors such as age and venous anatomy, tumor specific features such as histology, size, and location, and catheter characteristics, as well as the stringency of the diagnostic criteria [38]. Some studies required ultrasound or venography while others relied merely on clinical symptoms. Earlier studies observed a significant clinical benefit from low-dose warfarin or LMWH [39, 40] while recent randomized trials report a very low incidence of catheter thrombosis and no significant benefit from anticoagulant prophylaxis. Routine prophylaxis is therefore not currently recommended [36]. Certain patient groups, e.g. those with a personal history of thromboembolism or known hereditary thrombophilia, might be an exception. Risks and benefits of prophylaxis need to be discussed with these patients on an individual basis [41].

There is little information on how to treat central-line thrombosis in cancer patients if it eventually occurs. No randomized studies have validated type, duration and intensity of anticoagulant therapy in this situation. Attempting to maintain the catheter and providing at least 3 months of therapeutic dose LMWH seem to be reasonable [42].

6.8 Therapy

Every cancer patient with venous thromboembolism requires anticoagulation, usually starting with an LMWH. While non-cancer patients are often transitioned to an oral anticoagulant, this approach is associated with a higher risk of re-thrombosis and bleeding in cancer patients [43]. These complications, which are especially frequent during the 1st month of oral anticoagulant administration, cannot be explained by under- or over-anticoagulation but correlate with the activity of the malignancy. Therefore, a different approach is needed in cancer patients. Three large randomized studies have shown that continuous treatment with a LMWH is more effective than oral anticoagulation in reducing the risk of recurrent thromboembolism without increasing the risk of bleeding (Table 6.1) [44–46]. Anticoagulant treatment with a heparin also provides greater flexibility in dosing, and can more easily be adjusted to alternating clinical situations in tumor patients (cytopenias, altered food intake during chemotherapy, bleeding from surgery and metastases). In summary, vitamin K antagonists are only indicated in patients who refuse or are unable to perform s.c. injections or for patients with long-term stable cancer (e.g. breast and prostate cancer).

The duration of anticoagulation depends on the clinical situation. Treatment may be stopped after 3 months in patients whose thrombosis has been provoked by an intervention or therapy which has subsequently been discontinued. Otherwise, treatment is continued for at least 6 months if the tumor is in complete remission. On the other hand, indefinite treatment is selected for patients with persistent disease, particularly in the palliative setting where all the factors which initially led to thrombosis are still operative. However, long-term therapy with a LMWH in cancer patients poses problems, which are usually not encountered in non-cancer patients:

- LMWHs are more expensive than vitamin K antagonists. Many patients cannot afford the additional costs of long-term LMWHs or their health insurance does not cover these agents [47].
- LMWHs accumulate if creatinine clearance deteriorates. Renal function abnormalities are not uncommon in cancer patients on treatment with nephrotoxic agents such as cisplatin. This is especially true in multiple myeloma patients who are receiving LMWHs to counteract the

Table 6.1 Type of low-molecular-weight heparin, dose and duration of treatment for venous thromboembolism in cancer patients

LMWH	Dose and duration	Reference
CLOT Study: Dalteparin	1st month 200 anti-Xa U/kg s.c. qd 2nd month and following: 150 anti-Xa U/kg s.c. qd	[44]
CANTHANOX Study: Enoxaparin	1,5 mg/kg s.c. qd for at least 3 months	[45]
LITE Study: Tinzaparin	175 anti-Xa U/kg s.c. qd for at least 12 weeks	[46]

thrombogenicity of treatment with thalidomide or revlimide. While these new agents are very effective, they are not curative, and the myeloma will eventually progress. Progression is often associated with declining renal function, and if this is not detected early enough and the dose of LMWH decreased, the patient might experience bleeding from LMWH-accumulation.
- Thrombocytopenia from chemotherapy or underlying cancer requires frequent dose adjustments of anticoagulation.
- Brain metastases and primary CNS tumors may be complicated by hemorrhagic transformation, or the tumor may infiltrate the spinal cord with the risk of intraspinal bleeding. However, these patients with CNS tumors have a very high risk of thrombosis and need anticoagulant prophylaxis [48].
- Liver metastases may cause impaired synthesis of coagulation factors.
- Previously compensated bleeding from cancers of gastrointestinal or urogenital origin may be aggravated by anticoagulant therapy.

In these situations, an individual approach should be chosen. For example, in high-risk patients one might start anticoagulant therapy at reduced doses in the hospital and monitor closely for complications. If anticoagulation is not an option, alternative treatments (compression stockings, pneumatic compression, vena-cava filter) may be appropriate.

6.9 Cancer-Specific Thromboembolic Syndromes

6.9.1 Non-Bacterial Thrombotic Endocarditis

This is a rare thromboembolic complication mainly with advanced adenocarcinoma. Patients develop platelet- and fibrin-rich vegetations on mitral and aortic valve leaflets. The underlying vascular tissue seems to be unaltered. In contrast to infectious endocarditis, patients are usually asymptomatic and the vegetations are not detected until systemic embolism occurs. Therapy should be directed to the underlying cancer and systemic anticoagulation [49].

6.9.2 Hepatic Veno-Occlusive Disease (VOD)

VOD is a rare complication of high-dose chemotherapy with stem cell transplantation, also occurring after treatment with the anti-leukemic agent gemtuzumab ozogamicin (Mylotarg^{TM}). The occlusion of small hepatic veins causes painful hepatomegaly, hyperbilirubinemia and ascites. There is no standardized treatment for VOD. tPA, heparin, intrahepatic portosystemic shunting, and liver transplantation have been tried. Recently defibrotide has emerged as an effective and safe therapy [50].

6.9.3 *Thromboembolism with Myeloproliferative Syndromes*

The term myeloproliferative syndrome (MPS) is used for a group of diseases comprising chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF). Patients with MPS have a high risk of both thromboembolic and bleeding events. This Janus-faced behavior cannot solely be attributed to high or low platelet counts. There are alterations of thrombopoiesis, eventually leading to disturbed platelet function with hyper- and hyporeactivity. The presence of leukocytosis and of the JAK2-mutation also predisposes to thromboembolic complications [51, 52]. Besides venous thrombosis, many patients have symptoms of microangiopathy, e.g. headache, vertigo, visual disturbances, livedo, erythromelalgia, and acral gangrene. Treatment should be directed towards the underlying MPS. Prophylaxis with aspirin has been shown to be effective in patients with PV [53].

6.10 Summary

There are multiple and interacting mechanisms that induce coagulation abnormalities in malignant disorders. Cancer patients require special attention to early symptoms of thromboembolism. Prolonged prophylaxis is indicated in the perioperative setting. In addition, most non-surgical cancer patients will need LMWH prophylaxis as long as they are non-ambulatory or inpatients. Central venous lines do not require prophylactic anticoagulation any more except for certain high-risk situations. If thromboembolism has occurred, long-term therapy with a LMWH should be preferred to oral anticoagulants.

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Chapter 7
Risk Factors for Thrombosis in Cancer Patients

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7.1 Introduction

Venous thromboembolism (VTE), comprised of deep vein thrombosis (DVT) and its complication, pulmonary embolism (PE), is a multifactorial disease, involving complex interactions between environmental exposures and patients, including their hemostatic system and genetic predispositions. VTE is relatively common, with an overall average age- and sex-adjusted incidence of about 1.04–1.9 per 1000 person-years that rises dramatically with increasing age [1–4]. Active malignancy accounts for almost 20% of incident VTE events occurring in the community [5], and imparts a 4- to 6.5-fold higher VTE risk compared to non-cancer patients, depending on concurrent use of anti-cancer therapy [6]. The risk of VTE also varies by cancer type and stage [7–10]. The association between VTE and malignancy has been recognized since 1861 when Trousseau, in a lecture, described thrombophlebitis as the presenting sign of visceral malignancy [11]. In fact, idiopathic VTE may be a harbinger for an occult malignancy. Patients who present with an acute episode of idiopathic VTE have an approximately three- to fourfold increased likelihood of being diagnosed with a malignancy within a year of the VTE event [12–16], leading to a debate on whether cancer screening should be conducted in all individuals presenting with idiopathic VTE [17, 18]. While a clinical trial comparing extensive malignancy screening vs usual care for incident idiopathic VTE identified earlier stage malignancies in the extensive screening group and reduced the mean delay to cancer diagnosis from about 1 year to 1 month, 2-year cancer-related mortality did not differ between the two groups [19], thus making it challenging to justify aggressive screening.

VTE in cancer patients is associated with numerous negative implications, including significant morbidity and mortality. These patients may have their

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active anti-neoplastic therapy delayed and often require chronic anticoagulation. Compared to VTE patients without cancer, individuals with cancer and VTE have both a higher risk for hemorrhage with anticoagulant therapy and of recurrent VTE [20–22]. Moreover, patients diagnosed with cancer at the time of VTE diagnosis have worse survival compared to age-, gender-, cancer type-, and year of diagnosis-matched cancer patients without VTE (1-year survival rate 12% vs 36%, respectively; $p<0.001$) [15]. Furthermore, VTE is a leading cause of death in ambulatory cancer patients receiving chemotherapy [23, 24]. Thus, as cancer patients with VTE have a different natural history than such patients without VTE, more research is being conducted to identify VTE risk factors among active cancer patients in order to target high VTE-risk cancer patients for prophylaxis. In this review, the risk factors associated with VTE in cancer patients will be discussed.

7.2 General Risk Factors for Venous Thromboembolism

Symptomatic VTE is due to dysregulation of the normal hemostatic response to vessel wall "injury" that occurs with exposure to a clinical risk factor. However, the vast majority of individuals who are exposed to a clinical risk factor do not develop symptomatic thrombosis. Clinical VTE is now postulated to be a multifactorial disease which becomes manifest when a person with an underlying predisposition to thrombosis (i.e., a thrombotic diathesis) is exposed to additional risk factors. Please refer to Tables 7.1 and 7.2 for the lists of known acquired and hereditary thrombophilias, and to Table 7.3 for some of the independent risk factors associated with VTE.

Compared to individuals residing in the community, hospitalized patients have over a 130-fold increased incidence of acute VTE [25]. Hospitalization and nursing home residence together account for almost 60% of all incident VTE events occurring in the community [5]. Of note, hospitalization for medical illness and for surgery account for almost equal proportions of VTE (22% and 24%, respectively). The risk among surgery patients can be further stratified based on patient age, the type of surgery, and the presence of active cancer [26, 27]. The incidence of postoperative VTE is increased for surgery patients older than 65 years. High-risk surgical procedures include major orthopedic surgery of the leg; neurosurgery; thoracic, abdominal or pelvic surgery for malignancy; renal transplantation; and cardiovascular surgery [26]. After controlling for age, type of surgery and cancer, additional independent risk factors for incident VTE after major surgery include increasing body mass index, intensive care unit confinement for more than 6 days, immobility, infection and varicose veins [28, 29]. The risk from surgery may be less with neuraxial (spinal or epidural) anesthesia compared to general anesthesia [30]. Independent risk factors for incident VTE among patients hospitalized for acute medical illness include increasing age and body mass index, active cancer, neurological disease with

Table 7.1 Acquired or secondary thrombophilia

Strongly supportive data
Active malignant neoplasm
Chemotherapy (l-asparaginase, thalidomide, anti-angiogenesis therapy)
Myeloproliferative disorders
Heparin-induced thrombocytopenia and thrombosis (HITT)
Nephrotic syndrome
Intravascular coagulation and fibrinolysis/disseminated intravascular coagulation (ICF/DIC)
Thrombotic Thrombocytopenic Purpura (TTP)
Sickle cell disease
Oral contraceptives
Estrogen therapy
Pregnancy/post partum state
Tamoxifen and raloxifene therapy (selective estrogen receptor modulator [SERM])
Antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibody, anti-beta-2 glycoprotein-1 antibody)
Paroxysmal nocturnal hemoglobinuria (PNH)
Wegener's granulomatosis
Supportive data
Inflammatory bowel disease
Thromboangiitis obliterans (Buerger's disease)
Behçet's syndrome
Varicose veins
Systemic lupus erythematosus
Venous vascular anomalies (e.g., Klippel Trenaunay Syndrome)
Progesterone therapy
Infertility "therapy"
Hyperhomocysteinemia
HIV infection
Dehydration

extremity paresis, immobility, fracture and prior superficial vein thrombosis [29, 31, 32].

Active cancer accounts for almost 20% of incident VTE events occurring in the community [5]. VTE risk among active cancer patients can be further stratified by tumor site, presence of distant metastases and active chemotherapy, and is discussed in detail later in this chapter.

Central venous catheters or transvenous pacemaker account for about 9% of incident VTE occurring in the community [5]. Prior superficial vein thrombosis is an independent risk factor for subsequent DVT or PE remote from the episode of superficial thrombophlebitis [6]. The risk of DVT imparted by varicose veins is uncertain, but appears to be higher among persons less than age 40 years [6]. Long haul (>6 h) air travel is associated with a slightly increased risk for VTE [33, 34]. Among women, additional risk factors for VTE include oral contraceptive use and hormone replacement therapy [35],

Table 7.2 Hereditary (familial or primary) thrombophilia

Strongly supportive data
Antithrombin deficiency
Protein C deficiency
Protein S deficiency
Activated Protein C (APC) resistance
Factor V Leiden
Prothrombin G20210A
Homocystinuria
Supportive data
Increased plasma factors I (fibrinogen), II (prothrombin), VIII, IX, XI
Hyperhomocysteinemia
Dysfibrinogenemia
Hypoplasminogenemia and dysplasminogenemia
Hypofibrinolysis
Reduced protein Z and Z-dependent protease inhibitor [ZPI]
Reduced tissue factor pathway inhibitor (TFPI)
Weakly supportive data
Tissue plasminogen activator (tPA) deficiency
Increased plasminogen activator inhibitor (PAI-1) levels
Methylene tetrahydrofolate reductase (MTHFR) polymorphisms
Factor XIII polymorphisms
Increased thrombin-activatable fibrinolysis inhibitor (TAFI)

Table 7.3 Independent risk factors for deep vein thrombosis or pulmonary embolism [136]

Baseline Characteristic	Odds Ratio	95% CI	
Hospitalization			
Hospitalization for acute medical illness	7.98	4.49,	14.18
Hospitalization for major surgery	21.72	9.44,	49.93
Trauma	12.69	4.06,	39.66
Malignancy without chemotherapy	4.05	1.93,	8.52
Malignancy with chemotherapy	6.53	2.11,	20.23
Prior central venous catheter or transvenous pacemaker	5.55	1.57,	19.58
Prior superficial vein thrombosis	4.32	1.76,	10.61
Neurologic disease with extremity paresis	3.04	1.25,	7.38
Serious liver disease	0.10	0.01,	0.71

pregnancy and the postpartum period [36, 37]. The greatest risk may occur during early use of oral contraceptives [38] and hormone therapy [39]. This risk may be less for second generation oral contraceptives or progesterone alone compared to first or third generation oral contraceptives [35]. For women with disabling peri-menopausal symptoms that cannot be controlled with non-estrogen therapy, esterified oral estrogen or transdermal estrogen therapy may confer less risk than oral conjugated equine estrogen therapy [40–42]. Women receiving therapy with the selective estrogen receptor modulators, tamoxifen [43, 44] and raloxifene [45, 46] are also at increased risk for VTE.

Other conditions associated with VTE include heparin-induced thrombocytopenia [47], disseminated intravascular coagulation and fibrinolysis (DIC/ICF), nephrotic syndrome [48], paroxysmal nocturnal hemoglobinuria [49, 50], thromboangiitis obliterans (Buerger's disease), thrombotic thrombocytopenic purpura [51], Behçet's syndrome [52], systemic lupus erythematosus [53], Wegener's granulomatosis [54], inflammatory bowel disease [55], and homocystinuria [56–58]. Data on VTE risk with hyperhomocysteinemia [59, 60] are conflicting. Moreover, homocysteine-lowering therapy with vitamins B6, B12 and folic acid is ineffective for primary or secondary prevention of VTE, suggesting that homocysteine may not be playing a direct role in the pathophysiology of VTE [61, 62].

7.3 Genetic Risk Factors for Venous Thromboembolism

Recent family-based studies indicate that VTE is highly heritable and follows a complex mode of inheritance involving environmental interaction [63–65]. Inherited reductions in plasma natural anticoagulants (e.g., antithrombin III, protein C, or protein S) have long been recognized as uncommon but potent risk factors for VTE [66–68]. More recent discoveries of additional reduced natural anticoagulants (e.g., tissue factor pathway inhibitor and protein Z) [69–71] or anticoagulant cofactors (e.g., fibrinogen gamma') [72], impaired downregulation of the procoagulant system (e.g., activated protein C resistance, factor V Leiden) [73–76], increased plasma concentrations of procoagulant factors (e.g., factors I [fibrinogen], II [prothrombin], VIII, IX, and XI) [77–83] and increased basal procoagulant activity [84, 85], impaired fibrinolysis [86], and increased basal innate immunity activity and reactivity [87–91] have added new paradigms to the list of inherited or acquired disorders predisposing to thrombosis (thrombophilia). These plasma hemostasis-related factors or markers of coagulation activation both correlate with increased thrombotic risk and are highly heritable [63, 92–95].

Inherited thrombophilias interact with such clinical VTE risk factors (environmental exposures) as oral contraceptives [38, 96, 97], pregnancy [98], hormone therapy [99–101], surgery [102, 103] and cancer [8] to compound the risk of incident VTE. For example, among women factor V Leiden carriers of perimenopausal age, the relative risk of VTE associated with hormone therapy may be increased 7- to15-fold [99–101, 104]. Women factor V Leiden carriers who use oral contraceptives have a 35-fold increased risk of VTE compared to women who are non-carriers and are not using oral contraceptives [96]. Similarly, genetic interaction increases the risk of incident VTE. For example, factor V Leiden carriers with elevated factor VIII or thrombin activatable fibrinolysis inhibitor (TAFI) have a threefold higher risk for VTE compared to carriers of factor V Leiden mutation with normal factor VIII and TAFI [105]. Compared to individuals who are heterozygous for the factor V Leiden mutation, those with combined heterozygosity for factor V Leiden and prothrombin G20210A

mutations have a small, 1.3-fold increased risk for incident VTE, but those with combined heterozygosity for factor V Leiden and inherited protein C or protein S deficiency have a 17.5-fold increased VTE risk [106].

Data regarding the risk of recurrent VTE among isolated heterozygous carriers for either the factor V Leiden or the Prothrombin G20210A mutation are conflicting but the magnitude of increase in the risk is modest [107, 108]. In the meta-analysis by Ho et al. [107], pooled results from 10 studies involving 3,104 patients with incident VTE revealed that the factor V Leiden mutation was present in 21.4% of patients and associated with a 1.41-fold increased risk for recurrent VTE (95% CI, 1.14–1.75). Similarly, pooled results from 9 studies involving 2,903 patients revealed that the Prothrombin G20210A mutation was present in 9.7% and associated with a 1.72-fold increased risk of recurrence (95% CI, 1.27–2.31). In contrast, combined heterozygosity for factor V Leiden and prothrombin G20210A is associated with a greater risk (2.6- to 4.8-fold) for recurrent VTE [109, 110].

7.4 Cancer-Specific Risk Factors for Venous Thromboembolism

Active cancer accounts for almost 20% of incident VTE events occurring in the community [5]. VTE risk among active cancer patients can be further stratified by tumor site, presence of distant metastases and active chemotherapy. Although all active cancer patients are at risk, the risk appears to be higher for pancreatic cancer, lymphoma, malignant brain tumors, hepatocellular, leukemia, and colorectal and other digestive cancers [7–10, 111], and for patients with distant metastases [8–10].

Attempts to estimate the incidence of cancer-specific VTE have been made by linking data from cancer registries and either an anticoagulation clinic database or patient hospital discharge data [9, 10, 111, 112]. Blom et al. linked the Dutch cancer registry data to an anticoagulation clinic database to identify cancer patients with VTE between 1986 and 2002. The overall cumulative incidence of VTE in the first 6 months of cancer diagnosis was 12.3 per 1000 cancer person-years (95% CI: 11.5–13.0 per 1000). Patients with cancers of the bone, ovary, brain, and pancreas were associated with the greatest incidence of VTE (Table 7.4). The incidence of VTE was twofold higher in cancer patients with distant metastasis than those without, and in those undergoing chemotherapy compared to cancer patients who never received chemotherapy. Hormone therapy for breast cancer was associated with 1.6-fold higher risk for VTE. Surgery or radiation therapy for the management of cancer did not increase the risk of VTE in this study. In another study, data from the California cancer registry were linked to the California Patient Discharge data set to identify incident VTE cases occurring in cancer patients between 1993 and 1995 [10]. The overall 2-year cumulative VTE incidence in the cancer patients was 16.1 per 1000. Pancreatic, stomach, bladder, uterine, renal and lung cancer were

Table 7.4 Incidence of venous thromboembolism by cancer type

	Cumulative incidence of VTE per 1000 patients (95% CI)			
Type of malignancy	Dutch cohort study (0.5-year cumulative incidence) [9]	California Patient Discharge Data study (2-year cumulative incidence) [10, 137, 138]	National Hospital Discharge survey (inpatient rate of VTE) [111]	University Health System Consortium data (inpatient rate of VTE) [112]
Overall	12.3 (11.5–13.0)	16.1	20.3	41
Bone	37.7 (17.1–81.4)	NR	NR	29
Ovary	32.6 (24.5–43.1)	28.2	18.6	56
Brain	31.1 (23.1–44.6)	75.4	35.0	47
Pancreas	22.7 (16.6–31.0)	43.7	43.4	81
Non-Hodgkin's lymphoma	19.8 (15.0–26.2)	NR	24.8	48
CLL	17.2 (9.0–32.7)	NR	28.8[a]	NR
AML	16.9 (8.8–32.2)	36.1	[a]	[b]
Hodgkin's lymphoma	16.8 (8.0–34.8)	21.8	NR	46
Cervix	16.2 (9.6–27.1)	NR	16.0	35
Stomach	15.4 (11.1–21.3)	35.7	27.1	49
Lung	13.8 (11.6–16.4)	21.7	20.9	51
Leukemia	13.8 (9.1–20.9)	NR	16.9	42
Colon	13.4 (10.8–16.6)	20.4	19.1	40
Bladder	12.9 (9.0–18.5)	11.0	10.4	29
Kidney	12.6 (8.1–19.7)	24.9	20.2	56
Esophagus	12.5 (7.3–21.4)	NR	19.9	43
ALL	11.9 (3.0–46.3)	37.1	[a]	[b]
Multiple myeloma	11.1 (6.0–20.5)	NR	[a]	50
Uterus	10.5 (6.3–17.3)	16.2	22.0	35
Testes	10.4 (4.3–24.8)	NR	NR	33
Prostate	9.5 (7.3–12.3)	10.5	20.5	19
Rectum	8.9 (5.6–14.1)	NR	20.6	35
Breast	8.0 (6.4–9.8)	10.6	16.6	23
Liver	7.2 (3.4–15.1)	NR	18.5	NR
Melanoma	2.7 (1.2–6.0)	4.8	NR	NR

NR: Not reported
[a]Reported as myeloproliferative and other hematopoietic malignancies
[b]Reported as leukemia

associated with highest risk (Table 7.4). Metastatic disease was the strongest predictor for VTE. Adjusting for age, race and cancer stage, VTE was associated with decreased survival for all cancer types during the 1st year of diagnosis.

A study utilizing the National Hospital Discharge Survey data for short-stay hospital discharges from non-federal hospitals between 1979 and 1999 identified

incident VTE cases in hospitalized cancer patients [111]. The overall VTE rate in cancer patients was 20 per 1000 cancer person-years, which was twice the risk for patients without cancer. The highest rate of VTE was noted in patients with neoplasm of pancreas, brain, hematopoietic system, stomach and lymphoma (Table 7.4). One other study utilized the discharge database of 133 academic medical centers in the US participating in the University Health Consortium and reviewed discharge summaries of patients with cancer who were hospitalized between 1995 and 2003 to identify in-hospital incident VTE cases [112]. Patients with malignancy and VTE were identified using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). One-third of the patients had multiple hospitalizations in this time frame. VTE was reported in 4.1% of these individuals. Pancreatic, other non-colorectal abdominal, kidney, ovary, lung and stomach neoplasms, along with certain hematologic malignancies (i.e., multiple myeloma, non-Hodgkin and Hodgkin lymphoma) had the highest rates of VTE (Table 7.4). Individuals receiving active anti-cancer therapy had a higher rate of VTE compared to the rest of the study population. On reviewing the findings of these studies that estimated the incidence of thrombosis in cancer patients (Table 7.4), the thrombotic risk appears to be consistently higher for brain, pancreatic, hematologic, ovarian and stomach neoplasms.

All of the above- mentioned studies [9, 10, 111, 112] utilized cancer registries, databases and/or ICD-9-CM codes to identify VTE cases in cancer patients. It is unknown whether only symptomatic VTE cases were included in these databases, or whether it also includes cancer patients with asymptomatic, incidentally diagnosed VTE on cancer staging imaging studies or those who specifically underwent screening for VTE. Inclusion of asymptomatic VTE diagnosed either incidentally or on screening may have a profound effect on VTE frequency. Furthermore, as actual review of medical records were not performed, it is difficult to verify the accuracy of the data. Data evaluating the accuracy of VTE ascertainment via an administrative database from Minnesota Case-Mix-Review-Program (MCMRP) Public Research Files, and comparing it to the Rochester Epidemiology Project /medical record review-identified cases indicated significant discrepancies in identifying inpatient/hospital-acquired VTE [113]. For Olmsted County, MN, residents admitted to a hospital between 1995 and 1998, there were 53 MCMRP-identified VTE cases vs. 161 medical record review-identified cases; the proportion with PE was 21% vs. 62%, and 1-year mortality was 24% vs. 55%, respectively. Ascertainment of VTE from the administrative data was biased toward surviving cases, highlighting concerns regarding use of such data. Moreover, information regarding other known VTE risk factors is often lacking, thus making it difficult to distinguish whether it is the cancer per se, or the other coexisting risk factors that increases the risk of thrombosis.

In a large population-based, case-control study (Multiple Environmental and Genetic Assessment [MEGA] of risk factors for venous thrombosis) that included consecutive patients diagnosed with incident VTE between 1999 and

Table 7.5 Risk for venous thromboembolism by cancer type

Tumor type	MEGA case control study [8]	REP case cohort study [7]
	OR $_{adjusted}$ (95% CI)	SIR (95% CI)
Pancreatic	20.3 (4.9–83.0)[b]	37.3 (19.9–63.8)
Lymphoma	10.2 (1.4–76.9)	31.7 (17.7–52.3)
Brain	6.7 (1.0–45.4)	26.8 (5.5–78.3)
Leukemia	28.0 (4.0–199.7)[a]	21.2 (9.1–41.6)
Other digestive	[b]	17.4 (4.7–44.4)
Other gynecologic	2.9 (0.3–25.3)[c]	14.1 (5.7–29.1)
Multiple myeloma	[a]	12.3 (1.5–44.5)
Bladder	ND	11.9 (4.8–24.4)
Breast	4.9 (2.3–10.5)	11.5 (6.8–18.1)
Colorectal	16.4 (4.2–63.7)[b]	11.0 (6.9–16.7)
Ovary	3.1 (0.6–15.3)	10.6 (1.3–38.1)
Lung	22.2 (3.6–136.1)	10.5 (6.2–16.6)
Prostate	2.2 (0.9–5.4)	9.8 (6.1–15.0)

[a]Includes all hematological malignancies, including non-Hodgkin lymphoma, Hodgkin lymphoma, leukemia and multiple myeloma.
[b]Includes all gastrointestinal cancers, including bowel, pancreas, stomach and esophagus.
[c]Includes cervical cancer.
ND: Not determined.

2002, with their partners who acted as controls, the overall risk of VTE was sevenfold higher in patients with a malignancy (95% CI: 5.2–8.6) compared to individuals without malignancy [8]. Adjusted for age and gender, patients with hematological malignancies had the highest VTE risk (OR 28.0; 95% CI: 4.0–199.7) (Table 7.5), followed by lung cancer (OR 22.2; 95% CI 3.6–136.1) and gastrointestinal cancer (OR 20.3; 95% CI 4.9–83.0). Neoplasms of the brain and breast were associated with seven- and fivefold increased risk, respectively. The risk of VTE was highest in the first 3 months after the diagnosis of malignancy (OR 53.5; 95% CI: 8.6–334.3). Cancer patients with distant metastases had a higher VTE risk compared to patients without distant metastases (OR 19.8; 95% CI: 2.6–149.1).

In another large population based case-cohort study [7], incident VTE cases with an active cancer at the time of VTE diagnosis that occurred between 1991 and 1997 in Olmsted County, MN, were recorded. The Iowa State Surveillance, Epidemiology, and End Results (SEER) data was used to estimate the expected age- and sex-specific prevalence of cancer by tumor site in Olmsted County and calculate the expected age- and gender-group specific expected VTE incidence cases. All cancer sites were associated with a greater than fivefold increased risk for VTE (Table 7.5). Pancreatic cancer was associated with a 37-fold increased risk for thrombosis, followed by brain cancer (RR = 27). Surprisingly, lymphoma and leukemia had unusually high relative risks (RR = 31.7 and 21.2, respectively). Liver, other gastrointestinal (esophagus, small intestine, gallbladder, other biliary) and other gynecologic (primarily cervical) cancers were also associated with higher VTE risk ($17.0 < RR < 25.0$). On the other hand, the RR

for many common cancers (breast, colorectal, ovary, lung, prostate) were essentially the same as the overall baseline cancer associated VTE risk (all had 9.5<RR<12.0). However, the independent risk of VTE by tumor site after controlling for surgery, hospitalization and chemotherapy was not estimated, nor was the effect of tumor stage, tumor progression, and metastases.

Myeloproliferative disorders (MPD), especially polycythemia vera (PV) and essential thrombocythemia (ET), are risk factors for thrombosis [114–116]. In MPDs, arterial events are more common than venous thrombosis. At the time of diagnosis, the prevalence of thrombosis is ~34–39% in PV and 10–29% in ET. During the follow-up phase of MPD, the prevalence of thrombosis in PV is 8–19% and 8–31% for ET. MPD patients older than 60 years and those with prior history of thrombosis are at increased risk for recurrent thrombosis. For example, in the European Collaboration on Low-dose Aspirin in Polycythaemia Vera (ECLAP) epidemiology study of PV patients, the hazard ratio for vascular complications for patients older than 60 years was 8.6 (95% CI: 3.0–22.7) [117]. A previous thrombotic event in a patient with MPD increased the hazard for VTE recurrence by 4.85 (95% CI: 1.46–16.1). The interaction of age >60 years and a prior history of thrombosis increased the hazard for thrombosis 17.3-fold (95% CI 6.4–47). In addition, leukocytosis with WBC>15,000/mm^3 (but not thrombocytosis) has been identified as risk factor for thrombosis in both PV and ET, and may involve cross-talk between leukocytes and platelets and/or the endothelium. Other significant predictors of survival and cardiovascular morbidity in patients with MPD include smoking, diabetes, and congestive heart failure. More recently, a gain of function mutation has been identified in the Janus activating kinase-2 gene (JAK2 V617F) in >95% patients with PV and ~50% patients with ET [118]. However its association with thrombosis is controversial, with some studies reporting a positive association, whereas others do not [116, 118]. Data from studies evaluating the effect of JAK2 V617F allele burden (measured either by allele specific or quantitative PCR) on thrombosis are also conflicting. JAK2 has been associated with hepatic, portal and mesenteric vein thrombosis in patients with no overt clinical features of MPD, but with bone marrow histological features consistent with MPD [119–121]. However, for non-splanchnic venous thrombosis in the absence of clinically overt MPD, the JAK2V617F mutation was detected in less than 1% of patients and the mutant allele burden was correspondingly low (range: 2.2–7.5%) [122].

Cancer patients receiving cytotoxic therapy are at higher risk for VTE [6, 9]. Chemotherapeutic agents like L-asparaginase, cisplatinum, 5-fluorouracil, bleomycin, mytomycin, and anthracyclines (e.g., doxorubicin) are associated with an increased risk for VTE [123]. High rates of VTE have been reported in patients treated with immunomodulating drugs like thalidomide and lenalidomide, especially when used in combination with high-dose dexamethasone with or without chemotherapy [124]. A similar association with thrombosis has been reported for angiogenesis inhibitors like bevacizumab and sunitinib when used in combination with chemotherapy [125]. In addition, supportive therapy with

hematopoetic growth factors like recombinant human erythropoietins [126] and granulocyte-colony stimulating factor (G-CSF) [127] are associated with increased VTE risk. Hormonal manipulators like tamoxifen and aromatase inhibitors like anastrozole also increase risk for VTE, and the risk is enhanced when used in combination with chemotherapy. Data from randomized clinical trials in women with node negative, estrogen receptor positive breast cancer, indicate that the 5-year incidence of VTE in these women with early stage breast cancer treated with either placebo, tamoxifen or tamoxifen plus chemotherapy is 0.2%, 0.9% and 4.2% respectively [128, 129]. In women with node positive breast cancer treated with chemotherapy, the reported rates of thrombosis range from 2% to 9%, and up to 17.6% in women with metastatic stage IV disease [130]. Moreover, the risk of chemotherapy-associated VTE differs by cancer type, cancer patient baseline characteristics, and with supportive therapy with hematopoietic growth factors [127, 131]. Data from a prospective observational study of 3003 cancer patients who were treated with at least one cycle of chemotherapy and with a median follow-up of 2.4 months noted a 1.93% cumulative VTE incidence (0.8% per month) [127]. The highest incidence of VTE was noted in patients with upper gastrointestinal malignancies (2.3% per month), lung cancer (1.2% per month) and lymphoma (1.1%per month) ($p = 0.001$). A higher pre-chemotherapy platelet count was associated with increased VTE risk (1.66%per month for platelets $\geq 350{,}000/mm^3$ vs 0.52% per month for platelets $\leq 200{,}000/mm^3$; $p < 0.0001$). Pre-chemotherapy hemoglobin $< 10\,g/dL$ was associated with higher VTE risk (2.3% per month vs 0.71% per month; $p = 0.0003$). In addition, the use of white and red blood cell growth factors during cycle 1 were associated with increased VTE risk. Based on these findings and the results of multivariate logistic regression analysis, a predictive model for chemotherapy associated VTE was developed and validated [131]. Cancer sites associated with very high risk for VTE (i.e., stomach and pancreas) were assigned a risk score of two. A score of one was assigned to each of the following: cancer sites associated with high VTE risk (i.e., lung, lymphoma, gynecologic, bladder, and testicular); pre-chemotherapy platelet count $\geq 350{,}000/mm^3$; hemoglobin $< 10\,g/dL$ or use of red cell growth factors; pre-chemotherapy leukocyte count $> 11{,}000/mm^3$; and body mass index (BMI) $\geq 35\,kg/m^2$. The low-risk group (score = 0) was associated with 0.8% and 0.3% 2.5-month VTE rate in the derivation and validation cohorts, respectively, compared to 7.1% and 6.7% VTE rate the high-risk group (score ≥ 3).

Cancer patients who were carriers of the factor V Leiden and prothrombin G20210A mutations have a higher risk for VTE. In the MEGA study described above [8], carriers of the factor V Leiden mutation with no evidence of malignancy had a 3.3-fold increased risk for VTE compared to individuals with wild type factor V. Cancer patients with wild type factor V had a 5.1-fold higher risk for VTE compared to individuals with no evidence for malignancy. In contrast, cancer patients with factor V Leiden mutation had a 12.1-fold increased VTE risk compared to individuals without cancer and with wild type factor V.

Similar results were indirectly calculated for the prothrombin G20210A mutation in patients with malignancy.

Cancer patients undergoing surgery have a 1.7-fold increased risk of post-operative VTE compared to non-cancer patients who are undergoing similar procedures (95% CI: 1.6–1.8) [26]. The 30±5-day incidence of clinically overt VTE in cancer patients undergoing general, gynecologic or urologic surgery in a prospective observational study (@RISTOS registry) were 2.83%, 2.0% and 0.87%, respectively, despite 81.6% in-hospital and 30.7% post-discharge VTE prophylaxis rates [132]. Of VTE events, 40% occurred more than 3 weeks after the day of surgery. In a multivariable logistic regression analysis, age above 60 years (OR 2.6; 95% CI: 1.2–5.7), history of prior VTE (OR 6.0; 95%CI: 2.1–16.8), advanced cancer (OR 2.7; 95% CI: 1.4–5.2), anesthesia lasting for more than 2 h (OR 4.5; 95% CI: 1.1–19.0), and bed rest longer than 3 days (OR 4.4; 95% CI: 2.5–7.8) were identified as independent risk factors. Similarly, cancer patients with acute medical illness are at an increased risk for VTE. A post-hoc analysis of the MEDENOX study, a double blind randomized controlled trial that evaluated two different doses of low molecular weight heparin and compared it with placebo to reduce the risk of VTE in patients hospitalized for an acute medical illness [133], revealed that cancer was associated with a 1.6-fold increased risk for VTE (95% CI: 0.93–2.75) [31].

In the general population, the presence of a central venous catheter (CVC) is associated with a 5.5-fold increased risk for VTE [6]. In cancer patients, the incidence of clinically overt upper extremity DVT related to CVCs has been reported to vary between 0.3% and 28.3%, but the venographically diagnosed incidence is higher, and ranges between 27% and 66% [134]. About 15–25% patients with CVC-related upper extremity DVT have clinically overt PE, with an autopsy-proven PE rate up to 50% [134]. The Registro Informatizado de la Enfermedad TromboEmbólica (RIETE), a Spanish registry of patients with objectively confirmed, symptomatic acute DVT or PE that included 512 patients with upper extremity DVT, noted that cancer patients had a higher odds of CVC related thrombosis (OR 1.7; 95% CI: 1.2–2.5), bilateral upper extremity DVT (OR 5.1; 95% CI: 1.9–16.0), 3-month rate of major hemorrhage (OR 4.4; 95% CI: 1.2–21) and recurrent VTE (OR 2.2; 95% CI: 0.91–5.6) [135].

7.5 Summary

Active malignancy accounts for almost 20% of incident VTE events. The VTE risk varies by cancer type and stage, and anti-neoplastic therapy increases the risk for VTE. The thrombotic risk is higher for brain, pancreatic, hematologic, ovarian and stomach neoplasms. There is significant interaction between malignancy and traditional risk factors of VTE, including surgery, hospitalization, central venous catheter, and thrombotic diathesis (e.g., factor V Leiden and prothrombin G20210A mutations). Patients with VTE and cancer have a

different natural history than VTE patients without cancer, with increased risk for major hemorrhage, recurrent VTE, and mortality. Patients who present with an acute episode of idiopathic VTE have an approximately three- to fourfold increased likelihood of being diagnosed with a malignancy within a year of the VTE event.

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Chapter 8
Thrombotic Microangiopathy Syndromes

Anaadriana Zakarija

8.1 Introduction

Thrombotic microangiopathy (TMA) describes a spectrum of clinical syndromes all characterized by microvascular platelet thrombi with resultant thrombocytopenia and microangiopathic hemolytic anemia (MAHA). Organ dysfunction can commonly include renal failure or neurologic abnormalities such as mental status changes, confusion, or seizures. The most common TMA is idiopathic thrombotic thrombocytopenic purpura (TTP). Hemolytic-uremic syndrome (HUS) is commonly used to describe a TMA with associated renal insufficiency, and typically without neurologic sequelae. The distinction between TTP and HUS is not always clear, and therefore HUS can be reserved for the distinct TMA syndrome associated with bloody diarrhea due to shiga-toxin producing *Eschericia coli* 0157.H7. There are a variety of other conditions which can also be associated with a TMA including pregnancy, autoimmune diseases, cancers, drug-associated TMA and TMA associated with hematopoietic stem cell or solid organ transplantation. The diagnosis of TTP should be reserved for idiopathic cases, while the term TMA should be utilized for the many secondary causes of the syndrome.

Significant progress has been made in the last 20 years in understanding the pathophysiology of idiopathic TTP. Ultra-large von Willebrand (uLVWF) multimers were found to be present in patients with relapsing TTP [1]. In 1996, two groups described a vWF cleaving protease, which was responsible for cleavage of vWF under shear stress [2, 3]. This metalloprotease was subsequently named ADAMTS13 (*a d*isintegrin *a*nd *m*etalloprotease, with *t*hrombo*s*pondin-1 like domains). The protease is found to be deficient in patients with familial and acquired TTP [4, 5], and some patients with acquired TTP have autoantibodies to the protease [5, 6]. Therefore an autoimmune etiology

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appears to be important in a subset of patients with TTP. Despite this finding, deficient activity of ADAMTS13 or the presence of an inhibitor does not define all cases of TTP, and a significant proportion of patients with the disease and with a response to therapeutic plasma exchange have normal levels of ADAMTS13 [7, 8]. In addition, most cases of secondary TMA do not have severely deficient ADAMTS13 activity [7–9]. Therefore, despite these important advances, the pathophysiology of many TTP and TMA syndromes is still not well understood. This chapter will review the pathophysiology, clinical presentation and treatment of thrombotic microangiopathy syndromes seen in patients with cancer.

8.2 Cancer-Associated Thrombotic Microangiopathy

Thrombotic microangiopathy has been a well-recognized phenomenon in cancer patients for many years. Both the underlying malignancy and the chemotherapeutic agents used in disease treatment have been contributing factors. Another variant of TMA in these patients is disseminated intravascular coagulopathy (DIC), which is discussed in more detail in Chap. 9. Both syndromes present with microangiopathic hemolytic anemia and thrombocytopenia. Classically, the distinguishing feature is the coagulopathy that is present in DIC. Yet, in some cases both cancer-associated TMA and DIC can be present, which confounds the diagnosis. The prevalence of cancer-associated TMA is unknown, as no large prospective series have been conducted. The most frequent malignancies are adenocarcinomas, most commonly gastric cancer [10, 11]. Other cancers that have been described include breast cancer, colorectal cancer, small cell lung cancer, prostate cancer, squamous cell cancers, carcinoma of unknown primary, non-Hodgkin's lymphoma, and Kaposi's sarcoma [10, 12].

8.2.1 Epidemiology

A cancer-associated TMA syndrome can occur as the presenting symptom of malignancy, during treatment or at the terminal stage of disease. In some cases there is no evidence of active malignancy at the time of diagnosis [13]. Finally, cases have been described of an acute post-operative TMA syndrome that occurs shortly after resection of a tumor [14–16]. The Oklahoma TTP-HUS Registry, which has collected data on 351 consecutive patients diagnosed with TTP or hemolytic-uremic syndrome (HUS) since 1989, found that 3% of patients thought to have TTP actually had a disseminated malignancy [12]. The diagnosis of malignancy was made after further evaluation was prompted by a poor response to therapeutic plasma exchange (TPE). The clinical presentation of patients with malignancy was indistinguishable from those with idiopathic TTP, but treatment outcome was much worse [12]. Median survival

of patients with malignancy that presented as a TMA was 12 days, 30-day mortality was 90%, and only 10% responded to TPE, which is in stark contrast to idiopathic TTP cases that have a 20% early mortality and 82% response rate to TPE [12]. Therefore an underlying malignancy should be considered in patients who present with TTP, particularly if there is a poor response to treatment with plasma exchange. In a review of the Oklahoma Registry data and the literature, Francis et al. found that the diagnosis of disseminated malignancy was made by bone marrow biopsy in 13/29 patients and on autopsy in 8/29 patients whose initial presentation was with a TMA syndrome [12]. A bone marrow biopsy can be a valuable diagnostic tool in patients with TTP who do not respond to standard therapy.

8.2.2 Clinical Presentation

Features of cancer-associated TMA typically include thrombocytopenia, microangiopathic hemolytic anemia and normal coagulation studies (prothrombin time, partial thromboplastin time and fibrinogen). A reticulocytosis indicates adequate marrow production, while elevated lactate dehydrogenase (LDH) and indirect hyperbilirubinemia are consistent with intravascular hemolysis. A direct Coomb's test is negative. Organ dysfunction is due to microvascular thrombi, and can include the renal, cardiac and neurologic systems. Unlike idiopathic TTP, cancer-associated TMA is frequently associated with pulmonary symptoms such as dyspnea [10]. Finally, distinct syndromes have been described which involve thrombotic microangiopathy of either the renal or pulmonary systems without impressive hematologic abnormalities [17–19]. Pulmonary tumor thrombotic microangiopathy (PTTM) is most often associated with adenocarcinoma and is characterized by severe pulmonary hypertension, right-sided heart failure, and is frequently fatal [19]. Tumor cells metastasize to the pulmonary vasculature, which results in localized activation of coagulation and development of platelet and fibrin microthrombi, as well as fibrocellular subintimal proliferation [18, 19].

8.2.3 Pathophysiology

The pathologic cause of cancer-associated TMA is not well understood. Severe ADAMTS13 deficiency ($\leq$10%) is a factor in many cases of idiopathic TTP, but is not present in most cases of cancer-associated TMA [8, 9, 12, 14, 15, 20–27] (See Table 8.1). ADAMTS13 levels have been found to be decreased in a number of situations where TMA is not present including sepsis, liver disease, and DIC, although severe deficiency is reportedly found only in TTP [28, 29]. In patients with malignancy but no evidence of TMA, ADAMTS13 measurements have been performed by a few groups with varying results. Oleksowicz et al.

Table 8.1 ADAMTS13 activity in cancer and cancer-associated thrombotic microangiopathy

	No. of patients	TMA present	ADAMTS13 activity (%)	ADAMTS13 inhibitor	Clinical course
Oleksowicz et al. (1999) [30]	20	No	Localized tumor: 95±4.6% Disseminated malignancy: 4±4.9%	Negative	NA
Koo et al. (2002) [32]	28	No	Limited stage disease (n = 13): >50% Advanced stage (n = 15): 6–40% (mean 22.5%)	Negative	NA
Mannucci et al. (2003) [31]	49	No	Localized tumor(n = 29): 64±28% Disseminated malignancy (n = 20): 50±23%	NA	NA
Bohm et al. (2003) [33]	50	No	Benign brain tumor: 40–54% – (2/30); >60% (28/30) Malignant brain tumors: 42–59% (7/30); >60% (23/30) Local prostate cancer: 13–56% (4/10), >60% (6/10) Metastatic prostate cancer: 45–58% (4/10), >60% (6/10)	NA	NA
Veyradier et al. (2001) [8]	18	Yes	<5%, (7/18)	NA	Three patients with followup – ADAMTS13 activity improved with TPE from 0% to 50, 75 and 100%
Fontana et al. (2001) [21]	4	Yes	35%, 25%, 57%, 84%	NA	All four patients had metastatic malignancy
Blot et al. (2002) [22]	1	Yes	0%	Negative	Treated with chemotherapy only, resulted in improvement of ADAMTS13 to 75%

Table 8.1 (continued)

	No. of patients	TMA present	ADAMTS13 activity (%)	ADAMTS13 inhibitor	Clinical course
Studt et al. (2003) [23]	14	Yes	<5%, (2/14) 5–9%, (1/14), >25%, (11/14)	Positive in 1/2 cases with ADAMTS13 <5%	NA
Zheng et al (2004) [9]	2	Yes	48%, 66%	Negative	Both treated with TPE – 1 response, 1 no response. Died at 2 and 5 months after diagnosis of TMA
Rieger et al. (2005) [24]	1	Yes	8%	Negative	NA
Sugimoto et al. (2005) [25]	1	Yes	15–46%	NA	TPE and corticosteroids were ineffective
Cataland et al. (2006) [15]	1	Yes	2.3%	Positive	TPE resulted in remission, died of recurrent malignancy 3 months after diagnosis of TMA
Hamilton et al. (2007) [14]	1	Yes	26%	Negative	TPE resulted in thrombocytopenia improvement. Treatment with chemotherapy was initiated
Zupancic et al. (2007) [42]	1	Yes	Normal	Negative	No response to TPE, vincristine or IVIG. Response to splenectomy
Werner et al. (2007) [26]	1	Yes	Normal	NA	No response to TPE or corticosteroids. Died 2 weeks after TMA diagnosis
Morishita et al. (2007) [27]	1	Yes	48%	NA	Treatment with TPE resulted in partial response, complete response after chemotherapy initiated
Francis et al. (2007) [12]	8	Yes	Range:13–100% Median: 50%	NA	90% 30-day mortality despite TPE

Abbreviations: TMA (Thrombotic microangiopathy), TPE (therapeutic plasma exchange), NA (not available)

studied 20 patients with solid tumors and hematogenous spread of metastasis, but without TMA, and found that ADAMTS13 activity was $\leq$15% in all cases [30]. In 10 control patients with only localized tumors, ADAMTS13 activity was $\geq$88% in all cases. Other series have found that patients with both localized and advanced malignancy can have moderately decreased ADAMTS13 activity but none had severe ADAMTS13 deficiency [31–33]. When patients with cancer-associated TMA are studied, some have been found to have severe ADAMTS13 deficiency, although most have either mildly decreased or normal levels. ADAMTS13 antigen has not been measured in these patients, and the mechanism of decreased activity is not clear but may be due to decreased synthesis or an abnormal protein, but does not appear to be due to an autoantibody. Therefore although ADAMTS13 autoantibodies are not present, a modest decrease in ADAMTS13 activity may play a role in the thrombotic lesions present in cancer-associated TMA.

Other proposed mechanisms for cancer-associated TMA include endothelial cell injury, increased adhesion of tumor cells to the endothelium, circulating immune complexes, increased platelet aggregation, and impaired fibrinolysis [30, 34–37]. Elevated levels of von Willebrand factor (vWF) have been reported in some patients with malignancy [30]. Endothelial cells are one important source of vWF, and endothelial cell injury can result in release of stored uLVWF which may contribute to the hypercoagulability leading to TMA. Endothelial microparticles are found in plasma of patients with TTP, and are likely a manifestation of endothelial cell injury and activation [38]. In addition, tumor cells have been found to synthesize a protein which is similar to platelet glycoprotein Ibα, and has the ability to bind to von Willebrand factor and therefore play a role in platelet aggregation [37]. Further study into the mechanisms leading to cancer-associated TMA is warranted, and may provide insight into other treatment strategies.

8.2.4 Therapy

Treatment of idiopathic TTP with therapeutic plasma exchange (TPE) was demonstrated to be more effective than plasma infusion in 1991, and since then has become the treatment of choice [39]. Historically, mortality rates for TTP were greater than 80%, but recent series report mortality from idiopathic TTP to be 15–30% [7, 9]. Plasma exchange has been largely ineffective for treatment of cancer-associated TMA though controlled studies have not been conducted [9, 12, 25–27]. The only large consecutive series of patients is the Oklahoma TTP-HUS registry, in which all patients received TPE. Outcomes were poor, with a response rate of only 10% [12]. Anecdotal reports claiming benefit of TPE may be subject to publication bias, as those cases not responding to TPE are unlikely to be published. Furthermore, patients who improved with TPE

frequently received other treatments such as chemotherapy [14, 27]. Finally, the beneficial effects of TPE are likely due to removal of autoantibody and replacement of the absent ADAMTS13 protease. Since an autoantibody is not detectable in virtually all cases of cancer-associated TMA, even in those with moderately decreased ADAMTS13 activity (see Table 8.1), there is little rationale for plasma exchange or immunosuppression [12, 26]. Replacement of mildly deficient ADAMTS13 activity with plasma infusion has not been studied but could be a direction of future research in this area. Of course, plasma infusion carries with it risks including infusion of additional von Willebrand factor, allergic reactions and transfusion-related lung injury (TRALI), and therefore further study is necessary before it can be recommended. Finally, empiric use of TPE in this patient population should not be performed since TPE is not a benign procedure. As many as 30–40% of patients suffer serious adverse clinical events during the initial treatment period, including allergic reactions to plasma, citrate-related toxicity, and line-related complications, primarily bacteremia, sepsis or catheter-associated thrombosis [40, 41]. The evidence does not support the routine use of TPE for the treatment of cancer-associated TMA, and further treatment studies in this area are necessary.

Treatment of the underlying malignancy is of paramount importance for achieving hematologic improvement. Chemotherapy alone has resulted in partial correction of thrombocytopenia and anemia, and even a rise in the ADAMTS13 activity [22]. There is a case report describing the efficacy of splenectomy in the treatment of cancer-associated TMA [42]. The patient reportedly developed TMA in the setting of metastatic breast cancer; treatment with chemotherapy including gemcitabine and docetaxel may have contributed to the TMA. ADAMTS13 activity was normal and no inhibitor was detectable. Because treatment with TPE, vincristine and IVIG was unsuccessful, a splenectomy was performed 46 days after presentation with TMA. Post-splenectomy, thrombocytopenia and anemia improved along with a decline in lactic dehydrogenase. Pathology revealed a spleen with adenocarcinoma, suggesting that removal of tumor tissue as well as the spleen may have contributed to the favorable outcome.

A therapy of historical interest is extracorporeal immunoadsorption, using columns contained staphylococcal protein-A absorbent resin (PROSORBA®). Snyder and colleagues described the presence of circulating immune complexes (CIC) in chemotherapy-associated TMA, which they hypothesized triggered platelet aggregation in the renal vasculature [35]. Protein A immunoadsorption columns were utilized with an apheresis machine, and immunoglobulin G (IgG) and IgG-containing CIC were removed from the plasma of patients with TMA. Treatment of 55 patients with immunoadsorption resulted in improvement in TMA in 45%, and better survival rates and outcomes correlated with a decrease in CIC levels [35]. Few further studies with this therapy were performed, and the columns are no longer commercially available in the United States.

8.3 Drug-Associated Thrombotic Microangiopathies

A variety of medications have been associated with the development of TTP or HUS (Table 8.2) [10, 43, 44]. The most commonly reported agents are mitomycin-C, quinine, cyclosporine and ticlopidine [44]. Among the other chemotherapeutic drugs that have been implicated are daunorubicin, cytarabine, bleomycin, cisplatin, deoxycorformycin (pentostatin), arsenic, interferon-α [45, 46], gemcitabine [47], and bevacizumab [48]. In addition, patients undergoing hematopoietic stem cell transplantation (HSCT) can develop a thrombotic microangiopathy, which may be due to exposure to calcineurin inhibitors (cyclosporine or tacrolimus). Drugs may induce TMA by either an immune-mediated effect or a direct toxic effect [44]. Recent TTP case series demonstrate that most drug-associated TTP cases are not associated with severe ADAMTS13 deficiency or with the presence of an ADAMTS13 inhibitor [7–9]. Since TMA has been observed in patients with malignancy, particularly metastatic adenocarcinoma [10], it is difficult to determine whether drugs play an independent role in the development of the syndrome. In addition, although TMA typically develops during drug therapy, the syndrome has been described months after administration of chemotherapy, and frequently in the absence of active malignancy [17].

8.3.1 Mitomycin-C

The first significant association between a drug and thrombotic microangiopathy involved mitomycin-C, and was reported in the 1980s [11, 49]. Mitomycin-C is an alkylating agent which has been commercially available in the United States since 1974. It is used to treat a variety of malignancies, including gastric, pancreatic and anal cancers. The first case series describing an association between mitomycin-C and HUS included 12 patients and was published in 1985 [50]. Therefore a registry of patients with cancer-associated HUS was established [11]. A total of 85 patients were identified who had microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction. Of these

Table 8.2 Drugs associated with TTP/HUS

Chemotherapeutic agents	Others
Mitomycin-C	Quinine/quinidine
Gemcitabine	Ticlopidine
Bevacizumab	
Daunorubicin	Clopidogrel
Cytarabine	Tacrolimus
Bleomycin	Cyclosporine
Cisplatin	α-Interferon
Arsenic	
Deoxycoformycin	

patients, 89% had adenocarcinoma, and 99% had received mitomycin-C [11]. In clinical studies of patients receiving combination chemotherapy with mitomycin-C, the incidence of HUS has been 4–15% [49, 51]. In a study in which patients with metastatic colorectal carcinoma were randomized to receive a regimen containing mitomycin or one without this drug, the incidence of HUS was 15% vs 0%, respectively [51]. In addition, there is evidence of a dose-dependent response, with patients receiving higher doses having a higher incidence of thrombotic microangiopathy [11, 49]. Of cases in the Lesesne series, 89% received a cumulative dose of mitomycin greater than 60 mg [11]. Also suggestive of mitomycin's causative role is that at the time of HUS diagnosis 35% of the 85 patients had no evidence of active malignancy.

The mechanism by which mitomycin-C causes thrombotic microangiopathy is not understood, but is most likely due to a direct toxic effect on endothelium that is dose-dependent. Fibrin deposition and endothelial proliferation are seen in the renal vasculature [52]. When compared to patients with idiopathic TTP, mitomycin-C TMA cases do not demonstrate activation of the immune system or a decrease in von Willebrand factor antigen, but both syndromes show evidence of endothelial injury [53]. Circulating immune complexes have also been isolated from these patients. The antibodies from the complex do not react with mitomycin-C but recognize tumor antigens [11, 50]. One case in the literature provides data on ADAMTS13 activity in a patient with mitocmycin-associated TMA; no inhibitor was detected and ADAMTS13 activity was 29% [9].

The clinical characteristics and outcomes of mitomycin-C associated TMA differ from cases of idiopathic TTP. In the largest case series, evidence of renal dysfunction was required for the diagnosis, and 33% of the 85 cases required hemodialysis [11]. Neurologic symptoms were present in only 16% of cases. The median time from the last dose of mitomycin-C to development of HUS was 75 days. Of note, patients who received transfusions were at risk for adverse events. A total of 44% had a serious post-transfusion event, most commonly pulmonary edema. Despite therapeutic interventions, outcomes were poor: HUS-related mortality was 44%, while all-cause mortality was 74% [11]. Treatment with corticosteroids, TPE or anticoagulation has not been very effective. Of the 44% of patients treated with TPE, only 30% improved. The use of immunoadsorption by staphylococcal protein A column was the only therapy associated with a more positive outcome; 48% of the 21 patients who underwent this treatment improved [11]. This therapy has not been widely used in other series of drug-associated TTP-HUS, and is no longer available in the US.

8.3.2 Gemcitabine

Gemcitabine, approved by the FDA in 1996 for the treatment of metastatic pancreatic cancer, is currently used to treat a wide variety of malignancies. Gemcitabine-associated TMA was first reported in 1999 [54, 55]. A case series

by Fung et al. described 12 cases of HUS which occurred a median of 5.8 months after gemcitabine therapy was initiated [54]. HUS-related mortality was 17% in this series. The estimated incidence of gemcitabine-associated HUS is 0.015–1.4% [56, 57]. Humphreys et al. described nine cases of gemcitabine-associated TMA [47]. The majority of cases were patients with pancreatic cancer or sarcomas, although one patient was a young woman with Hodgkin lymphoma. An important clinical feature was the development of worsening hypertension, which preceded the diagnosis of TMA by 0.5–10 weeks in 78% of the cases. Gemcitabine was discontinued in all the cases, and five of nine patients also underwent TPE. The efficacy for TPE in patients with drug-induced TMA is not clear. In this case series, of the four patients who did not receive plasma exchange, only one died of progressive malignancy. There were no deaths attributable to TMA.

A more recent review of gemcitabine-associated TMA collected data on 56 patients with the syndrome [57]. The most frequent diagnosis was pancreatic cancer (43%), followed by small cell lung cancer (14%) and ovarian cancer (11%). The mean duration of gemcitabine therapy prior to diagnosis of the TMA was 7.6 months, and the median cumulative dose was 22 g, and the lowest dose was 2 g. Antecedent hypertension was noted in this large study, with 75% of patients developing new-onset hypertension or worsening of pre-existing hypertension prior to diagnosis [57]. In this series, treatment regimens varied and included discontinuation of gemcitabine, plasmapheresis (36%), corticosteroids (14%), and fresh frozen plasma (7%). Interestingly, three patients either continued or were reintroduced to gemcitabine. Treatment-specific outcomes were not reported, median survival was 16.5 months and 70% of patients had persistent chronic renal failure [57]. TTP-associated mortality is difficult to ascertain but was reported as 15% in another series [56].

Gemcitabine-associated TMA is a rare complication, but when it occurs the primary morbidity is renal failure, which does not usually improve even though the drug is discontinued. The majority of patients with this complication develop hypertension; therefore in patients treated with gemcitabine this symptom should prompt careful evaluation for microangiopathic hemolytic anemia, prior to continuation of therapy. At this time discontinuation of the drug is recommended if the TMA syndrome develops.

8.3.3 VEGF Inhibitors

Bevacizumab is the most recent anti-neoplastic drug to be linked to the development of TMA [48, 58]. It is a recombinant humanized monoclonal antibody that binds to vascular endothelial growth factor-A (VEGF-A) and its isoforms. In February 2004, bevacizumab was the first anti-angiogenic agent to be granted approval by the FDA for treatment of patients with metastatic colon cancer. Currently it is used for a variety of malignancies including lung and

breast cancer, and is in clinical trials for many other cancers. The first case of thrombotic microangiopathy with bevacizumab was reported in 2007 by Frangie et al., and was characterized by proteinuria, MAHA and thrombocytopenia after exposure to this agent [58]. Renal biopsy confirmed TMA, and ADAMTS13 activity was mildly decreased at 53%. Subsequent treatment of the same patient with sunitinib, an oral inhibitor of multiple tyrosine kinases, including VEGF-receptor (VEGFR), resulted in recurrence of the TMA. Discontinuation of each treatment led to resolution of symptoms.

Six additional cases of bevacizumab-associated TMA have been recognized [48]. All patients developed renal insufficiency and proteinuria while receiving bevacizumab, which led to renal biopsies that were consistent with a TMA. Only one out of six patients had evidence of MAHA and thrombocytopenia. The drug was discontinued in five out of six patients with improvement in renal function. One patient continued on bevacizumab for an additional 8 months, with stable proteinuria and renal function. Eremina and colleagues developed an animal model to evaluate the role of VEGF in the renal pathology. Knockout mice were created in which VEGF synthesis could be selectively turned off only in the glomerular podocytes. Once VEGF was eliminated, the mice developed proteinuria and a renal TMA, with evidence of intracapillary fibrin thrombi. Schistocytes were also noted in 58% of the blood smears from seven mice, though thrombocytopenia did not develop [48]. These elegant experiments demonstrate the importance of VEGF to glomerular microvasculature.

Finally, the FDA recently issued an alert regarding the incidence of TMA and concurrent administration of bevacizumab and sunitinib. (http://www.fda.gov/medwatch/safety/2008/MAHA_DHCP.pdf) In a Phase I clinical trial, 5 out of 12 patients that received the highest dose level of sunitinib (50 mg daily), in addition to bevacizumab every 2 weeks, developed clinical signs of a microangiopathic hemolytic anemia. Two out of five patients had evidence of TMA with thrombocytopenia, increased creatinine, proteinuria and hypertension. Discontinuation of the treatments resulted in resolution of symptoms.

Well-recognized toxicities of bevacizumab include proteinuria and hypertension [59]. The incidence of TMA is not well characterized but is likely low. Yet the cases above and the animal studies suggest that inhibition of VEGF may place some patients at risk for renal injury and TMA. In addition to renal injury, both hemorrhagic and thrombotic complications have been recognized in patients treated with bevacizumab [59]. A possible mechanism for these adverse events relates to the decreased ability of endothelial cells to respond to an injury in the face of VEGF inhibition, leading to an hemorrhagic tendency. On the other hand, if vascular injury occurs, the coagulation system may be activated by exposure of tissue factor, and a thrombotic event may result [60]. Therefore, the consequences of inhibition of VEGF signaling can be

complex, and it is not clear whether the thrombotic tendency may also have a role in the development of the TMA syndrome in these patients. The risk factors for these complications are not well understood, but further investigation of patients receiving anti-VEGF therapy is warranted. Until more data is available, the clinician must be aware of these rare complications, institute surveillance for renal dysfunction and thoroughly investigate patients that develop hematologic or renal abnormalities while receiving therapy.

8.3.4 Other Medications Associated with TMA

There are a few case reports of TMA in patients treated with interferon-α therapy, primarily for chronic myelogenous leukemia (CML) [45, 46, 61, 62]. The duration of interferon therapy prior to recognition of TMA was between 16–80 months, and in most cases the CML was in chronic phase at the time of diagnosis [45, 61]. The most common feature was renal failure, which did not completely resolve, although most patients did not require long-term hemodialysis [45]. Treatment included discontinuation of the drug, short-term hemodialysis and occasionally plasmapheresis. The true incidence of this complication is likely low, though it should be considered in the differential diagnosis, particularly in a patient with worsening renal function.

There has been a single case report of TTP in a patient treated with imatinib for hypereosinophilic syndrome (HES) [63]. This patient presented with constitutional symptoms and progressive eosinophilia which did not respond to treatment with hydroxyurea for 7 days. After 4 days of imatinib therapy, a microangiopathic hemolytic anemia and renal failure appeared, which were treated with discontinuation of the imatinib and TPE. Of note, ADAMTS13 activity was low (15%) and an ADAMTS13 autoantibody was detectable [63]. This case demonstrates the difficulty in assessing causality of a TMA. It is unlikely that imatinib resulted in the development of an ADAMTS13 autoantibody after only 4 days of drug exposure. Therefore caution must be exercised before attributing an adverse event to an agent. Rigorous post-marketing surveillance is necessary to identify rare but serious adverse events of new therapies.

Finally, patients with malignancies may receive other medications that can be associated with TMA syndromes (Table 8.2). Therefore careful review of all administered medications and supplements should be undertaken in patients who present with a TMA. A possible under-recognized exposure is quinine, which is available in tablet form, but is also found in herbal supplements and tonic water. Quinine is made from the bark of the Cinchona tree, and herbal supplements derived from it are known by a variety of names including Cinchona, Kinakina, Quina, Peruvian bark, and Jesuit's bark [64]. A careful exposure

history must be elicited from patients who present with TMA to identify any potential sources of quinine, so that future exposures can be avoided.

8.4 Thrombotic Microangiopathy Associated with Hematopoietic Stem Cell Transplantation

The lack of standard case definition makes it difficult to determine the true incidence of TMA in hematopoietic stem cell transplant (HSCT) patients, and estimates vary from 0.5% to 64% [65]. The largest report, which included 4,334 consecutive Italian patients undergoing HSCT, noted the incidence of TMA to be 0.13% in autologous HSCT, and 0.5% in allogeneic HSCT [66]. In order to standardize diagnostic criteria, particularly for clinical trials, two working groups developed definitions of transplant-associated TMA [67, 68] (Table 8.3). The criteria differed between the panels, most notably with respect to the extent of organ involvement. The European group required only evidence for a microangiopathic hemolytic anemia and thrombocytopenia [68], while the US group required evidence of either renal or neurologic dysfunction [67]. An attempt at arriving at a consensus definition is important although controversy remains, given that other causative factors can explain some of the hematologic abnormalities and even the organ dysfunction. In the HSCT population, other etiologies including infections, acute graft-versus-host disease (GVHD), chemotherapy or radiation-induced endothelial damage, may mimic a TMA making the diagnosis more difficult. In addition, the calcineurin inhibitors used for prophylaxis and treatment of GVHD may play a causative role by inducing direct toxic injury to the endothelium. A few groups have identified risk factors

Table 8.3 Definitions of transplant-associated TMA

International Working Group – European Group for Blood and Marrow Transplantation and the European LeukemiaNet [68] *All criteria must be present*	Blood and Marrow Transplant Clinical Trials Network Toxicity Committee Consensus Summary (United States) [67]
Increased percentage (>4%) of schistocytes in peripheral blood	Red blood cell fragmentation and ≥2 schistocytes per high power field on peripheral smear
De novo, prolonged or progressive thrombocytopenia (platelet count $<50\times10^9$/L or >50% decrease)	Concurrent increase in serum LDH above institutional baseline
Sudden or persistent increase in LDH	Concurrent renal and/or neurologic dysfunction without other explanation
Decrease in hemoglobin concentration or increased red blood cell transfusion requirement	Negative direct and indirect Coomb's test results.
Decrease in serum haptoglobin concentration	

for the development of TMA after HSCT; these include female sex, unrelated donor, total body irradiation during conditioning, high-dose busulfan, use of tacrolimus, GVHD grade II–IV, and hepatic veno-occlusive disease [69–71].

The pathophysiology of transplant-associated TMA (TA-TMA) is most likely related to endothelial injury. Autopsy findings in patients with HSCT-associated TMA do not demonstrate systemic microthrombi, which is the pathologic hallmark of classical TTP [65]. Severe deficiency of ADAMTS13 has not been found in patients with HSCT-associated TMA [7–9, 65, 72–74]. Yet in patients followed longitudinally after allogeneic or autologous HSCT, a decline in ADAMTS13 activity occurs after the conditioning regimen. In one study, the mean ADAMTS13 activity declined from 76% to 50% in allogeneic HSCT, while in autologous HSCT patients the activity nadir was 47% [74]. Levels returned to baseline by day 60 after transplantation. In the 46 patients followed by this group, only 3 developed TA-TMA, but ADAMTS13 activity was not different in this group as compared to the group that did not develop the TMA syndrome [74]. Therefore other factors independent of ADAMTS13 activity are likely important contributors to this complication. Evidence of endothelial injury in patients with TA-TMA includes absent endothelial prostacyclin, elevated vWF antigen but with normal vWF multimer pattern, and elevations of thrombomodulin, plasminogen activator inhibitor-1 (PAI-1) and soluble intercellular adhesion molecule-1 (ICAM-1) [73].

The treatment of HSCT-associated TMA is not well-defined. Poor prognostic factors in this population include age >18 years, unrelated or haploidentical donor, elevated LDH/platelet ratio, and nephropathy [69, 75]. The role of therapeutic plasma exchange is not clear, but it is much less effective than TPE in idiopathic TTP. A systematic review of the literature reported an 82% mortality in this population when treated with plasmapheresis [65], while mortality with idiopathic TTP is only 15–30% [7, 9]. Overall the efficacy of TPE appears low, and given the significant side effects discussed above, most experts do not recommend its use [65, 73, 76]. Treatment of HSCT-associated TMA has been based on anecdotal reports, and other agents that have been utilized in treatment include daclizumab, defibrotide, and rituximab. Clinical trials are necessary in order to determine the optimal mode of therapy in this population with a poor prognosis.

8.4.1 Cyclosporine-associated TMA

Since 1983, cyclosporine has been utilized for the prophylaxis and treatment of GVHD. Cyclosporine likely has a role in development of TMA given that cases of cyclosporine-associated TMA have been observed among stem cell and solid-organ transplant patients but also among patients treated for rheumatoid arthritis and uveitis [77, 78]. In addition, the incidence of TMA is higher in

patients treated with cyclosporine as compared to those receiving methotrexate for GVHD prophylaxis [79].

Patients with HSCT transplantation receiving cyclosporine have evidence of endothelial damage, even in the absence of a significant microangiopathy [79, 80]. Other potential mechanisms for TMA with cyclosporine include a decrease in endothelial prostacyclin synthesis [81] as well as reduced formation or release of activated protein C [82]. The severe ADAMTS13 deficiency observed in cases of idiopathic TTP has not been seen with cyclosporine-related TMA, although the number of reported cases with documented cyclosporine exposure is very small. A group from the Mayo Clinic reported eight allogeneic HSCT patients receiving cyclosporine who developed TMA, and found ADAMTS13 activity to be normal in all [72]. There is one case report of a renal transplant patient receiving cyclosporine who developed TMA 12 days after transplant, and was found to have an ADAMTS13 activity <5% along with the presence of an inhibitor [83]. Treatment with TPE led to resolution of the ADAMTS13 inhibitor, and 76 days after completion of plasmapheresis the ADAMTS13 activity was normal at 110% [83]. It is most likely that ADAMTS13 autoantibodies are not the primary mechanism for cyclosporine-associated TMA. The drug likely results in endothelial injury, and may be exacerbated by factors such as infection or GVHD.

Optimal therapy has not been adequately evaluated. The most commonly utilized strategy is the discontinuation of cyclosporine, with a switch to tacrolimus [84, 85]. Yet there are reports of successful resolution of the TMA with only a dose reduction of cyclosporine [84]. Therapeutic plasma exchange has been utilized, as well as other therapies including corticosteroids and intravenous immunoglobulin [86, 87]. Finally, the recognition and appropriate treatment of an underlying cytomegalovirus infection has resulted in successful resolution of TMA [88]. In many cases cyclosporine has been safely reintroduced after resolution of the TMA [89]. Therefore, despite a likely contribution of cyclosporine to the pathogenesis of TMA in this population, it is frequently not the only cause of TMA.

8.4.2 Tacrolimus-associated TMA

Although tacrolimus received FDA approval in 1994, 3 years earlier the first case of HUS was reported in a renal transplant patient who received the drug during a pre-marketing clinical investigation [90]. Since that time, cases have been observed in patients undergoing both solid organ and hematopoietic stem cell transplant, with an estimated incidence of 1–4.7% [91]. Tacrolimus is structurally different to cyclosporine, yet they both effect cytokine production by inhibiting calcineurin.

Although patients with elevated tacrolimus levels may develop TMA [92, 93], there is no clear dose-response association, since TMA has occurred with tacrolimus doses from 0.15 mg to 36 mg per day [91]. Just as with cyclosporine,

TMA patients receiving tacrolimus may have other contributing factors such as CMV infection [94]. Mechanisms for TMA include tacrolimus-associated endothelial cell injury, increased endothelin secretion, and decreased prostacyclin production [92]. Endothelin is a potent vasoconstrictor and may increase shear stress in the vasculature [95]. Tacrolimus has been associated with fibrin thrombi in the glomerular capillaries and arterioles in renal transplant patients without evidence of a systemic microangiopathy; this can improve with a reduction in drug dose [93]. The role for ADAMTS13 antibodies in tacrolimus-associated TMA is not known, but is unlikely to be a major factor. In two reported cases, severe ADAMTS13 deficiency was not observed (activity levels were 34% [9] and 17%) but a low-titer inhibitor was detected in the patient with the 17% activity level [96].

Tacrolimus associated TMA is most commonly seen in the first 6 months after HSCT or solid organ transplant [97], but there are cases, particularly after solid organ transplant, that occur 18–23 months after transplant [91]. The majority of reported cases have been in patients' post-renal transplants, but their outcomes appear to be better than cases in other solid-organ or HSCT transplants [98]. The clinical presentation of tacrolimus-associated TMA cases is quite variable, and although schistocytes are present, the LDH is often not elevated [91, 98]. Treatment strategies for tacrolimus-associated TMA are similar to those reviewed above for cyclosporine: discontinuation of the agent, dose reduction, substitution with cyclosporine, plasma exchange, corticosteroids or IVIG [91, 92, 94, 97–99]. Prospective studies comparing treatment modalities and outcome including mortality or graft function are needed in all transplant-associated cases of TMA.

8.5 Conclusion

Thrombotic microangiopathy syndromes in patients with cancer may be due to the underlying malignancy or treatments including chemotherapy or hematopoietic stem cell transplantation. Etiology of the disorders is thought to include endothelial cell injury, increased platelet aggregation, impaired fibrinolysis, and tumor adherence to microvascular endothelium. ADAMTS13 deficiency, an important factor in many cases of idiopathic TTP, is unlikely to play a major role in cancer-associated TMA. Further study is necessary to elucidate pathologic mechanisms, risk factors and optimal treatment for this syndrome which is associated with significant morbidity and mortality.

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Chapter 9
Disseminated Intravascular Coagulation (DIC) in Cancer

Hussain I. Saba, Genevieve A. Morelli, and Rashid I. Saba

9.1 Introduction

Venous thromboembolism (VTE) and disseminated intravascular coagulation (DIC) are important complications of cancer. The relationship was recognized as early as 1800 when Professor Armand Trousseau noted that patients with idiopathic VTE frequently harbor an occult cancer [1, 2]. Prandoni reported a 7.6% incidence of cancer in 145 patients following idiopathic VTE [3]. In 2000, Schulman reported that 13% of patients in his study developed cancer after their initial diagnosis of primary VTE [4]. We reported that 26.2% of veteran patients were diagnosed to have cancer within 6 months after initial VTE as compared to 11.5% of the control group [5]. These studies emphasize that there is an intimate relation between VTE and cancer.

Thrombogenesis and DIC in cancer patients involves defects at three different levels of the normal host defense against thrombosis [6]: (1) defects in the blood flow, resulting in stasis, (2) defects in the normal balance between procoagulant and anticoagulant proteins in blood, which leads to the activation of procoagulant circulating proteins, and (3) defects in the blood vessel wall which cause an increased procoagulant contribution by the vascular wall.

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9.2 Concept of DIC

In normal physiological states, there is continuous generation of small amounts of fibrin, which is needed to layer and seal the wear and tear of vascular endothelium. The fibrin is produced by the generation of small amounts of thrombin that converts the fibrinogen to fibrin on the endothelial cell surface of the vascular wall. Surface fibrin, along with the important initial role of platelets in this process, helps to arrest oozing and bleeding from the endothelial cell desmosomes. This physiological process has been referred to as "hemostasis".

DIC is induced by the abnormal activation of the clotting cascade. This over activation leads to the generation of pathologic levels of thrombin (i.e., a hyperthrombinemic state), and can induce exaggerated conversion of fibrinogen to fibrin resulting in widespread microvascular thrombosis which in turn leads to tissue hypoxia and organ damage. If the exaggerated conversion of fibrinogen to fibrin is excessive, consumption and depletion of important procoagulant clotting factors can occur and cause a state of hypocoagulability due to depletion of clotting procoagulants. This hypocoagulable state thereby leads to bleeding. In DIC, as microthrombosis occurs on the vascular surface, secondary fibrinolysis sets in as a result of the release of plasminogen activator, activating plasminogen to plasmin and inducing fibrinolysis of the clot (i.e., secondary fibrinolysis).

DIC can therefore be referred to as a "two phase" thrombo-hemorrhagic syndrome: the development of a hypercoagulable (thrombotic) phase which, if not interrupted, can slip into a second hypocoagulable or bleeding phase due to consumptive coagulopathy [7] and secondary fibrinolysis.

9.3 Pathophysiology of Cancer Associated DIC

Mediators and contributors of DIC in cancer include: procoagulants released from cancer cells and adjacent tissues (tissue factor, cancer procoagulant), abnormalities of fibrinolytic mediators, the role of cancer-related cytokines, tumor-associated endothelial cell alterations, their interactions with blood cells and contribution from compromised state of normal defense mechanisms of hemostasis [8].

9.4 Procoagulant Mediators

A variety of procoagulant molecules have been associated with cancer cells and tumor associated macrophages, including tissue factor (TF) and cancer procoagulant (CP), as well as other procoagulants (i.e., factor V receptor associated with shed tumor cells plasma membrane vessels, facilitating the assembly of prothrombinase complex) [9] and a factor XIII-like activity promoting cross-linking of fibrin as reported in human breast cancer cells [10].

9.4.1 Tissue Factor (TF)

Tissue factor, which is considered to be the important primary cellular activator of normal blood coagulation, has been found to be over-expressed in many cancer cells and to be involved in the development of thrombosis and DIC [11–16].

Factors VII and VIIa can be bound to several high affinity sites of tissue factor, and this complex can then activate factors X and IX, as shown by Nemerson [11]. Tissue factor expression is tightly controlled and not generally expressed in resting endothelium or in normal monocytes and macrophages. Pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and bacterial liposaccharides can induce expression of TF procoagulant activity (PCA) in these cells. TF has also been found to be essential for embryonic development of vasculature and in tumor angiogenesis and cell adhesion interactions [17–19]. Cross-linked fibrin (xLF) can be localized with TF in both tumor associated macrophages and within the endothelium of tumor associated blood vessels in human breast and lung cancer [20, 21]. TF expression and xLF deposition has only been seen within or in close proximity to growing tumors. Such TF expression supports the concept that new and angiogenic vessels during tumor growth are more susceptible to thrombogenesis.

9.4.2 Cancer Procoagulant (CP)

Cancer procoagulant (CP) is another important mediator of thrombosis and DIC in cancer patients. CP is synthesized by cancer cells and its activity has been found in the sera from cancer patients and in tumor bearing animals [22–25]. CP was found in the extract of tumor cells but not in that of normally differentiated cells. CP levels have been shown to be elevated in the sera of approximately 85% of cancer patients [22, 24]. Donati et al. have emphasized the role of CP in thrombogenesis observed in leukemia, and have shown CP activity paralleled the course of the disease [26]. Studies have shown that in promyelocytic leukemia, a leukemic disease with a high propensity for DIC and thrombo-hemorrhagic syndrome, ATRA (all-trans retinoic acid) modulates PCA expression on the APL leukemic cell [27–30]. Both TF and CP of APL marrow blast cells are progressively reduced in patients receiving ATRA. The demonstration of this effect parallels the improvement of plasma markers for hypercoagulability. This observation is strong evidence of PCA involvement in the clotting complications associated with malignancy [31].

9.4.3 Alteration of Fibrinolytic Activities in the Mediation of Thrombosis and DIC in Cancer

Components of fibrinolysis such as tissue plasminogen activator (t-PA) and urokinase-type activator (u-PA) have been found on cancer cells. Urokinase-type

plasminogen activator (u-PA) is widely associated with cancer cells and may be linked to tumor progression and/or prognosis [32–34]. Plasminogen activator inhibitor 1 (PAI-1) and plasminogen activator inhibitor 2 (PAI-2) are also found on cancer cells. Specific receptors on tumor cells favor the assembly of all fibrinolytic components, thus facilitating the activation of the fibrinolytic system [35]. They may have a role in the pathogenesis of bleeding in APL patients. On the other hand, alteration of these fibrinolytic components on cancer cells could also play a role in thrombogenesis and ensuing DIC in solid tumor patients.

9.4.4 Cytokines in Cancer as Mediators of Thrombogenesis

Malignant cells produce important cytokines which can elicit procoagulant effects on the vascular endothelium. Some of these, such as TNFα and IL-1β, induce expression of procoagulant TF from vascular endothelial cells [36, 37]. These cytokines also down regulate endothelial thrombomodulin, which could compromise the activation of Protein C, leading to reduced inhibition of factors Va and VIIIa. This down regulation of endothelial thrombomodulin and upregulation of TF converts the normal anticoagulant endothelium to a prothrombotic state [38]. TNF-α and IL-1β have also been shown to stimulate the vascular endothelium to produce t-PA inhibitor PAI-1, further enhancing this prothrombotic activity [39]. Administration of TNF-α and LPS to normal human volunteers has been shown to increase the release of tPA followed by a prolonged release and a larger increase of PAI-1 [40]. Thus, the net effect of cytokines TNF-α and IL-1β is prolonged reduction of fibrinolytic activity. Leukemic promyelocytes in patients with DIC secrete more IL-1β than blast cells in APL, suggesting that tumor derived IL-1β has more of a role in the onset and pathogenesis of DIC in leukemic states [41].

9.4.5 Interaction of Tumor and Host Cells and Their Influence on Thrombogenesis

The interaction of tumor cells with monocytes, platelets and endothelium, has been reported to increase the thrombogenic potential of the host cells. Monocytes are present at the margin and vicinity of tumor masses. In their native state, these cells have very little to no procoagulant activity. However, as they come in contact with cancer cells, these monocytes acquire the ability to produce tissue factor and other direct factor X activators [42–44]. Increased platelet reactivity after tumor-platelet interaction has been observed and substances obtained from various tumors have been shown to aggregate platelets directly [45, 46].

With tumor cell contact, endothelial cells can become procoagulant as well as thrombogenic under the influence of cytokines. The release of IL-1 and TNF after tumor contact with endothelial cells may increase the expression of leukocyte adhesion molecules, platelet activating factor and tissue factor. TNF suppresses the endothelial fibrinolytic activity and down regulates thrombomodulin expression, thus diminishing activation of anticoagulant Protein C. These reactions enhance the procoagulant properties while suppressing anticoagulant properties of endothelial cells [47–49].

Apoptosis involves a series of biochemical events leading to characteristic cell morphological changes (e.g., cell membrane changes, cell shrinkage, nuclear fragmentation, chromatin and chromosomal DNA changes) leading to cell death [50, 51]. Apoptosis has been associated with the development of thrombosis [52]. Tumor lysis syndrome is a serious and life-threatening condition normally associated with large bulky tumors with rapidly dividing cells and with tumors that respond rapidly to therapeutic intervention such as chemotherapy, antibody therapy and/or radiotherapy [53, 54]. This condition involves a large and acute release of intracellular contents into the blood stream following significant cytotoxic malignant cell death. Tumor lysis syndrome can occur in response to therapeutic intervention or can occur spontaneously in such malignancies as Burkitt's lymphoma and acute leukemias including acute promyelocytic leukemia [54]. The thrombosis sometimes observed as a result of apoptosis such as that seen in tumor lysis syndrome has been linked to release of procoagulants such as tissue factor from the interior of the cell. It has been suggested that external exposure of cell membrane phosphatidylserine during apoptosis is linked to thrombin generation and to the activation of tissue factor, resulting in a greater risk of thrombosis [52].

9.5 Diagnosis of DIC in Cancer

It has been suggested that nearly all patients with cancer have subclinical activation of chronic DIC in the absence of active bleeding and/or thrombosis [8, 55]. Levi reported that DIC can be a complication in 7–20% of patients with cancer [56]. Wada et al. (1998) published results of a Japanese Ministry of Health and Welfare on frequency of underlying malignancies in patients diagnosed with DIC [12]. These were non-Hodgkins lymphoma (23.7%), hepatoma (17.4%), acute myeloid leukemia (14%), lung (12.6%), APL (10.9%), gastric (7.2%), leukemia (13.1%), acute lymphoblastic leukemia (6.9%), chronic myelocytic leukemia (3.4%), acute myelomonoblastic leukemia (1.7%), acute monoblastic leukemia (1.1%) and breast (1.1%). Blom et al. investigated 3,220 patients with venous thrombosis and found that 389 had one or more previous malignancies. These malignancies included lung (14.1%), hematological malignancies (15.4%), gastrointestinal (21.6%), urinary/prostate (17.8%),

female malignancies such as breast, ovarian and other (24.5%), brain (4.6%) and other (16.2%) [57]. Sack et al. studied Trousseau's syndrome and DIC coagulopathy in 182 different cancer patients, and found that 24% had cancer of the pancreas, 20% lung cancer, 13% prostate, 12% stomach, 9% had acute leukemia and 5% were diagnosed with colon cancer [58].

Chemotherapy and/or surgical interventions in the treatment of solid tumors can induce DIC. Normal risk factors for VTE such as immobility or stasis, advanced age, history of previous thrombosis and sepsis can further enhance this thrombo-hemorrhagic problem seen in cancer [2]. In many cases, the actual incidence and frequency of DIC in cancer subtypes is often difficult to assess due to the presence of these risk factors, problems in diagnosis and complications resulting from treatment.

DIC in cancer patients can be classified as either acute or chronic, and laboratory parameters and presenting symptoms could be different in each. Bleeding could be a common presenting symptom in DIC and has been observed to be as high as 64% in one series of 118 patients reported by Siegal et al, and thrombosis in 7% of patients [59].

Chronic DIC occurs when small amounts of tissue factor are released and the hemostatic mechanism is more capable of correcting the activation of coagulation that occurs. Thrombosis is common, but not exclusive to malignancy patients with chronic DIC [60], and it may be merely thrombotic with organ dysfunction or, more frequently, may involve venous thromboembolism [61]. It is most commonly associated with solid tumors (e.g., lung, breast, prostate, pancreatic cancer, etc.) (Table 9.1). Laboratory assessments and commonly expected results in chronic DIC can include: variable platelet counts (could be high with sequential counts over time showing variable decreases); normal to mildly prolonged PT, APTT, TT; normal to elevated fibrinogen; normal levels of factors V and VIII and high FDP and D-dimer levels.

Table 9.1 Cancers commonly associated with DIC

Clinical parameter	Cancer type
Acute DIC (localized or systemic bleeding, echymosis)	Acute promyelocytic leukemia (APL)
	Acute non-M3 myeloid leukemia (AML)
	Acute lymphocytic leukemia (ALL)
	Prostate cancer[a]
	Mucin-producing adenocarcinomas (e.g., pancreatic[a], gastrointestinal, ovary, thyroid, gallbladder)
	Lymphoma (e.g., Stage IV, natural killer)
	Chronic myelocytic leukemia (CML)
Chronic DIC (Thrombosis)	Solid tumors (e.g., lung, breast, prostate[a], pancreatic cancer[a])

[a] Can be associated with either chronic or acute DIC

Acute DIC develops when excessive tissue factor is released over a relatively brief time frame leading to immediate and excessive thrombin development. Acute DIC is most commonly associated with hematologic malignancies (e.g., acute promyelocytic leukemia, acute non-M3 myeloid leukemia (AML), chronic myelocytic leukemia (CML), acute lymphocytic leukemia (ALL) and in lymphomas) (Table 9.1). It can also occur in some solid tumor malignancies, such as prostate cancer and mucin producing adenocarcinomas (e.g., pancreatic, gastric, thyroid, gallbladder). Laboratory assessments and commonly expected results in acute DIC can include: low platelet counts; prolonged APTT, PT and TT; low levels of fibrinogen, factors V and VIII; high FDP and D-dimer with reduced levels of antithrombin, Protein C and Protein S.

Several diagnostic algorithms for DIC have been developed. One of these is the International Society of Thrombosis and Hemostasis Scoring Systems as outlined in Tables 9.2 and 9.3 [62–66]. Figure 9.1 illustrates a general algorithm outlining clinical presentation and testing of cancer associated DIC. It should be kept in mind that DIC is a spectrum with a range in clinical laboratory parameters observed in patients. Although the diagnostic algorithm outlined in Fig. 9.1 is useful as a guide, it should not be considered to be absolute.

Global laboratory tests of coagulation including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin clotting time (TT), platelet count, and fibrinogen are more commonly used in the initial evaluation of DIC in cancer patients. Other more specialized tests include coagulation factors V and VIII levels, fibrin split products (FSP), D-dimers and prothrombin fragment 1 + 2, thrombin-antithrombin complex (TAT), soluble fibrin monomers and fibrinopeptides A and B [67–69]. Although these tests have their own problems of specificity and sensitivity, in combination they have been useful in assessing the status of DIC in cancer patients. Abnormalities are more commonly found in the consumptive phase of DIC as compared to the thrombotic phase [70–73]. The progressive fall in platelet count remains a more sensitive DIC marker if the baseline platelet count is known. The importance of accurately monitoring platelet counts and recognition of relative changes in suspected DIC patients cannot be overly emphasized. Circulating platelets are extremely sensitive to activation and subsequent aggregation by small amounts of thrombin and drops in their count occur early in DIC [74, 75]. Obtaining repeat platelet counts can illustrate this drop.

Fibrinogen levels in patients with cancer-induced DIC can vary, and could have a correlation with clinical outcome in some patients [76]. Many of these tests are not specific only to the diagnosis of DIC. For example, detection of fibrin breakdown products are positive in DIC, as well as in renal failure/disease and other disorders [77, 78]. Positive D-dimers and prothrombin fragments F1 + 2 may be indicative of DIC, but have been associated with other disorders as well [79, 80].

Table 9.2 Overt DIC scoring system – International Society of Thrombosis and Hemostasis (ISTH)

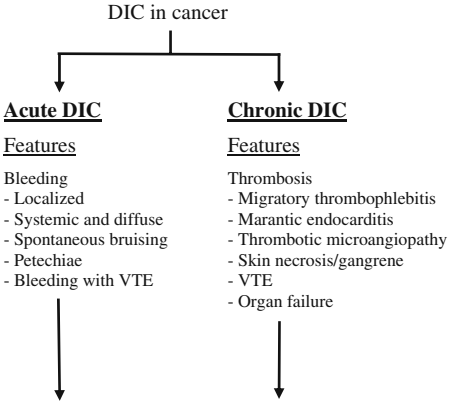
Laboratory test	Result	Score
Platelet Count ($\times 10^3$/L)	>100	0
	>50 <100	1
	<50	2
Increase in Fibrinogen and Fibrin-related markers (i.e., FDP)	None	0
	Moderate	2
	Strong	3
Prolongation of Prothrombin Time (PT) over upper limit of normal range (in seconds)	<3	0
	>3–<5.9	1
	>6	2
Fibrinogen level (g/dL)	≥1	0
	<1	1

Scores can range from 0 to 8, with scores ≥5 compatible with overt DIC. Scores <5 are suggestive of non-overt DIC and scoring should be repeated every 1–2 days [62]

Table 9.3 Non-overt DIC scoring system – International Society of Thrombosis and Hemostasis (ISTH)

Laboratory test	Result	Score
Other diagnosis	Not associated with DIC	0
	Associated with DIC	2
Platelet Count ($\times 10^3$/L)	>100	0
	<100	1
Trend in platelet count	Rising	–1
	Stable	0
	Falling	1
Prolongation of Prothrombin Time (PT) over upper limit of normal range (in seconds)	≤3	0
	>3	1
Trend in PT prolongation	Falling	–1
	Stable	0
	Rising	1
FDP or soluble fibrin monomers	Normal	0
	Increased	1
Trend in FDP or soluble fibrin monomers	Falling	–1
	Stable	0
	Rising	1
Antithrombin	Normal	–1
	Low	1
Protein C	Normal	–1
	Low	1
TAT (Thrombin-Antithrombin)	Normal	–1
	Elevated	1

Scores can range from –6 to 1. The higher the score, the more likely non-overt DIC is present [63]



Laboratory Parameter	Most commonly Observed Results	
Relative Platelet count	Low	Variable
Peripheral Smear RBC	Schistocytes	Schistocytes
PT, APTT, TT	Prolonged	Normal to mildly prolonged
Fibrinogen	Low	Normal to elevated
Factors V, VIII	Low	Normal
FDP	High	High
D-dimers	High	High
Prothrombin frag. F1+2	High	High
Proteins C and S	Low	Normal to low
Antithrombin	Low	Normal to low

Fig. 9.1 Clinical and laboratory features of DIC in cancer patients

9.6 Management of DIC in Cancer

9.6.1 Treatment of the Underlying Malignancy

The cardinal rule for the management of DIC is treatment of the underlying condition [81]. This concept also holds true in the management of DIC in cancer patients. It has been reported in one study that 85% of patients with severe DIC died due to their underlying disease and not due to DIC [82]. Although this may not be immediately possible in all cancer patients (i.e., solid tumors, metastatic cancers, G.I. malignancies, lung masses), it is still quite applicable in hematological malignancies and some solid tumors. Schlaeppi et al. reported successful treatment of DIC in a metastatic melanoma patient with chemotherapy [83].

Huang et al. reported some success with weekly infusions of 5-fluorouracil for acute DIC associated with advanced gastric cancer [84]. Other reports have also been published regarding successful treatment of DIC in cancer patients with chemotherapy or, in the case of prostate cancer, hormone therapy [85–88]. Treatment of acute promyelocytic leukemia with all-trans retinoic acid or arsenic trioxide in the presence of DIC has also been noted to correct the thrombo-hemorrhagic condition [89].

While chemotherapy may alleviate DIC, it has also been reported to induce it in some patients [90–92]. Sarris et al. found that DIC was diagnosed in 12% of 58 acute lymphoblastic leukemia (ALL) patients before treatment and a total of 78% during remission induction [92]. If chemotherapy can be temporarily held, reduced or changed, it may be possible to correct the thrombo-hemorrhagic disorder.

9.6.2 Use of Heparin in Treatment of Cancer-Related DIC

Generally speaking, the use of unfractionated heparin in DIC has been controversial and discouraged due to its potential to induce bleeding [93]. However, some evidence may indicate that heparin may provide some benefit in the treatment of DIC in the face of some malignancies. Hoyle et al. assessed therapeutic approaches in 115 APL patients with DIC, and found that 85% of patients who received heparin corrected the DIC vs 49% of the patients who did not receive heparin therapy for DIC in APL [94]. This difference appeared to be related to a decrease in the number of hemorrhagic deaths in the heparin group. Oguma et al. reported successful use of low molecular weight heparin (FR-860) in DIC patients at a dose of 75 U/kg/day [95]. Shirai and Chaudhary recently reported the successful treatment of a patient with chronic DIC associated with prostate cancer using low molecular weight heparin (LMWH) and aminocaproic acid [96]. It has been considered that the thrombotic state at the initial stage of DIC could be interrupted by the cautious use of either unfractionated or low molecular weight heparin.

Heparin usage could slow the consumption of clotting factors and/or platelets and save some hypercoagulable DIC patients from entering into the consumptive and hypocoagulable phase of DIC and, therefore, from bleeding. For example, patients with mucin-producing adenocarcinoma of the GI tract or ovary, who represent a high risk group for the development of VTE and DIC, may benefit from prophylaxis with heparin. Although the true incidence of reoccurrence is unknown in patients who have already experienced one episode of VTE, it has been reported to reach as much as 50% in some studies [97, 98]. The ACCP recommends that these patients could be on some level of anticoagulant prophylaxis [99, 100]. Such high recurrence rates of VTE have raised concern and stimulated consideration of low molecular weight heparin for prophylaxis in these patients.

If unfractionated heparin in used in DIC patients, it is advisable to use it at low dose (500–750 units/h without a bolus injection) [7]. Patients receiving

heparin must be cautiously monitored for bleeding complications and/or benefit (e.g., increase in platelet count, correction of other laboratory parameters and clinical symptoms). Treatment may be continued if benefit occurs, with continued monitoring. If benefit is not observed or if bleeding occurs, treatment should be discontinued.

If LMWH is used in DIC, thromboprophylactic doses are recommended, and the dose titrated to response (correction of the coagulation parameters and platelet count) [101, 102].

9.6.3 Use of Warfarin in Treatment of Cancer-Related DIC

The use of warfarin in cancer related DIC is debatable and not generally recommended. It may, in some situations, have a place in the treatment of some subacute and chronic DIC patients who are unable to tolerate low molecular weight heparin, but some investigators have reported that warfarin is ineffective in thrombotic DIC patients [103, 104]. It is not indicated in DIC associated with bleeding. Warfarin decreases Vitamin K dependent factors, which could further increase the bleeding seen with acute DIC or could actually further the imbalance from thrombosis to bleeding seen as DIC progresses. Certain cancers (e.g., prostate) have been reported to be associated with increased warfarin sensitivity [105]. Patients must, therefore, be carefully monitored.

9.6.4 Replacement Therapy in Cancer-Related DIC

Patients in acute DIC (i.e., consumptive coagulopathy and bleeding) may have low factor levels due to over utilization. Replacement of fibrinogen and or factor VIII with fresh frozen plasma or factor concentrates are the appropriate management [106, 107]. Platelet transfusions may be beneficial if platelet counts are severely low due to DIC-related over-utilization and the patient is bleeding.

9.6.5 Other Therapeutic Modalities in Cancer-Related DIC

Fibrinolytic inhibitors such as epsilon aminocaproic acid and tranexaminic acid have been used in cancer-related DIC patients during the acute (bleeding) stage of DIC [108]. However, careful monitoring is needed in these patients as well, since there have been reports suggesting that antifibrinolytics may not be effective and may lead to fatal thromboembolism [109]. Treatment of malignancy-related DIC with hirudin, antithrombin and Protein C concentrates has also been reported and are suggestive of effectiveness, but the clinical benefit has not been established [110]. There have been isolated cases in which activated factor VII has been used to treat life-threatening bleeding in cancer patients with DIC [111, 112]. Further study is necessary prior to advocating the use of

Table 9.4 Therapeutic approaches in DIC with cancer: advantages and disadvantages

Treatment	Advantage(s)	Disadvantage(s)	Useful in
Treatment of the underlying condition (such as chemotherapy, surgery, radiation, hormonal therapy)	1. Recommended first line therapy for DIC related to cancer 2. Exposes patients only to treatment they would receive for their underlying malignancy	1. Does not specifically address immediate thrombo-hemorrhagic complications 2. May take time to observe benefit 3. May stimulate DIC 4. May worsen bleeding due to treatment-related thrombocytopenia	1. Hematological malignancies 2. Some solid tumors
Anticoagulation			
Heparin (unfractionated)	1. Inexpensive 2. Short half-life 3. Can be neutralized with protamine sulfate	1. Need for close laboratory monitoring 2. Risk of bleeding 3. Risk of cancer-related heparin resistance 4. Risk of heparin-induced thrombocytopenia (HIT) 5. Effectiveness depends upon adequate antithrombin levels	1. Mucin producing adenocarcinoma of the GI or ovary 2. Some solid tumors (e.g., prostate)
Low molecular weight heparin	1. No need for close laboratory monitoring 2. May have beneficial advantage on tumor biology 3. Ease of administration 4. Not influenced by dietary deficiencies or liver dysfunction	1. Cost	1. Mucin producing adenocarcinoma of the GI or ovary 2. Some solid tumors (e.g., prostate)
Warfarin	1. Inexpensive 2. Biological advantage as anticancer agent	1. Need for close laboratory monitoring 2. Warfarin-induced necrosis 3. May lead to increased bleeding and/or acute DIC	1. Good agent in some subacute and chronic DIC patients

Replacement therapy			
Platelets, FFP, cryoprecipitate	1. Re-establishes adequate levels in order to attempt to rebalance hemostasis	1. Blood borne pathogen exposure (e.g., HIV, hepatitis) 2. FFP or cryoprecipitate may provoke massive thrombosis	1. Useful in consumptive coagulopathy with diffuse bleeding.
Thrombolytic therapy (e.g., urokinase, streptokinase, t-PA)	1. Clot lysis	1. Risk of bleeding outweighs use in all but a few patients	1. Some chronic DIC patients with massive thrombosis where rapid flow restoration is needed
Direct thrombin inhibitors (e.g., hirudin, argatroban)	1. Inhibition of fibrin-bound thrombin 2. More predictable dose response due to lack of plasma protein binding 3. Does not induce HIT	1. Effectiveness not yet established 2. Requires close monitoring	1. May be effective in some chronic DIC patients
Antifibrinolytic agents (e.g., aminocaproic acid)	1. When combined with LMWH, may be effective for chronic DIC in prostate cancer (fibrinolysis with bleeding)	1. Only isolated reports in literature 2. Should always be used with caution and in combination with anticoagulation due to risk of thrombosis	1. May be effective in some acute DIC patients
Activated factor VII	1. Strong thrombotic agent but could possibly be used as a last resort in life-threatening CNS bleeds	1. Limited studies done 2. Requires close monitoring 3. Expensive 4. Can induce paradoxical thrombosis	1. Could be useful in consumptive coagulopathy with life-threatening, diffuse bleeding

activated factor VII in this patient population. In some situations, such as life-threatening central nervous system bleeds, therapeutic modalities such as activated factor VII and fibrinolytic inhibitors may be therapeutic options for patients in which other treatments have failed but these patients must be carefully and closely monitored. Having stated this, it must be again emphasized that these treatment modalities are not advocated in cancer patients with DIC and may be contraindicated in all except dire, life-threatening refractory bleeds.

Treatment of DIC should be tailored to the specific cancer and patient involved. Pros and cons of the various treatments for DIC in cancer are summarized in Table 9.4 [113].

9.7 Summary

DIC and thromboembolic events are important complications of cancer. Significant amounts of knowledge have evolved in the understanding of the pathophysiology of these thrombo-hemorrhagic disorders. The process appears complex and represents interplay of many mediators from the cancer cells, the host cells around the cancer and alterations in the circulating blood. It has been suggested that every cancer patient is in a hypercoagulable state and at the risk of DIC and thrombosis. This thrombo-hemorrhagic syndrome in cancer patients can be subclassified into the chronic phase and the acute phase. With some overlap, chronic DIC is usually seen in solid tumors and acute DIC is more common in the hematological malignancies. There has not been a single precise diagnostic tool for DIC, but a series of global laboratory tests and some specialized laboratory evaluations can help make the diagnosis. A trend of falling platelets counts in cancer patients could be a sensitive laboratory marker for impending DIC in cancer. Management challenges appear somewhat different in solid tumor DIC vs DIC in hematological malignancies. The cardinal rule still remains the management of the underlying cause; however, in some cases, the sequelae of DIC can be decreased with the use of replacement therapy, careful use of heparin in the appropriate setting or other therapeutic modalities.

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Chapter 10
Myeloproliferative Neoplasms: Thrombophilic Clonal Stem Cell Diseases

Ayalew Tefferi

10.1 Introduction

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are currently classified as *BCR-ABL*-negative classic myeloproliferative neoplasms (MPN). According to the revised 2008 World Health Organization (WHO) classification system, adult chronic myeloid neoplasms are now divided into four broad categories; myelodysplastic syndrome (MDS), MPN, "MDS/MPN overlap" and "myeloid or lymphoid neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*" [1]. The WHO MPN category includes the four classic MPN: chronic myelogenous leukemia (CML; *BCR-ABL*-positive), PV, ET, and PMF. All three *BCR-ABL*-negative MPN are uncommon and incidence figures are estimated at 0.2–2.5/100,000 for ET [2–4], 0.4 to 1.5/100,000 for PMF [2, 3] and 0.8–2.6/100,000 for PV [5, 6]. The median age at diagnosis for all these three MPN is approximately 60 years [7].

10.2 Pathogenesis

Fialkow and colleagues were the first to establish PV (1976) [8], PMF (1978) [9] and ET (1981) [10] as clonal stem cell diseases. In 2005, an activating Janus kinase 2 mutation (*JAK2*V617F) was discovered in the majority of patients with PV and a substantial fraction of those with ET or PMF [11–14]. In 2006 and 2007, additional *JAK2* and *MPL* (thrombopoietin receptor) mutations were described in these diseases and some have been shown to induce PV- (*JAK2*) or PMF-like (*MPL*) phenotype in mice [15, 16]. *JAK2*V617F is by far the most frequent mutation in these disorders; mutational frequency is estimated at over 95% in PV and 50% in ET or PMF. *JAK2* exon 12 mutations (e.g.,

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N542-E543del) are relatively specific to PV and occur in virtually all *JAK2*V617F-negative PV cases (i.e., approximately 3% of all PV cases) [15, 17]. *MPL* mutations (e.g., *MPL*W515L/K) occur in both PMF and ET with an estimated incidence of 5–10% in PMF and 1–5% in ET [16, 18–22].

The precise pathogenetic contribution of *JAK2* and *MPL* mutations is currently under intense investigation. However, variable degrees of genotype-phenotype correlations have been recognized. In PV, where virtually all patients carry a *JAK2* mutation [17], *JAK2*V617F "homozygous" as opposed to "heterozygous" state has been associated with a higher hemoglobin level, higher leukocyte count, lower platelet count and presence of pruritus [23, 24]. A somewhat similar set of correlations were made for higher mutant allele burden in PV, measured by quantitative assays [25–27]. In ET, the presence of *JAK2*V617F has been associated with advanced age, higher hemoglobin level, increased leukocyte count and decreased platelet count [28–31]. Similarly in PMF, the presence of *JAK2*V617F has been associated with an older age at diagnosis and higher leukocyte count [32]. In addition, *JAK2*V617F "homozygous" PMF patients displayed an even higher incidence of leukocytosis, marked splenomegaly and pruritus [33].

Recent studies have raised a possible association between leukocytosis and thrombosis, in both ET [34, 35] and PV [36]. Specific mechanisms, in this regard, appear to involve crosstalk between granulocytes and platelets and/or endothelial cells. Compared to healthy controls, patients with ET or PV display increased baseline [37, 38] and induced (via thrombin, arachidonic acid) platelet P-selectin expression, [39] platelet-granulocyte [37–39] and platelet-monocyte [39] complexes, granulocyte activation (increased CD11b, fibrinogen, leukocyte alkaline phosphatase but lower myeloperoxidase expression) [37–41], baseline and lipopolysaccharide-induced expression of tissue factor (TF) by both monocytes [39] and neutrophils [37], and plasma evidence of endothelial damage and hypercoagulable state [40]. Furthermore, these abnormalities might be more pronounced in patients with history of thrombosis and in those with *JAK2*V617F, according to some studies [39, 41].

A recent study suggested in vivo down-regulation of both neutrophil TF expression and number of neutrophil-platelet complexes, but not platelet P-selectin expression, by hydroxyurea therapy, in patients with either ET or PV [37]. This in vivo phenomenon was recapitulated in vitro where hydroxyurea inhibited both P-selectin-mediated neutrophil TF expression and mixed aggregate formation in healthy subjects [37]. It is to be noted that earlier studies in healthy volunteers have shown P-selectin-mediated in vitro induction of TF synthesis and expression by neutrophils [42]. Also, in vitro induction of platelet-neutrophil complex formation is decreased in ET patients receiving aspirin therapy [38]. These observations provide additional novel mechanisms of action for both hydroxyurea and aspirin that might contribute to their proven efficacy in the treatment of high-risk patients with MPDs [43–45].

10.3 Diagnosis

Table 10.1 outlines the current WHO diagnostic criteria for PV, ET and PMF [46, 47]. Figures 10.1–10.3 provide WHO-based diagnostic algorithms for these diseases [1]. Virtually all patients with PV carry a *JAK2* mutation (either *JAK2*V617F or *JAK2* exon 12 mutations) [15, 17]. Therefore, peripheral blood *JAK2*V617F screening is currently the preferred initial test for evaluating a patient with suspected PV (Fig. 10.1) [48–53]. The concomitant determination of serum erythropoietin (Epo) level is encouraged in order to minimize the consequences of false positive or false negative molecular test results [54–58]. Mutation screening for an exon 12 *JAK2* mutation and bone marrow examination should be considered in a *JAK2*V617F-negative patient who displays subnormal serum Epo level (Fig. 10.1) [15, 57]. Because *JAK2*V617F also occurs in approximately 50% of patients with either ET or PMF [59], it is reasonable to include mutation screening in the diagnostic work-up of both thrombocytosis (Fig. 10.2) and bone marrow fibrosis (Fig. 10.3). However, although the presence of the mutation excludes the possibility of reactive myeloproliferation [58], its absence does not exclude an underlying MPN.

10.4 Clinical Features

At presentation, microvascular symptoms are found in a substantial proportion of patients with ET or PV. These include headaches, lightheadedness, visual symptoms such as blurring and scotomata, palpitations, chest pain, erythromelalgia, and distal paresthesias. The underlying pathology might involve abnormal platelet-endothelium interactions [60]. Erythromelalgia is the most dramatic vasomotor symptom, characterized by erythema, warmth, and pain in distal extremities; this symptom is rare but not entirely specific for ET or PV [61]. Other non-life threatening complications in MPN include constitutional symptoms, superficial thrombophlebitis, minor mucocutaneous bleeding and increased propensity for first trimester miscarriage.

PV and ET are associated with an increased risk of thrombosis and bleeding. Table 10.2 (at diagnosis) [4, 31, 34, 35, 62–69] and Table 10.3 (during follow-up) [4, 31, 34, 35, 62–70] present incidence figures of "major" thrombotic events in a selected series of large studies in PV and ET. These tables also include incidence figures for bleeding and microvascular events. In general, major thrombosis at diagnosis ranges from 9.7% to 29.4% for ET and 34% to 38.6% for PV; the corresponding figures for major thrombosis during follow-up are 8–30.7% for ET and 8.1–19% for PV. Arterial events are more prevalent than venous events and thrombosis more frequent than major bleeding.

Most investigators consider strokes, transient ischemic attacks, myocardial infarctions, angina pectoris, peripheral artery occlusions, pulmonary embolism and DVT as major thrombotic events in ET or PV [34, 44, 69]. Superficial

Table 10.1 The 2008 World Health Organization diagnostic criteria for polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [46]

2008 WHO Diagnostic Criteria			
	PV[a]	ET[b]	PMF[c]
Major criteria	1 Hgb >18.5 g/dL (men) >16.5 g/dL (women) *or* Hgb >17 g/dL (men), or >15 g/dL(women) if associated with a sustained increase of ≥2 g/dL from baseline that can not be attributed to correction of iron deficiency *or*[e]	1 Platelet count ≥ 450×10^9/L	1 Megakaryocyte proliferation and atypia[d] accompanied by either reticulin and/or collagen fibrosis, *or* In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (i.e. pre-fibrotic PMF).
	2 Presence of *JAK2*V617F or similar mutation	2 Megakaryocyte proliferation with large and mature morphology. No or little granulocyte or erythroid Proliferation.	2 Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm
		3 Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm	3 Demonstration of *JAK2*V617F or other clonal marker *or* no evidence of reactive marrow fibrosis
		4 Demonstration of *JAK2*V617F or other clonal marker *or* no evidence of reactive thrombocytosis	

Table 10.1 (continued)

2008 WHO Diagnostic Criteria			
	PV[a]	ET[b]	PMF[c]
Minor criteria	1 BM trilineage myeloproliferation		1 Leukoerythroblastosis
	2 Subnormal serum Epo level		2 Increased serum LDH
	3 EEC growth		3 Anemia
			4 Palpable splenomegaly

[a]Diagnosis of PV requires meeting either both major criteria and one minor criterion *or* the first major criterion and 2 minor criteria.
[b]Diagnosis of ET requires meeting all 4 major criteria
[c]Diagnosis of PMF requires meeting all 3 major criteria and two minor criteria.
[d]Small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.
[e]*or* Hgb or Hct >99th percentile of reference range for age, sex, or altitude of residence *or* red cell mass > 25% above mean normal predicted.

Key: Hgb, hemoglobin; Hct, hematocrit; Epo, erythropoietin; EEC, endogenous erythroid colony; WHO, World Health Organization; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; LDH, lactate dehydrogenase

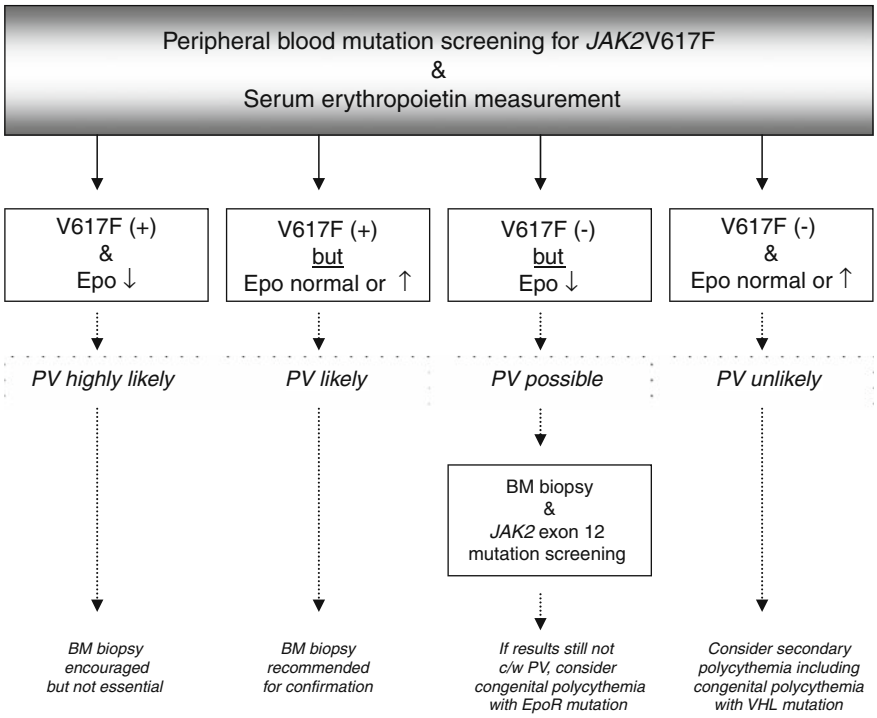

Fig. 10.1 Diagnostic algorithm for suspected polycythemia vera. (With permission from Tefferi and Vardiman, Leukemia 2008) [1] Key: PV, polycythemia vera; SP, secondary polycythemia; CP, congenital polycythemia; BM, bone marrow; V617F, *JAK2*V617F; Epo, erythropoietin; EpoR, erythropoietin receptor; VHL, von Hippel-Lindau; c/w, consistent with

thrombophlebitis is not considered as a major thrombotic event [71]. The same is true for erythromelalgia, which represents a separate platelet-vessel pathology that often responds to aspirin therapy [60]. DVT in MPN includes sometimes catastrophic abdominal vein thrombosis (AVT) [72].

In addition to thrombohemorrhagic events, other life-threatening complications in ET and PV include transformation of the disease into either a fibrotic phase resembling PMF or acute myeloid leukemia (AML). Fibrotic and leukemic evolution of ET are rare events (<5% of patients) during the first 10 years after diagnosis [62, 73]. At least two-thirds of PV patients have splenomegaly at diagnosis [74]. Pruritus is common in PV and may be provoked by warm water ("aquagenic") [75, 76].

Most patients with PMF have anemia and marked splenomegaly at presentation. Spleen and liver enlargement in PMF is secondary to extramedullary hematopoiesis (EMH) and may be associated with hypercatabolic symptoms (profound fatigue, weight loss, night sweats, low-grade fever), peripheral edema (from venous compression), diarrhea, early satiety (from gastric compression),

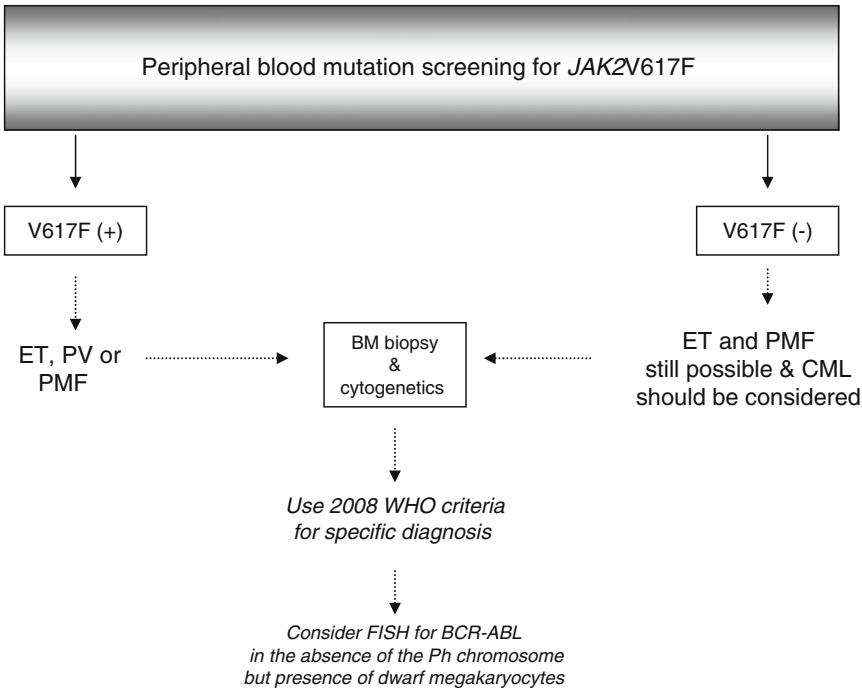

Fig. 10.2 Diagnostic algorithm for suspected essential thrombocythemia. (with permission from Tefferi and Vardiman, Leukemia 2008) [1] Key: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; WHO, World Health Organization; RT, reactive thrombocytosis; FISH, fluorescent in situ hybridization; Ph, Philadelphia; BM, bone marrow; V617F, *JAK2*V617F

and, occasionally, portal hypertension. Splenomegaly in PMF may be complicated by splenic infarction manifested by LUQ pain and referred left shoulder pain [77]. EMH might also occur at other sites including lymph nodes, skin, pleura, peritoneum, lung, and the paraspinal and epidural spaces. The latter may result in spinal cord and/or nerve root compression, which is a medical emergency requiring corticosteroids to reduce edema and immediate radiotherapy [78]. Localized EMH responds promptly to low doses of gamma irradiation (100–150 cGy). AML occurs in approximately 20% of PMF patients over the first 10 years of disease [79].

10.5 Prognosis

Median survival in both ET and PV exceeds 15 years and the 10-year risk of developing either myelofibrosis (MF; <4% and 10%, respectively) or acute myeloid leukemia (AML; <2% and 6%, respectively) is relatively low

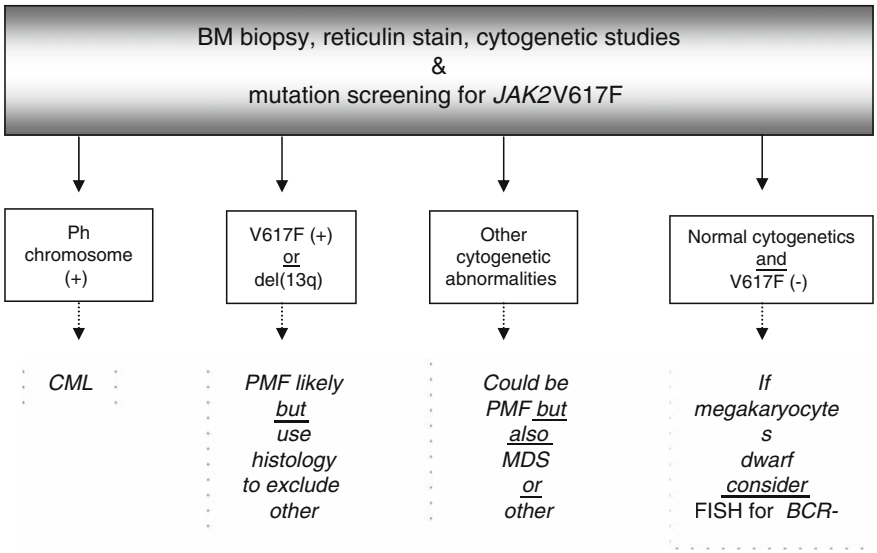

Fig. 10.3 Diagnostic algorithm for suspected primary myelofibrosis. (with permission from Tefferi and Vardiman, Leukemia 2008)[1]Key: PMF, primary myelofibrosis; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; FISH, fluorescent in situ hybridization; Ph, Philadelphia; BM, bone marrow; V617F, *JAK2*V617F

[34, 80, 81]. Compared to both PV and ET, PMF has a significantly worse prognosis with a median survival of 6 years and 10-year risk of AML estimated at 20% [82, 83].

Several studies have consistently identified advanced age (>60 years) and thrombosis history as risk factors for thrombosis in both PV [81, 84, 85] and ET (Table 10.4) [34, 63, 86]. In ET, two recent large single institutional studies (n = 322 and n = 439, respectively) [34, 35] confirmed the prothrombotic effect of advanced age (≥60 years) and history of thrombosis, although the latter association was significant in regards to arterial but not venous events in one of the two studies [34]. In addition, both studies identified leukocytosis ($\geq 15 \times 10^9$/L in one study [34] and $>8.7 \times 10^9$/L in the other) [35], but neither thrombocytosis nor the presence of *JAK2*V617F, as an additional independent risk factor for thrombosis. Similarly, the presence of cardiovascular risk factors did not modify thrombosis risk in one of the two studies [34], as well as in another recent study [87].

In PV, a series of reports from the European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) group have addressed multiple clinical issues including thrombotic complications. In their most recent report (n = 1638), Landolfi and colleagues, on behalf of ECLAP, confirmed the strong association between advanced age and thrombosis and, in addition, identified leukocytosis ($>15 \times 10^9$/L as opposed to $\leq 10 \times 10^9$/L) as an independent

Table 10.2 Thrombotic, hemorrhagic, and microvascular events in polycythemia vera (PV) and essential thrombocythemia (ET) reported at diagnosis (references are given in the text). (With permission from Tefferi and Elliott. Seminars in Thrombosis and Haemostasis 2007) [160]

ET	n	Major thrombosis (%)	Major arterial thrombosis[a] (%)	Major venous thrombosis[a] (%)	MVD (%)	Total bleeds % (major%)
Fenaux, 1990	147	18	83	17	34	18 (4)
Cortelazzo, 1990	100	11	91	9	30	9 (3)
Colombi, 1991	103	23.3	87.5	12.5	33	3.6 (1.9)
Besses, 1999	148	25	NA	NA	29	6.1 (NA)
Jensen, 2000	96	14	85	15	23	9 (5.2)
Chim, 2005	231	13	96.7	3.3	5.6	3 (1.7)
Wolanskyj, 2006	150	21.3	NA	NA	13.3	9.3
Campbell, 2005	776	9.7	82.7	17.3	NA	NA
Carobbio, 2006	439	29.4	68.2	31.8	NA	NA
PV						
GISP[b], 1995	1213	34	~66[b]	~33[b]	NA	NA
Passamonti, 2000	163	34	64	36	24	3 (NA)
Marchioli, 2005	1638	38.6	~75	~25	5.3	8.1 (4.8)

MVD, microvascular disturbances; NA, not available
[a]% of total major thrombotic events
[b]Estimate per Gruppo Italiano Studio Policitemia (GISP)

Table 10.3 Thrombotic and hemorrhagic events in polycythemia vera (PV) and essential thrombocythemia (ET) reported at follow-up (references are given in the text). (With permission from Tefferi and Elliott. Seminars in Thrombosis and Haemostasis 2007) [160]

ET	n	Major thrombosis (%)	Major arterial thrombosis (%)[a]	Major venous thrombosis (%)[a]	Total bleeds % (major %)	% of deaths from hemorrhage	% of deaths from thrombosis
Fenaux, 1990	147	13.6	86	14	NA (0.7)	0	25
Cortelazzo, 1990	100	20	71	29	NA (1)	0	100
Colombi, 1991	103	10.6	91	9	8.7 (5.8)	0	27.3
Besses, 1999	148	22.3	94	6	11.5 (4.1)	0	13.3
Jensen, 2000	96	16.6	69	31	13.6 (7.3)	3.3	16.7
Chim, 2005	231	10	91.3	8.7	6.5 (5.2)	10	10
Passamonti, 2004	435	10.6	71.7	28.3	NA	1	26
Wolanskyj, 2006	150	30.7	NA	NA	10	NA	NA
Campbell, 2005	776	8	74.2	25.8	4.1 (3.5)		
Carobbio, 2006	439	17.8	65.4	34.6	NA	NA	NA
PV							
GISP[b], 1995	1213	19	62.5	37.5	NA	2.6	29.6
Passamonti, 2000	163	18.4	80	15	NA (1.8)	6	19
Marchioli, 2005	1638	13.4	57.1	42.9	2.9 (0.8)	4.3	41
Passomonti, 2004	396	8.1	59.4	40.6	NA	2	20

[a]Percent of total major thrombotic events
[b]GISP, Gruppo Italiano Studio Policitemia.

Table 10.4 Current management and risk stratification in essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF)

			PMF	
Risk Categories	ET	PV	Age < 50 years	Age ≥ 50 years
Low	Low-dose aspirin	Low-dose aspirin + Phlebotomy	Observation *or* Experimental drug therapy	Observation *or* Experimental drug therapy
Low but with extreme thrombocytosis[a] for ET and PV	Low-dose aspirin[b]	Low-dose aspirin[b] + Phlebotomy	Experimental drug therapy *or* RIC[c] transplant	Experimental drug therapy *or* Conventional drug therapy
Intermediate for PMF				
High	Low-dose aspirin + Hydroxyurea	Low-dose aspirin + Phlebotomy + Hydroxyurea	Experimental drug therapy *or* Full transplant	Experimental drug therapy *or* RIC[c] transplant

[a]Extreme thrombocytosis is defined as a platelet count of 1000×10^9/L or more
[b]Clinically significant acquired von Willebrand syndrome (ristocetin co-factor activity <30%) should be excluded before the use of aspirin in patients with a platelet count of over 1000×10^9/L
[c]RIC, reduced intensity conditioning

Risk stratification for ET and PV:

High risk: Age ≥60 years *or* previous thrombosis

Low-risk: Neither of the above

Risk stratification of PMF according to the Mayo Prognostic Scoring System:[161]

(One point each for hemoglobin <10 g/dL, leukocyte count <4 or >30×10^9/L, platelet count <100×10^9/L, or monocyte count ≥1×10^9/L)

Low-risk: score 0

Intermediate-risk: score 1

High-risk: score ≥2

predictor of myocardial infarction [36]. History of arterial or venous events predicted recurrence of a similar vascular event. In contrast, neither the platelet count nor the hematocrit level affected thrombosis risk. Similarly, controlled prospective studies are needed to clarify the prognostic relevance of hereditary and acquired causes of thrombophilia [88], pattern of X chromosome inactivation in granulocyte-derived DNA (i.e., monoclonal vs polyclonal) [89–92], and altered PRV-1, platelet Mpl, or endogenous erythroid colony (EEC) expression [90].

Current evidence is inconclusive regarding the prognostic relevance of *JAK2* or *MPL* mutations in MPNs. In ET, overall or leukemia-free survival does not appear to be affected by either the presence of *JAK2*V617F or its allele burden [28, 29]; the impact on the risk of thrombosis or fibrotic transformation is less clear [24, 28, 29, 93]. Equally unclear is the prognostic relevance of *JAK2*V617F allele burden in PV where a higher mutant allele burden is implicated by some but not by others as an adverse prognostic factor for fibrotic transformation, thrombosis and need for chemotherapy [23–25,27]. In PMF, *JAK2*V617F presence was associated with inferior survival in one but not in another study [32, 94]. Similarly divergent results were reported in terms of leukemic transformation rate and need for chemotherapy or splenectomy [33, 95]. The most recent study on the subject matter revealed shortened overall and leukemia-free survival in PMF patients with lower as opposed to higher quartile *JAK2*V617F allele burden [95].

10.6 Treatment

10.6.1 Polycythemia Vera and Essential Thrombocythemia

Controlled studies have shown significant reductions in the incidence of thrombotic complications in patients with PV treated with low-dose aspirin [43] and in high-risk patients with ET treated with hydroxyurea [45]. Also, there is compelling, although not controlled, evidence to support the use of phlebotomy in all patients with PV [96] and hydroxyurea in those with high-risk disease [84]. Taken together, current recommendations for treatment in PV include phlebotomy and low-dose aspirin in all patients and the addition of hydroxyurea in high-risk disease (Table 10.4) [97]. In this regard, it is generally recommended but not mandated to keep the hematocrit level below 45% in men and 42% in women during phlebotomy for PV [98]. This treatment strategy, with the exception of phlebotomy, also applies to ET – low-dose aspirin in all patients [99–101] and the addition of hydroxyurea for high-risk disease [102, 103]. Finally, new evidence suggests that aspirin therapy in PV and ET might be most effective in preventing strokes whereas cytoreductive therapy and systemic anticoagulation might be needed for minimizing the risk of coronary event and DVT, respectively [87].

The use of aspirin in both PV and ET requires the absence of clinically-relevant acquired von Willebrand syndrome (AvWS), which might occur in patients with extreme thrombocytosis (platelet count $>1000 \times 10^9$/L) [104]. On the other hand, extreme thrombocytosis neither defines high-risk disease nor warrants the use of cytoreductive therapy [105]. The frequently cited association of extreme thrombocytosis with gastrointestinal bleeding is based on anecdotal observation and may, in some instances, be attributed to occult AvWS [106].

Very few studies in PV or ET have directly compared the efficacy of other cytoreductive agents with that of hydroxyurea. In ET, hydroxyurea (plus aspirin) was shown to be superior to anagrelide (plus aspirin) in terms of preventing arterial thrombosis and anagrelide performed better in terms of venous thrombosis; in addition, anagrelide therapy was less tolerated and was associated with significantly more occurrences of severe hemorrhage and fibrotic transformation [44]. In PV, patients randomized to treatment with hydroxyurea or pipobroman exhibited similar survival, thrombosis risk and leukemic transformation rate [107]. However, treatment with pipobroman was associated with a lesser incidence of transformation into post-PV MF [107]. Several single arm studies have confirmed the efficacy of pipobroman [68, 108–110] or busulfan [111–113] in both PV and ET. Single agent activity, sometimes associated with modest reductions in *JAK2*V617F allele burden, has also been demonstrated for alfa interferon (α-IFN) in PV and ET [114–119].

There is an increased rate of first-trimester miscarriages (approximately 30%) in both ET and PV [120–122] and a recent study suggested that this risk might be higher in *JAK2*V617F-positive patients [121]. However, there is no controlled evidence to suggest that specific treatment influences outcome [120, 123]. Other pregnancy-associated complications in ET and PV are infrequent and platelet count usually decreases substantially during the second and third trimesters [123]. Therefore, at present, low-risk pregnant patients with ET or PV might be managed the same way as their non-pregnant counterparts – no cytoreductive therapy and aspirin use is optional. In high-risk disease, α-IFN is the drug of choice in women of childbearing age wishing to be pregnant, because of the theoretical risk of teratogenicity associated with the use of other cytoreductive agents [115].

10.6.2 Myelofibrosis

At present, only allogeneic hematopoietic stem cell transplantation (ASCT) is considered potentially curative in MF [124]. In this regard, both myeloablative and reduced intensity conditioning (RIC) strategies have been employed. Regarding the former, a retrospective study of 66 patients revealed 5-year survival of 62% in patients younger than 45 years of age and 14% in those that were older [125], although other investigators have reported better survival figures in older patients [126]. In the most recent communication of RIC transplant in MF, 1-year mortality was 19% and 32% of the patients

experienced chronic graft-versus-host disease. The 3-year overall survival, event-free survival, and relapse rate were 70%, 55%, and 29% [127]. Taken together, it is reasonable to consider ASCT in high-risk MF: full myeloablative conditioning in patients below 45 years of age and RIC in older patients (Table 10.4). The decision in all other instances should be individualized and balanced against possible participation in experimental drug therapy (Table 10.4).

Drug therapy in PMF is usually considered in the presence of either symptomatic anemia or splenomegaly. Conventional therapy for the former include androgen preparations [128], prednisone [128], erythropoiesis stimulating agents (ESAs) [129–131] and danazol [132, 133]. Also, low-dose thalidomide in combination with prednisone has recently been identified as an effective drug for MF-associated anemia, thrombocytopenia, and splenomegaly with an approximately 50% overall response rate [134–138]. Lenalidomide, a thalidomide analog, has also been evaluated in MF where 20–30% response rate in both anemia and splenomegaly were documented [139]. Lenalidomide response rates were higher and quality of responses most impressive in MF patients with the del(5q) abnormality [140].

Hydroxyurea is the current drug of choice for controlling splenomegaly, leukocytosis, or thrombocytosis in PMF [141]. Other drugs that have been used in a similar setting include busulfan [142], melphalan [143] and 2-chlorodeoxyadenosine [144]. In contrast, alfa interferon has limited therapeutic value in MF [145]. Drug-refractory symptomatic splenomegaly may necessitate splenectomy that often alleviates mechanical symptoms and may also benefit approximately 25% of patients with transfusion-dependent anemia [146]. However, splenectomy in MF is associated with an approximately 9% procedure-related mortality and up to 25% of patients may experience accelerated hepatomegaly and extreme thrombocytosis after splenectomy [146, 147]. Radiation therapy is most useful in the treatment of non-hepatosplenic extramedullary hematopoiesis [148–151].

10.6.3 Anti-JAK2 Investigational Therapy

In less than 3 years from the first description of *JAK2*V617F [11–14], and in accordance with the CML-imatinib paradigm [152–154], small molecule JAK2 inhibitor drugs have been developed and are already undergoing clinical trials [155]. Among these, some are *JAK2* selective ATP-mimetics (e.g., TG101209, TG101348, INCB018424, XL019) and others were developed with other targets in mind but later were found to be potent JAK2 inhibitors as well: the FLT3 inhibitor CEP-701, certain aurora kinase inhibitors and the histone deacetylase inhibitor ITF2357. These JAK2 inhibitors have been reported to have in vitro or in vivo (i.e., mouse models) activity against *JAK2*V617F-driven cell proliferation and signal transduction. Among the aforementioned JAK2 inhibitor ATP mimetics, INCB018424, XL019, CEP-701 and TG101348 are currently

undergoing clinical trials in patients with advanced stages of PMF (regardless of *JAK2* or *MPL* mutation status), post-PV/ET MF, PV, and *JAK2*V617F-positive ET [156–159]. At present, it is too early to make valid statements about effect and toxicity of these drugs.

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Chapter 11
Chemotherapy-Associated Thrombosis

Aneel A. Ashrani and S. Vincent Rajkumar

11.1 Introduction

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a multifactorial disease, involving complex interactions between individuals (including their hemostatic system and genetic predispositions) and their environmental exposures. Active malignancy is a well recognized risk factor for VTE and accounts for almost 20% of incident VTE events occurring in the community [1], with chemotherapy independently adding to that risk [2, 3]. As compared with the general population, the risk of VTE is increased fourfold in patients with cancer and this risk is further elevated to more than sixfold when these patients are receiving chemotherapy [2]. Moreover, there is a twofold increase in recurrent VTE in patients with malignancy and a fourfold increase in this risk for those receiving chemotherapy [2]. In addition, the risk of VTE varies by cancer type and stage [3–6]. The type of chemotherapy administered, hormonal treatments, immunomodulating and anti-angiogenesis agents (e.g., thalidomide, lenalidomide, bevacizumab), and supportive therapy with hematopoietic growth factors like recombinant human erythropoietins have been implicated in alterations in hemostasis and with increased VTE risk.

VTE in cancer patients is associated with significant morbidity. These patients may have their active anti-neoplastic therapy delayed or modified, and often require chronic anticoagulation. Compared to VTE patients without cancer, individuals with cancer and VTE have both a higher risk for hemorrhage with anticoagulant therapy and of recurrent VTE [7–9]. Moreover, patients diagnosed with cancer at the time of VTE diagnosis have a poorer survival in contrast with age-, gender-, cancer type-, and year of diagnosis-matched cancer patients with no history of VTE [10], and VTE has been noted

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to be a leading cause of death in ambulatory cancer patients receiving chemotherapy [11, 12]. In this review, antineoplastic therapy associated thrombosis in cancer patients will be discussed. As the best evidence supporting a causal relationship between antineoplastic agents and VTE comes from breast cancer and multiple myeloma clinical trials, special emphasis will be given to these neoplasms.

11.2 Pathogenesis/Pathophysiology of Chemotherapy-Associated Thrombosis

The pathogenesis of chemotherapy-associated thrombosis is poorly understood. However, there are several different mechanisms via which chemotherapy can potentially provoke a prothrombotic state [13] (see Fig. 11.1). These drugs can directly damage the vascular endothelium [14–19], increase procoagulant proteins and/or reduce endogenous anticoagulants [20–23]. Chemotherapy can also induce tumor and endothelial cell apoptosis and cytokine release, which in turn can lead to increased expression of the endothelial and monocyte tissue factor (TF), the physiologic initiator of coagulation [24–27]. Finally, chemotherapy can lead to activation of platelets [28], and induce expression of monocyte TF [29].

11.3 Therapy-Associated Thrombosis in Breast Cancer

The increased risk of thrombosis in breast cancer patients receiving hormonal and/or chemotherapy is well characterized. Patient's age, post-menopausal status, recent surgery/mastectomy, high body mass index, and central venous catheter are additional risk factors impacting the risk of thrombosis in this population [30]. In patients with early stage breast cancer not receiving adjuvant therapy, the risk for thrombosis is low (<1%) [31, 32], compared to up to 17.6% in women with metastatic stage IV disease receiving therapy [33].

11.3.1 Tamoxifen

Tamoxifen is an orally active selective estrogen receptor modulator (SERM) used in treating pre- and post-menopausal women with both early and advanced estrogen receptor positive (ER+) breast cancer. Tamoxifen competitively binds to the estrogen receptors on ER+ breast cancer cells, producing a nuclear complex that decreases DNA synthesis and thus inhibits estrogen effects. The risk of thrombosis with tamoxifen may be related to its role in reducing the levels of protein C, protein S and antithrombin [21, 34, 35], and inducing activated protein C resistance [35]. Data from randomized clinical

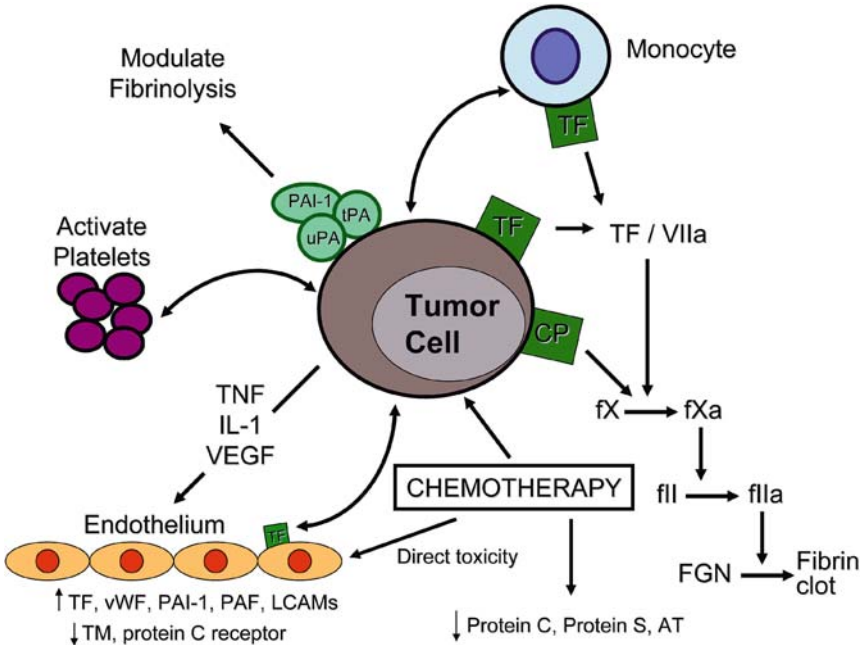

Fig. 11.1 Pathophysiology of chemotherapy-associated thrombosis, AT: Antithrombin, CP: Cancer procoagulant, FGN: Fibrinogen, IL-1: Interleukin-1, LCAMs: Leucocyte adhesion molecules, PAF: Platelet activating factor, PAI-1: Plasminogen activator inhibitor-1, TF: Tissue factor, TM: thrombomodulin, TNF: Tumor necrosis factor, t-PA: Tissue plasminogen activator, u-PA: Urinary plasminogen activator, VEGF: Vascular endothelial growth factor, vWF: von Willebrand factor

trials in women with node negative, estrogen receptor positive breast cancer, indicate that the 5-year incidence of VTE in these women with early stage breast cancer treated with either placebo, tamoxifen or tamoxifen plus chemotherapy is 0.2%, 0.9% and 4.2% respectively [31, 36]. In the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (NSABP P-1), the rates of DVT and PE in older women receiving tamoxifen were increased 1.6- and 3-fold respectively [37]. In the International Breast Cancer Intervention Study (IBIS-1), the risk of VTE was 2.1-fold higher in women treated with tamoxifen compared to placebo [38]. In a pooled analysis of 13 NSABP breast cancer prevention and treatment trials, the risk of DVT, PE and superficial thrombophlebitis was increased 2- to 3-fold in those treated with tamoxifen, and was increased 11- to 15-fold in those treated with tamoxifen plus chemotherapy [39]. In another systematic review, the relative risk of VTE was noted to be 3- to 8-fold greater in women who were treated with concurrent tamoxifen and chemotherapy than those on tamoxifen alone, 3- to 5-fold greater than women treated with chemotherapy alone, and 20-fold greater than women taking placebo or being observed [40].

11.3.2 Aromatase Inhibitors

Aromatase inhibitors (AI) are a class of drugs used in the treatment of breast cancer in postmenopausal women. They inhibit aromatase, a cytochrome P450 enzyme which catalyzes the conversion of androgens into estrogens. As peripheral conversion of androgens is the main source of estrogen in postmenopausal women, and AIs do not block ovarian estrogen synthesis, these agents are used in managing ER + breast cancer in postmenopausal women. In the Arimidex, Tamoxifen Alone or in Combination (ATAC) trial, the risk of DVT or PE in women receiving anastrozole alone was lower (1.0%), compared to those receiving tamoxifen (1.7%) or a combination of anastrozole and tamoxifen (2.0%; $p = 0.02$) [41]. However, the absolute risk of VTE (approximately 3.6 per 1000 individuals per year) with anastrozole is still greater compared with the overall estimated risk in untreated, healthy women (1.1 per 1000 individuals per year) [42].

11.4 Therapy-Associated Thrombosis in Multiple Myeloma

The cumulative incidence of VTE in individuals with monoclonal gammopathy of unknown significance (MGUS) has been estimated at between 6.1 and 7.5% [43, 44]. However, it is unclear whether the VTE risk is truly increased with MGUS, as one could speculate that the VTE risk may solely be related to the underlying medical problems that prompted testing for monoclonal paraproteinemia. In our population-based studies, we have not found an increase risk of thrombosis in individuals with MGUS compared with those without MGUS. Similarly, the incidence of VTE in patients with multiple myeloma (MM) is difficult to estimate and ranges from 3% to 10% [44, 45]. Data from clinical trials where MM patients were treated with dexamethasone alone [45] or melphalan/prednisone [46, 47], show that the risk of VTE is 2–4%. In general, the baseline risk of VTE within the first 4 months of therapy in newly diagnosed myeloma in the absence of immunomodulatory agents or doxorubicin is less than 5%. The risk appears lower in patients with relapsed, refractory myeloma.

11.4.1 Thalidomide

Thalidomide was initially prescribed during the late 1950s and early 1960s to pregnant women as an antiemetic to combat morning sickness and as a sedative-hypnotic. It was withdrawn from the market in 1962 due to its severe teratogenic effects. However, thalidomide's antiangiogenic and immunomodulatory properties were subsequently discovered, and led to clinical trials in

myeloma and several other malignancies. Currently thalidomide is approved in the United States for the treatment of patients with newly diagnosed MM, and for patients with erythema nodosum leprosum. In addition, it has been used off-label for the management a number of other malignant and non-malignant disorders. Thalidomide inhibits the synthesis of tumor necrosis factor-α (TNF-α), blocks activation of nuclear factor-$\kappa\beta$ kinase (NF-$\kappa\beta$) activity, co-stimulates proliferation of T-cells (especially CD8+ T cells), stimulates interleukin-2 (IL-2) and interferon γ production (IFN-γ) [48], and downregulates expression of cell surface molecules like VCAM-1, E-selectin, and L-selectin [49].

Thalidomide administered as a single-agent does not increase the risk of VTE when compared to other treatment modalities like dexamethasone or melphalan/prednisone; in patients with newly diagnosed MM the incidence of VTE was 3–4% [50–52] and in the relapsed/refractory MM setting, it was 2–4% [53–55]. In contrast, the combination of thalidomide with dexamethasone significantly increases the risk of VTE to 14–26% in newly diagnosed MM patients [45, 52, 56, 57] and to 2–8% in relapsed/refractory patients [58, 59]. A similar increased risk is seen when thalidomide is used in combination with melphalan +/- steroids (either prednisone or dexamethasone), 10–20% in newly diagnosed patients [46, 47, 60] and 11% in relapsed/refractory patients [61]. The risk of VTE further increases when thalidomide is combined with doxorubicin-containing regimens, [62–64] up to 58% in a phase II study which included both newly diagnosed and relapsed multiple myeloma patients [65] (see Table 11.1).

The increased risk of thrombosis with thalidomide containing regimens is predominantly seen in the first few months following the initiation of therapy, with the median time to onset of a thrombotic event being around 3 months. It is unclear why the risk of VTE is higher in patients with newly diagnosed MM as compared to those with relapsed MM. To further characterize the risk of VTE in patients with MM treated with thalidomide, Zangari et al. conducted a multivariate analysis on 535 patients treated either with thalidomide in combination with multi-agent chemotherapy or with dexamethasone only [66]. Overall, the incidence of VTE was 15%. Doxorubicin containing regimens were associated with a 4.3-fold increase in VTE, while newly diagnosed disease was associated with a 2.5-fold increase of VTE and chromosome 11 abnormalities were associated with a 1.8-fold increased risk. Interestingly, unlike other malignancies, the MM patients who develop VTE may not have an adverse survival as compared to similarly staged MM patients without VTE [66, 67].

A high VTE rate with thalidomide in combination with chemotherapy has also been reported in other malignancies. In a phase II trial of thalidomide with gemcitabine and continuous infusion fluorouracil in the treatment of metastatic renal cell carcinoma, the risk of VTE was noted to be 43% [68].

Table 11.1 Therapy associated venous thromboembolism (VTE) in multiple myeloma (MM)

	VTE incidence (%)								
	No thromboprophylaxis				Thromboprophylaxis				
Treatment regimen	Newly diagnosed MM	References	Relapsed / refractory MM	References	LMWH	Fixed low-dose warfarin	Dose adjusted warfarin (INR 2–3)	Aspirin	References
Dexamethasone	3	[45]			–	–	–	–	–
Melphalan + prednisone	2–4	[46, 47]			–	–	–	–	–
Bortezomib ± dexamethasone	0	[138]	0	[139]	–	–	–	–	–
Thalidomide	3–4	[50–52]	2–4	[53–55]	–	–	–	–	–
Thalidomide + dexamethasone	14–26	[45, 52, 56, 57]	2–8	[58, 59]	8[a]	13–25	8[a]		[51, 57, 140]
Thalidomide + melphalan	10–20	[46, 47, 60]	11	[61]	3				[46]
Thalidomide + doxorubicin	10–27	[62–64]	58[b]	[65]	9			18	[65, 127]
Thalidomide + cyclophosphamide	3–11	[141, 142]	4–8	[143–146]					
Thalidomide + multi agent chemo	16–34	[129, 147]	15	[148]	15–24	31			[128, 129]

Table 11.1 (continued)

	VTE incidence (%)								
	No thromboprophylaxis				Thromboprophylaxis				
Treatment regimen	Newly diagnosed MM	References	Relapsed / refractory MM	References	LMWH	Fixed low-dose warfarin	Dose adjusted warfarin (INR 2–3)	Aspirin	References
Lenalidomide	–	–	0	[70]	–	–	–	–	–
Lenalidomide + dexamethasone	8–75	[76, 149]	9–15	[73, 74]	–	–	–	3–14	[75, 76]
Lenalidomide + melphalan								6	[130]
Lenalidomide + doxorubicin	–	–	–	–	–	–	–	9	[131]
Lenalidomide + cyclophosphamide	–	–	14	[150]	–	–	–	–	–
Lenalidomide + bortezomib	0	[151]	0	[152]	–	–	–	4	[153]

– Data not available

[a]Patients were prescribed either therapeutic doses of LMWH or warfarin to maintain INR between 2.0 and 3.0 [140]

[b]Trial included both newly diagnosed and relapsed multiple myeloma patients

11.4.2 Lenalidomide

The exploration of the antiangiogenic and immunomodulatory activities of thalidomide has led development of thalidomide analogs. In 2005, lenalidomide (Revlimid) was approved in the United States for treatment of relapsed or refractory multiple myeloma and myelodysplastic syndromes with deletion of long arm of chromosome 5. Lenalidomide is substantially more potent than thalidomide and has fewer gastrointestinal and neurologic side effects, although it has greater myelosuppression. Lenalidomide's mechanism of action is similar to that of thalidomide, and it is a potent immunomodulator. It acts by stimulating T-cell proliferation, increasing IL-2 and IFN-γ production, and augmenting the cytotoxic activity of natural killer cells [69]. It inhibits the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-12 and augments the anti-inflammatory cytokine, IL-10. It also inhibits angiogenesis by decreasing secretion of VEGF and basic fibroblast growth factor (FGF) [69].

As with thalidomide, there were no reported cases of VTE in early phase studies of lenalidomide when used as a single agent for the treatment of relapsed or refractory multiple myeloma, myelodysplastic syndromes and advanced solid tumors [70–72]. However, when administered concurrently with dexamethasone, the incidence of VTE was higher, ranging from 9% to 15% in phase III studies [73, 74]. The reasons for the high VTE rate seen with thalidomide and lenalidomide in combination with steroids are unknown, although endothelial damage may be playing a role. In a Mayo Clinic study, the VTE rate associated with lenalidomide plus dexamethasone was only 3% when daily aspirin 80–325 mg prophylaxis was given concomitantly [75].

The dose of dexamethasone and concomitant erythropoietin may be risk factors for VTE associated with lenalidomide. In a phase III trial of lenalidomide plus high-dose dexamethasone (40 mg 4 times a week for 3 weeks in a 4-week cycle) vs lenalidomide plus low-dose dexamethasone (40 mg once a week), the risk of thrombosis was significantly higher in the high-dose dexamethasone group as compared to the low-dose dexamethasone group (25% vs 9% respectively) [76]. In phase III trials investigating lenalidomide plus dexamethasone vs placebo plus dexamethasone, concomitant administration of erythropoietin increased the risk of thrombosis in patients receiving lenalidomide [77].

11.5 Other Anti-Neoplastic Agents Associated with VTE

11.5.1 Cisplatin-Based Regimens

Cisplatin is used to treat various neoplasms, including sarcomas, carcinomas such as small cell lung cancer and ovarian cancer, lymphomas and germ cell

tumors. Platinum complexes are formed in cells, which bind to DNA and lead to DNA cross-linking, resulting in apoptosis. The exact mechanism by which Cisplatin promotes thrombosis is unclear. In vitro data indicate that it induces platelet activation, increases monocyte TF activity, and elevates von Willebrand factor [13].

Cisplatin based combination chemotherapy regimens have been associated with both arterial and venous thrombotic events. Thrombosis has been observed in 8.4–17.6% of patients with urothelial transitional cell carcinoma, germ cell tumors, advanced (stage III or IV) non-small cell lung cancer and ovarian cancer [78–81]. A thrombosis rate of 16.7% was reported for invasive cervical cancer patients treated with concurrent radiation therapy and low-dose weekly cisplatin [82].

11.5.2 L-Asparaginase

L-Asparaginase is an enzyme used for the management of acute lymphoid leukemia (ALL). L-Asparaginase catalyzes the hydrolysis of asparagine to aspartic acid, thus depleting plasma asparagine and in turn depriving the leukemic cells of circulating asparagine. Leukemic cells are unable to synthesize asparagine. Normal cells, on the other hand, are able to make their own asparagine. By depleting plasma asparagine, L-asparaginase inhibits protein synthesis, including synthesis of procoagulant factors II, VII, VIII, IX, X, XI, fibrinogen and α-2 antiplasmin [83, 84]. As a result, the prothrombin time, activated partial thromboplastin time and thrombin time are prolonged. However, the risk of bleeding complications is low. More importantly, the naturally occurring anticoagulant proteins, antithrombin, protein C, protein S and plasminogen are also decreased with L-asparaginase use [84–86]. Following the cessation of L-asparaginase therapy, recovery of the procoagulant proteins (fibrinogen, factors VII, IX, X and XI) occurs sooner than the recovery of anticoagulant proteins; thus, the hemostatic balance is shifted towards a prothrombotic predisposition.

L-Asparaginase is associated with a 1–2% incidence of thrombosis in children and around 4–14% in adults [13, 33, 87, 88]. However, in a prospective study of children treated for ALL with L-asparaginase, a significantly higher rate (36.7%) of thrombosis was reported [89]. Most of the thrombotic episodes were asymptomatic and occurred in the upper extremities, suggesting that the use of central venous access catheters was a contributory factor. Other case series have reported a high proportion of CNS complications (either thrombosis or hemorrhage) [90, 91]. Thus, monitoring of coagulation parameters is recommended in individuals receiving L-asparaginase therapy along with replacement of deficient coagulation factors (especially antithrombin).

11.5.3 5-Fluorouracil

5-Fluorouracil (5-FU), an antimetabolite, is a pyrimidine analog, and inhibits thymidylate synthase. Thymidylate synthase methylates deoxyuridine monophosphate (dUMP) into thymidine monophosphate (dTMP), which is a nucleotide required for DNA replication. Furthermore, as a pyrimidine analogue, 5-FU is transformed inside the cell into cytotoxic metabolites which are then incorporated into DNA and RNA, inducing cell cycle arrest and apoptosis by inhibiting the cell's ability to synthesize DNA.

The prothrombotic property of 5-FU is related to a decrease in the levels of protein C and an increase in thrombin activity, leading to increased levels of fibrinopeptide A, a proteolytic product of fibrinogen produced by thrombin [92, 93]. Exposure to 5-FU also results in endothelial damage that is accompanied by platelet accumulation and fibrin formation [17]. The cumulative incidence of VTE in patients with colorectal cancer treated with fluorouracil and leucovorin is estimated at 15–17%, especially when used in combination with the hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor (GM-CSF) [20, 94].

11.5.4 Bevacizumab

Bevacizumab (Avastin) is a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), and inhibits tumor growth by inhibiting angiogenesis. It is used in combination with chemotherapy for the treatment of metastatic breast, colorectal and non-small cell lung cancer. Treatment with bevacizumab is associated with an increased risk of arterial and venous thromboembolic events, particularly in patients over 65 years of age or with a history of thromboembolic events. In addition, these patients are predisposed to hemorrhagic complications. The predisposition to both bleeding and thrombosis following VEGF inhibition reflects the diverse actions of VEGF on vascular walls. VEGF stimulates endothelial cell proliferation, promotes endothelial cell survival and helps maintain vascular integrity [95, 96]. Thus, inhibition of VEGF could decrease the regenerative capacity of endothelial cells and lead to vessel wall defects that expose the pro-coagulant phospholipids on the luminal surface or underlying matrix, leading to thrombosis or hemorrhage [97]. VEGF also increases the production of nitric oxide (NO) and prostacyclin (PGI_2), suppresses pathways involved in endothelial cell activation and apoptosis, and inhibits proliferation of vascular smooth muscle cells [98]. Therefore, VEGF inhibition and the resulting increase in TF expression, along with a reduction in endothelial NO and PGI_2, may predispose to thromboembolic events.

A phase II randomized trial of patients with metastatic colorectal cancer comparing 5-FU and leucovorin with or without bevacizumab revealed that both the thrombotic and hemorrhagic complications were significantly higher

in the bevacizumab-treated patients [99]. Thrombotic events occurred in 9% of the patients treated with 5-FU and leucovorin versus 19% of the patients treated with 5-FU, leucovorin and bevacizumab. However, in a large phase III trial comparing irinotecan, fluorouracil and leucovorin with or without bevacizumab for the treatment of metastatic colorectal carcinoma, the thromboembolic rates were similar (19.4% and 16.2%, respectively) [100]. The relatively high frequency of thrombosis in these patients is probably due to the combination chemotherapy, with a modest additional contribution by bevacizumab. Another phase II trial on patients with gastric cancer utilizing bevacizumab with irinotecan and cisplatin for the treatment of patients with metastatic or unresectable gastric cancer noted a 25% incidence in VTE [101]. Another phase III randomized trial conducted on patients with metastatic breast cancer who were treated with paclitaxel with or without bevacizumab reported low rates of thromboembolic events (2.1% in the bevacizumab arm vs 1.5% in the control arm), but the incidence of cerebrovascular ischemia was significantly higher in the bevacizumab arm (1.9% vs 0%, respectively) [102]. In patients with metastatic non-small lung cancer, the risk of thrombosis does not appear to be increased by adding bevacizumab to chemotherapy; however it significantly increases the risk of hemorrhage [103].

11.6 Supportive Therapy with Hematopoietic Growth Factors and Risk for VTE

11.6.1 Recombinant Human Erythropoietins

Erythropoietin or EPO is a glycoprotein hormone synthesized by the kidney that is a cytokine for bone marrow erythrocyte precursors. Recombinant erythropoietin has been approved for the treatment of anemia associated with renal disease, chemotherapy-associated and zidovudine-associated anemia. It is also used to treat anemia associated with chronic inflammation/disease.

Erythropoietin use, especially in cancer patients undergoing chemotherapy, has been implicated with increased risk for VTE [104, 105]. In a meta-analysis, 7.5% of patients with cancer chemotherapy-associated anemia who were treated with either erythropoietin or darbepoetin developed VTE, compared to 4.9% control group (RR 1.57; 95% CI: 1.31–1.87) [105]. This increased risk is not limited just to cancer patients, but is also observed in other patient groups, such as those with chronic kidney disease [106] and end stage renal disease [107], especially those receiving recombinant human erythropoietin to achieve a hemoglobin target levels of 13–15 g/dL as compared to 10.5–11.5 g/dL.

The mechanism by which EPO promotes VTE is still under investigation. Erythropoietin has been reported to activate platelets and increase vWF, factor VIII and thrombin generation (measured indirectly by measuring thrombin-antithrombin complexes), and decreases protein C and protein S [108]. Moreover,

erythropoietin triggers signaling pathways in endothelial cells, increasing their thrombogenicity by inducing expression of tissue factor [109]. The increased risk of thrombosis is unlikely due to the higher hemoglobin, as the mean hemoglobin target is usually less than 13.5 g/dL, but may be secondary to the role of erythropoietin in inducing chronic inflammation and inhibiting fibrinolysis [110].

11.6.2 Leucocyte Growth Factors (G-CSF and GM-CSF)

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein produced predominantly by the endothelium and macrophages. It stimulates the bone marrow to produce and release granulocytes and stem cells into the circulation. It also enhances the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is another cytokine that stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes.

G-CSF may be prothrombotic, as it is associated with an increase in the markers of activation of coagulation, such as prothrombin fragment 1.2 (F1 + 2), TAT and D-dimer, suggesting increased thrombin and fibrin formation [111].

In addition, G-CSF and erythropoietin stimulate the release of PAI-1 from human umbilical vein endothelial cells [112].

The overall prevalence of venous and arterial thrombosis in cancer patients treated with GM-CSF is estimated at 4.2% and 1.2% for G-CSF [33]. There may be synergistic interaction for the use of the growth factors and site and type of cancer. Data from a prospective observational study of 3003 cancer patients who were treated with at least one cycle of chemotherapy and with a median follow-up of 2.4 months noted a 1.93% cumulative VTE incidence (0.8% per month) [113]. The highest incidence of VTE was noted in patients with upper gastrointestinal malignancies (2.3% per month), lung cancer (1.2% per month) and lymphoma (1.1% per month) ($p = 0.001$). Patients with these high VTE risk sites of cancer had a significantly increased risk of VTE associated with the use of growth factors during cycle 1 (VTE rate 5.9% vs 1.52% without growth factor use, $p = 0.0001$; OR 4.0 [95% CI: 1.8–8.7]). In contrast, the use of growth factors did not appear to increase the risk of VTE in patients with cancer in other sites (VTE rate of 1.31% with vs 1.42% without growth factor use) [113].

11.7 Other Agents

11.7.1 Corticosteroids

The prothrombotic effects of corticosteroids may be related to their action to increase coagulation factors VII, VIII, XI and vWF [114]. Furthermore, long-term steroid use leads to a hypercoagulable, hypofibrinolytic state by

suppressing tissue plasminogen activity and increasing PAI-1 synthesis in addition to increasing the procoagulant clotting factors [115].

High-dose corticosteroids, given for their anti-emetic activity, are an independent risk factor for VTE in patients with germ cell tumors (OR 3.5; 95% CI: 1.2–10.3) [79]. Furthermore, the combination of steroids with thalidomide or lenalidomide, with or without chemotherapy, is associated with an increased risk for thromboembolic events (see preceding section on MM).

Gemtuzumab ozogamicin (Mylotarg) is a monoclonal antibody to CD33 linked to a cytotoxic agent, calicheamicin and is used to treat acute myelogenous leukemia. It is associated with an increased risk for veno occlusive disease (VOD). Possible mechanisms for development of VOD include injury by free radicals due to glutathione deficiency and inflammatory cytokine-induced endothelial activation [84]. When used a single agent, the frequency of VOD is 0.9%. In contrast, the risk for VOD increases to 5% when this agent is combined with other chemotherapy, and reaches 19% following stem cell transplantation [116].

11.8 Prevention of VTE in Patients Receiving Chemotherapy

11.8.1 Prophylaxis in Solid Tumors

Pharmacologic VTE prophylaxis is very effective in reducing the risk of VTE for cancer patients hospitalized either for surgery or for medical illness [117–120], but is controversial for prevention of VTE in patients with solid tumors undergoing outpatient chemotherapy. Only one clinical trial thus far has demonstrated a benefit of VTE prophylaxis in cancer patients undergoing outpatient chemotherapy [121]. In this study, stage IV breast cancer patients were randomized to either low-dose warfarin (i.e., 1 mg daily for 6 weeks followed by dose adjustments to maintain the International Normalized Ratio [INR] between 1.3 and 1.9) or placebo [121]. The rate of thrombosis in the low-dose warfarin group was 0.7% vs 4.4% in the placebo group. The risk of bleeding was not statistically different in the two groups. However, none of the other clinical trials have shown reduced VTE events with pharmacologic prophylaxis of cancer patients undergoing chemotherapy on an outpatient basis [122–125], but there may be a survival advantage of using LMWHs in combination with chemotherapy. Thus, there is no clear role for administering routine thromboprophylaxis to all cancer patients undergoing outpatient chemotherapy [126].

11.8.2 Prophylaxis in Multiple Myeloma

In multiple myeloma, given the high risk of VTE in patients receiving combination regimens that include thalidomide or lenalidomide, all newly diagnosed

patients should be considered for thromboprophylaxis (Table 11.1). In one study, without any VTE prophylaxis, the thalidomide-dexamethasone combination for management of newly diagnosed multiple myeloma was associated with a 26% incidence of VTE [57]. The study protocol was then amended to include low-fixed-dose prophylactic warfarin (1.25 mg daily), and following that the incidence of VTE declined to 13%. However, in another study of thalidomide and dexamethasone, low-fixed-dose warfarin was administered to all study participants and the incidence of VTE was 25%, showing that low fixed-dose warfarin does not impact VTE risk [51].

In a randomized trial where melphalan, prednisone, and thalidomide were administered as initial therapy for newly diagnosed multiple myeloma, the first 65 patients who did not receive any anticoagulant prophylaxis had a 20% incidence of VTE, but this risk fell to 3% when the protocol was amended to include the low molecular weight heparin (LMWH) enoxaparin, 40 mg subcutaneously daily for the first 4 months of therapy [46]. Of note, all thromboembolic events occurred within 2 months after the discontinuation of enoxaparin. In another randomized study of patients with newly diagnosed multiple myeloma receiving vincristine-doxorubicin-dexamethasone (VAD) or thalidomide-doxorubicin-dexamethasone (TAD), patients randomized to the thalidomide arm were given prophylactic doses of the LMWH, nadroparin [127]. The incidence of VTE in the VAD group was 5% and 9% in the TAD group, with all events occurring in the first 6 months of therapy. In another study, patients with newly diagnosed or relapsed multiple myeloma received a combination of pegylated doxorubicin, vincristine, thalidomide, and dexamethasone, and the VTE incidence was 58% in the 19 patients who did not receive any VTE prophylaxis [65]. The study protocol was then amended and the ensuing 58 patients received aspirin 81 mg once daily, reducing the VTE incidence to 18% (HR 0.24; $p<0.001$).

In a phase III study, newly diagnosed myeloma patients were randomized to receive multi-agent chemotherapy including doxorubicin, dexamethasone, etoposide, cyclophosphamide and cisplatin with or without thalidomide [128]. Without anticoagulant prophylaxis, VTE rate was 14% in patients who received chemotherapy alone vs 34% in those who received chemotherapy plus thalidomide. The VTE risk remained unchanged by adding low, fixed-dose warfarin (1 mg/day) to the chemotherapy plus thalidomide arm (31%); however with the addition of enoxaparin (40 mg/day) to the chemotherapy plus thalidomide arm, the VTE risk declined to that of the chemotherapy alone arm (15% each). In another study, the VTE incidence without any prophylaxis in patients randomly assigned to multi-agent chemotherapy plus thalidomide vs no thalidomide was 34% vs 18%, respectively [129]. The protocol was subsequently amended and prophylactic LMWH was added to the chemotherapy plus thalidomide arm, but the VTE rate remained high (24%) compared to the chemotherapy alone arm (15%). These data demonstrate that low, fixed-dose warfarin prophylaxis provides no protection against VTE in multiple myeloma patients treated with thalidomide, but aspirin seems to the reduce the VTE risk

somewhat (although its mechanism in doing so remains unclear), and that LMWH provides the best protection, although in spite of its use, the risk of VTE remains quite high when thalidomide is used in combination with multi-agent chemotherapy.

As discussed earlier, in newly diagnosed multiple myeloma patients who were treated with lenalidomide and dexamethasone, and received concomitant aspirin (80–325 mg/day) prophylaxis, the rate of VTE was as low as 3% [75]. In a larger phase III trial of lenalidomide plus high-dose dexamethasone (40 mg four times a week for 3 weeks in a 4-week cycle) vs lenalidomide plus low-dose dexamethasone (40 mg once a week), the risk of thrombosis was higher in the high-dose dexamethasone group as compared to the low-dose dexamethasone group without any prophylaxis (23% vs 8% respectively), but was reduced to 14% and 5% respectively after the addition of aspirin [76]. Furthermore, the overall survival was noted to be better in the low-dose dexamethasone arm. The above data indicate that high-dose dexamethasone by itself is a major risk factor for VTE, and that aspirin prophylaxis, especially in patients treated with low-dose dexamethasone, may be reasonable. With aspirin prophylaxis, multiple myeloma patients treated with melphalan, prednisone, and lenalidomide had a VTE rate of 5.7% [130]. The use of aspirin (81 mg/day) in patients with relapsed or refractory multiple myeloma given lenalidomide, liposomal doxorubicin, vincristine and dexamethasone, was associated with a VTE rate of 9%, even though 85% of patients received erythropoietin therapy [131].

Although dose-adjusted warfarin therapy with a target INR of 2.0–3.0 and LMWH are both highly effective in the prevention of VTE in multiple myeloma patients receiving lenalidomide or thalidomide, they are cumbersome and require close monitoring, especially as these patients can become thrombocytopenic, are on multiple medications (that can interact with warfarin), and often have renal insufficiency (which can be an issue with LMWH). Moreover, elderly patients may have difficulty complying with these strategies and there is a financial burden, especially for those who are on Medicare and do not have a supplemental drug coverage plan. On the other hand, aspirin is a less effective but simpler option. The type of prophylaxis for prevention of VTE in myeloma patients receiving thalidomide or lenalidomide therapy is therefore based on the expected baseline risk of such thrombotic events. When low doses of steroids are used, especially in the absence of erythropoietin, the risk of VTE is less than 10%. Given this baseline risk, the choice of thromboprophylaxis is then personalized and modified depending on patient characteristics, and risk associated with a given treatment regimen and schedule. Although aspirin is appealing because of its convenience and ease of administration, the rate of thrombosis is relatively high, especially in patients treated with thalidomide-based combinations, and therefore is insufficient in high-risk patients. LMWH (at a dose equivalent to enoxaparin 40 mg daily) or dose-adjusted warfarin to maintain an INR between 2.0 and 3.0 should be considered in patients receiving thalidomide or lenalidomide who are treated with concomitant high-dose dexamethasone, doxorubicin, or multi-agent

chemotherapy, or those individuals who have additional VTE risk factors. The duration of prophylaxis may vary according to the length of treatment. In multiple myeloma, the vast majority of VTE occur within the first 6 months of therapy and almost all episodes are reported within the first 12 months [46, 128]. It may be reasonable to provide VTE prophylaxis for 4–6 months, while longer periods may be considered in the presence of additional patient- or treatment-specific risk factors [132].

11.8.3 Prophylaxis in Myeloproliferative Disease

Myeloproliferative disorders (MPD), especially polycythemia vera (PV) and essential thrombocythemia (ET), are associated with thrombosis [133–135]. In MPDs, arterial events are more common than venous thrombosis. At the time of diagnosis, the prevalence of thrombosis is ~34–39% in PV and 10–29% in ET. During the follow-up phase of MPD, the prevalence of thrombosis in PV is 8–19% and 8–31% for ET. MPD patients older than 60 years and those with prior history of thrombosis are at increased risk for recurrent thrombosis. Aspirin in PV was very effective in reducing nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes [136]. However, the risk of VTE was not significantly lower with aspirin use (1.6% vs 3.8%; $p = 0.28$), and cytoreductive therapy and/or anticoagulation might be necessary to reduce its risk.

11.9 Predictive Model for Chemotherapy Associated Thrombosis

In a prospective observational study of 3,003 cancer patients who were treated with at least one cycle of chemotherapy and with a median follow-up of 2.4 months, a cumulative VTE incidence of 1.93% (0.8% per month) was noted [113]. The highest incidence of VTE occurred in patients with upper gastrointestinal malignancies (2.3% per month), lung cancer (1.2% per month) and lymphoma (1.1% per month) ($p = 0.001$). Higher pre-chemotherapy platelet count was associated with increased VTE risk (1.66% per month for platelets $\geq 350{,}000/mm^3$ vs 0.52% per month for platelets $\leq 200{,}000/mm^3$; $p < 0.0001$). Pre-chemotherapy hemoglobin <10 g/dL was associated with higher VTE risk (2.3% per month vs 0.71% per month; $p = 0.0003$). In addition, the use of white and red blood cell growth factors during cycle 1 was associated with increased VTE risk. Based on these findings and the results of multivariate logistic regression analysis, a predictive model for chemotherapy associated VTE was developed and validated [137]. Cancer sites associated with a very high risk for VTE (i.e., stomach and pancreas) were assigned a risk score of two. A score of one was assigned to each of the following: cancer sites associated with high VTE risk (i.e., lung, lymphoma, gynecologic, bladder, and testicular); pre-chemotherapy platelet count $\geq 350{,}000/mm^3$; hemoglobin <10 g/dL or use of

red cell growth factors; pre-chemotherapy leukocyte count $>11{,}000/mm^3$; and body mass index (BMI) $\geq 35\,kg/m^2$. The low-risk group (score = 0) was associated with 0.8% and 0.3% 2.5-month VTE rate in the derivation and validation cohorts, respectively, compared to 7.1% and 6.7% VTE rate in the high-risk group (score ≥ 3). This predictive model needs to be validated in an independent cohort of cancer patients, but the data is provocative, as the predictive model discriminated the high VTE risk group from the low VTE risk group, and future trials could be designed to test the role of thromboprophylaxis in high VTE risk cancer patients undergoing outpatient chemotherapy.

11.10 Summary

Active malignancy is a well recognized risk factor for VTE, and chemotherapy independently adds to that risk. The type of chemotherapy administered, hormonal treatments, immunomodulating and anti-angiogenesis agents, and supportive therapy with hematopoietic growth factors, have been implicated in alterations in hemostasis and with increased VTE risk. There is no clear role for administering routine thromboprophylaxis to all cancer patients undergoing outpatient chemotherapy, but to target high VTE-risk groups, including patients with multiple myeloma receiving thalidomide or lenalidomide in combination with chemotherapy and/or steroids.

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Chapter 12
Catheter-Related Thrombosis

César O. Freytes

The use of central venous catheters (CVC) has greatly facilitated the delivery of chemotherapeutic agents and is one of the most important aspects of the supportive care of patients with cancer. The most frequently utilized central venous catheters in cancer care include totally implanted catheter systems and single or multilumen tunneled catheters. Despite the convenience of use of CVC, thrombosis and infection remain frequent and serious complications of these devices [1]. This chapter will address the different types of catheter-related thrombosis as well as their diagnosis and management.

12.1 Thrombotic Complications of Central Venous Catheters

Thrombotic complications of CVC include fibrin sheath formation, intraluminal thrombosis and catheter-related deep vein thrombosis (DVT). Although these thrombotic complications have different natural histories and require different treatments, they are frequently confused in clinical practice.

Most patients experience formation of a fibrin sheath around the catheter shortly after insertion of the catheter [2–4]. Experimental studies in animals have demonstrated that days after catheter insertion, the fibrin sheath clot is replaced by stable cellular collagen covered by endothelium [5, 6]. This collagen matrix is formed by smooth muscle and other connective tissue cells migrating from the injured vein wall into the nascent fibrin sheath. Formation of a fibrin sheath is considered a predictable response to endothelial injury and the presence of a thrombogenic material inside the vascular bed. Fibrin sheath formation is rarely associated with deep vein thrombosis. A prospective study utilizing venography demonstrated that only 6% of patients that had fibrin sheaths around the CVC developed thrombosis [7]. Fibrin sheaths can result in withdrawal occlusion, the inability to draw blood from the catheter because of a

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"ball-valve" effect of the sheath at the distal end of the catheter. In addition to withdrawal occlusion, some authors believe that fibrin sheaths serve as a substrate for catheter mural thrombus and infections. In contrast to intraluminal thrombosis in which pathologic studies demonstrate increased incidence of catheter infections, there are no clinical studies that have clearly established the role of fibrin sheath in promoting bacteremia [8]. Nevertheless, experimental studies have demonstrated that fibrin sheath formation enhances central venous catheter infection and that the use of the low molecular weight heparin, enoxaparin, inhibits sheath formation and decreases central venous catheter colonization following bacteremic challenge [9, 10].

Intraluminal thrombosis, or clotting within the lumen of the catheter, is another frequent complication observed in cancer patients with CVC [11]. Intraluminal thrombosis should be suspected when it becomes difficult to aspirate blood and infuse fluids through the catheter. It is frequently assumed that the inability to draw blood is solely a consequence of intraluminal thrombus but it has to be kept in mind that it can be due to other causes including catheter malposition, catheter kinking or suture compression. A large study that utilized radiographic contrast injections to determine the cause of the failure to aspirate blood from CVC demonstrated that, although this was most frequently related to intraluminal catheter thrombosis, mechanical dysfunction was also observed in a significant number of patients [12]. This study demonstrates that imaging studies should be considered in patients in whom the use of thrombolytic agents fail to restore patency of a central venous catheter since mechanical problems could be responsible for the catheter malfunction.

Catheter-related deep vein thrombosis (CRDVT) is the most important thrombotic complication of CVC. Currently, the most common cause of upper extremity deep vein thrombosis is the use of CVC. Sequelae of CRDVT include pulmonary embolism, the postphlebitic syndrome and increased incidence of infection [8, 13–16]. The prevalence of pulmonary embolism varies greatly among published reports – from 2% to 36% – but most studies report a figure of approximately 17%. The frequency of venous thromboembolism (VTE) depends greatly on the population studied, the presence of symptoms, the study design, the time interval between CVC insertion and imaging studies utilized to establish the diagnosis of thrombosis, as well as the time period when the study was performed [17]. Prospective studies performed in the 1980s and 1990s demonstrated an incidence of CRDVT in cancer patients that varied from 30% to 74% [3, 4, 18–25]. Nevertheless, most of the cases of CRDVT were asymptomatic. In a comprehensive review of this topic, Kuter estimated that approximately one third of all CRDVT cause symptoms [26].

Recent clinical trials have shown a much lower incidence of CRDVT. Symptomatic CRDVT was seen in 4.3% of cases in two recent prospective studies in which imaging studies were performed only if patients develop symptoms suggestive of CRDVT [27, 28]. Two other recent studies, in which venography or color Doppler ultrasound were performed in all patients at predetermined time intervals, demonstrated an incidence of venous thrombosis of 6% in one

study and 16% in the other. Nevertheless, only 2% of all episodes of CVDRT were symptomatic in both studies [29, 30]. In another recently published thromboprophylaxis study, the incidence of catheter-related thrombotic complications was only 3.6% [31]. These recently published studies suggest that the incidence of catheter-related thrombosis has decreased significantly in recent years. The reason for the decreased incidence of CRDVT is unclear. Possible explanations include improvement in biocompatible materials, better catheter insertion techniques and better catheter maintenance [32]. Another possible explanation is a difference in patient population, since most of those who participated in recent clinical trials had solid tumors in contrast to earlier studies that included large numbers of patients with hematologic malignancies. It also has to be kept in mind that many of these studies were performed at centers with a special interest in vascular access. These centers have personnel who are expert in inserting and maintaining catheters, which may result in a lower rate of complications.

12.2 Diagnosis of Central Venous Catheter-Related Deep Vein Thrombosis

Symptoms of CRDVT are nonspecific and include swelling of the upper extremity, pain, functional impairment of the arm, discoloration of the skin, and distended collateral veins over the shoulder girdle and chest [15, 16]. Objective methods of examination yield negative findings for this complication in more than 50% of the patients in whom these symptoms are present [33].

Venography is the reference method for the diagnosis of deep vein thrombosis. However, venography is an invasive procedure that requires contrast agents that can cause allergies and nephrotoxicity. Venography also exposes the patient to ionizing radiation, is costly and may be extremely difficult to perform in patients with limited access to the veins in the upper extremities. These disadvantages limit the role of venography as the initial imaging method for CRDVT. Other imaging studies utilized for the evaluation of deep vein thrombosis include compression ultrasonography, Doppler ultrasonography and color flow Doppler imaging. Ultrasonography is noninvasive, has a lower cost, is portable and does not require ionizing radiation. Several prospective studies of patients with signs or symptoms of upper extremity thrombosis have compared ultrasonography, color flow Doppler imaging, and Doppler ultrasonography prior to venography (Table 12.1) [15, 33–37]. Sensitivity and specificity of compression ultrasonography and color flow Doppler imaging were comparable and better than those of Doppler ultrasonography.

After an extensive review of the literature, Gaitini et al. concluded that color Doppler duplex ultrasonography was the imaging test of choice for the diagnosis of CRDVT. The authors based this conclusion on the high sensitivity (78–100%) and specificity (82–100%) of this procedure [32]. The main

Table 12.1 Clinical utility of imaging studies in diagnosing upper extremity deep-vein thrombosis using venography as the standard reference

Diagnostic test	Sensitivity [1] (%)	Specificity [1] (%)
Compression ultrasonography [15]	96	93.5
Doppler ultrasonography [15]	81	77
Color flow Doppler [15]	100	93
Color flow Doppler [33]	94	96
Color flow Doppler [34]	78	92
Color flow Doppler [35]	82	82
Color flow Doppler [36]	100	100

limitation of color Doppler duplex ultrasonography to diagnose CRDVT is the presence of overlying bones on the medial subclavian vein and on centrally located veins including the brachiocephalic and superior vena cava confluence.

Based on the available data, it is reasonable to perform color flow Doppler imaging or compression ultrasonography on patients in whom CRDVT is suspected. Venography should be reserved for those patients in whom the diagnosis of CRDVT remains doubtful after noninvasive testing or in cases in which the diagnosis is strongly suspected after a normal or indeterminate noninvasive test. This is of particular importance when thrombosis of the medial subclavian vein or other central veins is suspected.

The diagnostic approach to CRVT differs in children. In a study of CVC in pediatric acute lymphocytic leukemia (ALL), it was shown that ultrasound was relatively insensitive in diagnosing DVT in the upper body of children (79%) but appeared more sensitive than venography for jugular vein thrombi. The authors concluded that a combination of venography and ultrasound was required for screening for DVT in the upper venous system of children [38].

Despite the fact that computerized tomography and magnetic resonance imaging have been utilized in the diagnosis of central vein thrombosis with encouraging results, these imaging modalities have not been compared rigorously with venography, the reference standard to diagnose CRDVT of the central veins [39, 40].

12.3 Risk Factors for the Development of Central Venous Catheter-Related Deep Vein Thrombosis

Multiple patient- and catheter-related risk factors for CRDVT have been proposed [41]. Patients with cancer have a sevenfold risk of thrombosis compared to patients without cancer [42]. Patient-related factors that influence the probability of thromboembolic disease in cancer patients include the type and stage of cancer, the time interval from diagnosis of the cancer to the development of thromboembolic disease, surgical intervention and therapy with antineoplastic and hormonal agents. In one study, the platelet count was found to

influence the incidence of thrombotic complications in cancer patients. Patients with a low platelet count had a lower risk of developing CRDVT [25]. The role of thrombophilic mutations in promoting CRDVT is unclear. Some authors have reported an increased incidence of factor V Leiden mutation in patients with thrombosis of CVC but other authors have not found any correlation between the factor V Leiden mutation and CRDVT [43–46]. The presence of prothrombin G20210A mutation has been implicated as a risk factor in some studies but not in others [45, 47, 48]. A recent meta-analysis of 10 studies with a total of 1,000 patients reported that the pooled odds ratio for CRDVT was 4.6 in patients with factor V Leiden and 4.9 for the prothrombin gene mutation [49].

It is possible that differences in the incidence of thrombosis observed in studies of patients with thrombophilic mutations is influenced by the type of tumor or its therapy. Wermes et al. reported that genetic mutations appeared to be additional risk factors for the development of thrombosis in patients with ALL but not in a small number of children with other malignant diseases [50]. These authors hypothesized that the difference could be due to the asparaginase and corticosteroids used to treat ALL. Similar observations have been made in patients treated with interleukin-2 and in patients undergoing peripheral blood stem cell mobilization with granulocyte-monocyte colony stimulating factor [51, 52]. Large studies in well-defined populations will be necessary to clarify the influence of thrombophilic mutations on CRDVT.

The insertion of CVC further increases the risk of thromboembolism in patients with cancer. Venous stasis, direct endothelial injury and exposure to thrombogenic surfaces result in an increased incidence of thrombotic complications. Catheter-related factors also influence the incidence of CRDVT. In experimental studies silicone and polyurethane catheters appear to be less thrombogenic than polyvinylchloride and polyethylene catheters [53]. Two studies in children utilizing heparin-bonded catheters demonstrated a lower incidence of thrombosis suggesting that the heparin coating is able to decrease thrombus formation [54, 55]. It is of interest that, despite the fact that one of these studies was randomized, it has not generated great interest in studying heparin-bonded catheters in adults. The number of catheter lumens also influences the incidence of thrombosis. Triple-lumen catheters have a significantly higher incidence of thrombosis compared to double-lumen catheters [56]. The insertion site is also an important risk factor in the development of CRDVT. Catheters inserted on the left side have a higher incidence of thrombosis compared to catheters inserted in the right side [4, 57, 58]. This is probably a reflection of the anatomy of the upper venous system [59]. The position of the catheter has been found to be an important factor in the development of CRDVT. A significantly lower rate of CRDVT was observed when the catheter tip was positioned between the superior vena cava and the right atrium [60]. Another study demonstrated that patients in whom the catheter tip was above the upper half of the superior vena cava had a significantly higher probability of developing CRDVT [17]. Other factors that influence the incidence of CRDVT

include insertion technique, previous CVC insertion, more than one CVC insertion attempt and CVC related infection [8, 18, 26, 28, 61, 62].

Some veins may be more vulnerable to CRDVT. In a retrospective analysis of more than 200 patients, a higher incidence of symptomatic thrombosis was found in those who underwent subclavian compared to internal jugular catheter insertion [62]. McDonald et al. reached the same conclusion after a prospective observational study [63]. Male et al. also found a higher incidence of CRDVT in children when the subclavian vein was catheterized [59]. A large, prospective, observational study performed at a tertiary cancer center was recently published that demonstrated a lower incidence of thrombosis when the CVC were inserted in the internal jugular vein (0.6%) compared to the subclavian vein (2%) [64]. However, other studies have failed to show differences in CRDVT based on the catheterized vein [41, 65].

Despite the fact that the risk factors discussed are well recognized, particular clinical situations frequently dictate the type of catheter utilized and the particular vein catheterized in the individual patient.

12.4 Prophylaxis for Catheter-Related Deep Vein Thrombosis

Prophylactic anticoagulation for patients with CVC has been studied for more than two decades. During this period of time, multiple clinical trials have been performed with varying results. It is important to realize that the available published reports include heterogeneous populations of patients with different types of vascular access devices inserted in different central veins. Published studies also have utilized different imaging modalities to establish CRDVT. In addition, multiple anticoagulants have been studied for varying duration, and the study endpoints have also varied greatly from study to study.

One of the earliest and most influential studies was performed by Bern et al [20]. In this small study, prophylaxis with 1 mg of oral warfarin daily decreased the incidence CRDVT from 25% to 10% and without hemorrhagic complications. With this low dose of warfarin, there were no measurable changes in the coagulation parameters studied except in patients who became anorectic because of chemotherapy or their disease. Based on this study, many clinicians utilized low-dose warfarin for CRDVT prophylaxis since it appeared to be safe for most patients. However, questions have been raised about its effectiveness. Multiple prospective studies have been published since the report by Bern evaluating the efficacy of different anticoagulant regimens in preventing CRDVT [21, 27, 31, 66–68] and several of these studies addressed the use of low-dose warfarin. A small study by Heaton et al. failed to demonstrate a lower incidence of CRDVT in patients given low-dose warfarin compared to controls [67]. A recently reported, well-executed prospective randomized trial that included more than 200 patients failed to demonstrate a reduction in the incidence of symptomatic CRDVT [27]. However, a very low incidence of

CRDVT was observed in this study as compared with studies performed years earlier. The authors concluded that, in view of the low overall incidence of CRDVT, an even larger study would be required to determine if low-dose warfarin is effective in preventing CRDVT. A recently published meta-analysis that took into account 4 studies that included over 1,000 patients did not demonstrate a reduction of CRDVT in patients who received warfarin [69].

Several studies have been performed to determine if prophylaxis with low molecular weight heparins (LMWH) decrease the incidence of CRDVT. The earliest prospective study was reported by Monreal et al. [21] This group found a marked difference in CRDVT between patients who received dalteparin LMWH vs patients who did not receive the drug (6% vs 62%). Strengths of this study include the utilization of only one type of totally implanted catheter system and mandatory evaluation by venography at predetermined time intervals. The main drawback of the study was its small size because the study was terminated early by recommendation of the ethics committee of their institution. Two excellent studies evaluated the efficacy of enoxaparin and dalteparin in preventing CRDVT [30, 31]. Both of these studies were large, double-blind and placebo-controlled. Neither found any difference in the rate of CRDVT. The incidence of CRDVT also was lower than in earlier studies: the rate of symptomatic thrombosis in both studies was only 3% and 4%. A more recently published randomized, placebo controlled clinical trial in patients with hematologic malignancies undergoing chemotherapy also failed to show a statistically significant difference in the incidence of CRDVT in patients receiving dalteparin [70].

As the above-mentioned studies illustrate, recent well-executed clinical trials of prophylaxis for CRDVT have shown a low incidence of CRDVT and failed to show a significant reduction in the incidence of symptomatic or asymptomatic catheter-related thrombosis. Several meta-analyses have been published that also do not support the routine use of thromboprophylaxis in cancer patients with CVC [71–73]. Akl et al., as part of the Cochrane Collaboration, concluded that use of heparin was associated with a trend towards reduction in symptomatic CRDVT but the data did not show any significant effect on mortality, infection, major bleeding or thrombocytopenia [72]. The effect of warfarin on symptomatic CRDVT was not statistically significant. Of interest, when studies assessing different types of anticoagulants were pooled, symptomatic DVT rates were significantly lower. Another meta-analysis by Chaukiyal et al. concluded that thromboprophylaxis has no significant effect on the risks of catheter related thrombosis or bleeding [71].

Despite the results of these recent clinical trials, significant questions remain regarding thromboprophylaxis for CVC [32, 74]. It is unknown whether higher doses of current anticoagulants or new anticoagulant agents can prevent CRDVT. More relevant is the possibility that patients at very high risk of CRDVT can benefit from thromboprophylaxis [75]. As discussed earlier, it remains to be seen whether the low incidence of CRDVT observed in recent studies translates into a lower incidence of catheter-related thrombosis in

general practice and outside of centers specialized in this area of supportive care. In order for future studies to assess the efficacy of anticoagulation in the prevention of CRDVT, a very large number of patients will have to be recruited given the low incidence of this complication. In addition, future clinical trials will have to include homogenous populations with similar diagnoses, vascular access devices, risk factors and endpoints in order to clarify the role of thromboprophylaxis for CRDVT.

12.5 Management of the Thrombotic Complications of Central Venous Catheter in Cancer Patients

Intraluminal thrombosis and fibrin sheath formation occur frequently and can be treated effectively with small doses of alteplase or recombinant urokinase (not currently available in the United States for this purpose) without hemorrhagic or embolic complications [11, 76–78]. As mentioned earlier, imaging studies should be considered in patients in whom the use of thrombolytic agents fails to restore patency of a central venous catheter, since malposition or other mechanical problems are frequently responsible for the catheter malfunction. However, there is an association between catheter occlusion and CRDVT [28].

The management strategies of CRDVT are less well defined than the management of intraluminal thrombosis or fibrin sheath formation because there are no prospective controlled studies to guide the therapy of this complication. The goals of therapy of CRDVT are to prevent propagation of the clot, pulmonary embolism, postphlebitic syndrome and recurrent thrombosis [79]. Another important goal of therapy is to maintain adequate vascular access in order to continue antineoplastic treatment or supportive care. Because of the paucity of information in this area, most physicians have treated CRDVT in a similar fashion to lower extremity DVT.

Anticoagulation has been the most utilized therapy for CRDVT. The rationale for this approach includes the fact that pulmonary embolism complicates a significant proportion of cases of upper extremity DVT and is more frequent after CRDVT [16]. In addition, recurrence of the thrombus is frequent in patients with upper extremity DVT. A recent report from an ongoing registry of consecutive patients with objectively confirmed, symptomatic, acute upper extremity DVT demonstrated that 38% of patients with upper extremity thrombosis had cancer and 45% of all patients had CRDVT [80]. In this registry report, patients with CRDVT and cancer had an increased incidence of major bleeding, recurrent thromboembolism and death when compared with patients without cancer. Patients with CRDVT and cancer also had a higher incidence of composite events defined as any combination of recurrent DVT, symptomatic pulmonary embolism or major bleeding. This study clearly demonstrates the potential consequences of CRDVT in patients with cancer.

Several studies have demonstrated that the use of LMWH followed by the administration of warfarin is a reasonable therapy for patients with CRDVT. In a prospective cohort study of 46 patients receiving dalteparin followed by warfarin, only one patient developed recurrent DVT and no patient developed pulmonary embolism. Only one patient experienced major bleeding, suggesting that this approach is safe and effective in the management of patients with upper extremity DVT [81]. In a prospective study of patients with CRDVT, patients who underwent anticoagulation either with LMWH only or LMWH followed by warfarin were able to retain their catheters with a low incidence of long-term complications, suggesting that anticoagulation was safe and effective in the few symptomatic patients on this study [28]. A more recent study using the same approach of administering dalteparin followed by warfarin corroborated previous results [82]. In this study there were no episodes of recurrent venous thromboembolism and no lines were removed because of infusion failure or recurrence or extension of the CRDVT. Nevertheless, there was a 4% incidence of major bleeding. An open question is the duration of anticoagulation after CRDVT; recommendations have varied from 3 months to indefinite duration in the presence of active tumor [79].

The role of catheter removal in the management of CRDVT is unclear. In a retrospective analysis of patients with thrombosis of CVC, 20 patients with CRDVT who were treated only by removing the CVC did not experience recurrent thrombosis but the follow-up after removing the catheter was short [83]. In this study, 25% of patients who had their CVC removed due to thrombosis and had another catheter inserted without anticoagulation experienced recurrent thrombosis. It has to be emphasized that the safety of catheter removal without anticoagulation has not been well studied. It is very relevant that, in this study, anticoagulation with or without catheter removal resulted in resolution of clinical symptoms in all patients.

The precise role of systemic thrombolysis in the management of CRDVT has not been established, at least in part due to its potential adverse effects. In a retrospective study of the long-term outcome of patients with subclavian and axillary vein thrombosis, patients who underwent systemic urokinase thrombolysis had a 21% rate of bleeding complications [84]. In this study, patients treated with systemic thrombolysis exhibited a 60% reduced risk for a thrombosed subclavian vein at the time of follow-up compared to patients treated with anticoagulation only. However, the frequency of symptomatic postthrombotic syndrome after thrombolysis and anticoagulation was similar. Since there is no clear benefit of systemic thrombolysis and a higher risk for bleeding, this modality of therapy should not be utilized except in unusual circumstances such as when the upper extremity circulation is at risk or in patients without other therapeutic alternatives.

There is limited information regarding the role of catheter-directed thrombolysis in the management of patients with CRDVT [85]. In a small study of patients undergoing high-dose chemotherapy, all 18 patients treated with catheter-directed thrombolysis had a partial or complete resolution of clinical signs

and symptoms. Of the patients, 50% also achieved a partial radiographic response defined as clot lysis with irregular canalization of the vein. In this study, therapeutic doses of heparin for 5–7 days and warfarin for at least 3 months were commenced at the conclusion of the thrombolytic therapy. Twelve catheters were salvaged and utilized subsequently until no longer required. Six catheters were removed because of poor catheter function or rethrombosis. The median interval from diagnosis of the thrombus until extraction of the 12 salvaged catheters was 3 months (range 1–8 months). Discontinuation of the procedure was required in only one patient who developed gastrointestinal bleeding. Another small study in which this approach was utilized in patients with CRDVT complicating peripherally inserted central catheters described resolution of CRDVT in all patients [86]. Important differences from the previously described study were that all the central venous catheters were removed during the procedure and that no long term anticoagulation was given. Current experience suggests that this therapeutic approach requires further study in patients with CRDVT. This is especially relevant after a recent report of catheter-directed thrombolysis for upper and lower extremity DVT demonstrated not only that complete clot lysis was achieved in two thirds of the patients but also that the complication rate observed in patients with cancer was similar to that in patients without malignancy.

Superior vena cava vein filters have been utilized in the therapy of patients with upper extremity deep vein thrombosis who have contraindications to anticoagulant therapy or in patients in whom anticoagulant therapy has failed. Spence et al. reported on 91 patients who underwent percutaneous filter placement in the superior vena cava for prevention of pulmonary embolism due to acute upper extremity DVT [87]. In this study a CVC was present at the site of thrombosis at the time of diagnosis or within the previous 2 weeks in a total of 88% of patients, and one third of the patients had carcinomas. With a median follow-up of 12 weeks, no complications such as filter migration, dislodgement or fracture was observed. In addition, no patients developed clinical evidence of pulmonary embolism due to upper extremity thrombosis or superior vena cava syndrome. Central venous catheters were inserted subsequent to filter placement in half the patients without complication. Another study corroborated these findings [88]. Additional studies will be necessary to establish firmly the role of filter insertion in patients with CRDVT since there has never been a randomized controlled trial to examine objectively the use of this modality in patients with CRDVT.

It is encouraging that the importance of CRDVT has been recognized by an alliance of leading cancer centers. A panel of experts from The National Comprehensive Cancer Network (NCCN) recently issued practice guidelines based on the available information and expert opinion [89]. These guidelines are periodically updated and take into account new information published in this field.

Evidence of the increasing importance of vascular access in the management of patients with multiple medical problems is illustrated by the fact that the New England Journal of Medicine recently published instructions of how to insert central venous catheters via the subclavian vein as part of their “Videos on

Clinical Medicine" series [90]. Although variations of this technique exist, and not all clinicians agree with every recommendation, this video provides a detailed illustration of how to accomplish central venous vascular access [91–94]. It must be emphasized that vascular access insertion should be performed by properly trained personnel that have experience in the insertion of central venous catheters and are familiar with the complications and their management.

In summary, recent well-designed prospective controlled trials have demonstrated a lower incidence of catheter-related deep vein thrombosis (CRDVT) compared to earlier studies but this complication remains an important limitation for the delivery of drugs and supportive care to cancer patients. Prophylaxis of CRDVT with antithrombotic agents was not supported by recent prospective randomized trials, and their routine use is not recommended. Larger studies will be required to determine if cancer patients at particularly high risk for CRDVT can benefit from prophylaxis with antithrombotic agents. There are no prospective controlled studies to guide the therapy of CRDVT but treatment with anticoagulants remains the mainstay of therapy. While recently published guidelines might help standardize the therapy of patients with CRDVT, prospective controlled studies are needed to determine the optimal therapy of this important complication of central venous catheters in cancer patients.

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Chapter 13
Thrombosis in Childhood Cancer

Geoffrey A. Allen and Rukhmi Bhat

13.1 Introduction

Over the past five decades, the survival rate for children diagnosed with oncologic disease has dramatically increased from less than 20% to almost 80%. Accordingly, an area of growing interest and concern is the long-term effects of the patients' disease and therapy. Thromboembolic events (TEE) occur with greater frequency in children and adults with cancer and have been extensively studied in the latter population. However, given the relative infrequency of TEE in the general pediatric population and paucity of coordinated efforts, information on the incidence, nature and impact of thrombosis in children with cancer has been lacking until recently. In this chapter, we address the salient features of TEEs in the pediatric cancer patient.

13.2 Epidemiology

13.2.1 Incidence in the General Pediatric Population

Previous efforts to document the incidence of TEE in the general pediatric population have consisted primarily of literature reviews and retrospective compilations of single institution experiences. More recent efforts, including prospective studies and registries, have made valuable contributions to the knowledge base. Arterial thromboembolic events are relatively rare in children, as compared with venous thromboembolism and are primarily related to therapeutic interventions with arterial catheters. For this reason, the primary focus will be on venous TEEs.

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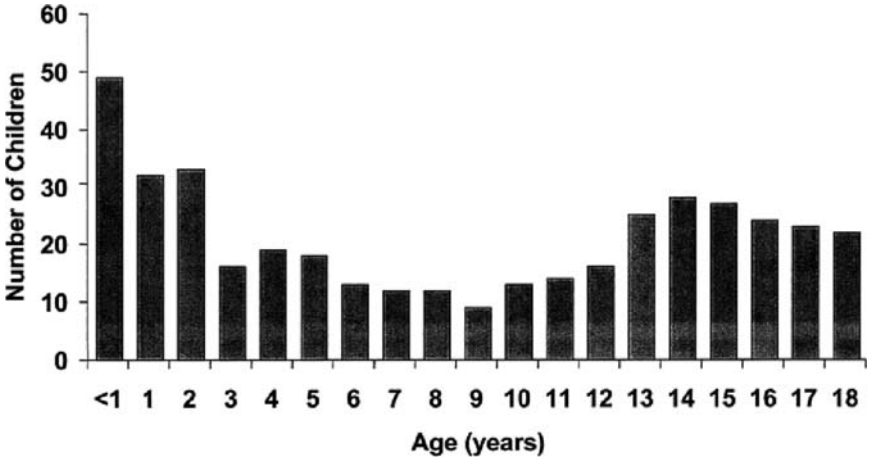

Fig. 13.1 Age distribution of 405 children with PE, or DVT in the upper or lower venous system reported to the Canadian Registry of Venous Thromboembolic Complications in Children. Reprinted with permission [108]

Compared with an estimated average annual incidence of 1–2/1000 in the adult population, the incidence of venous thromboembolism in the pediatric population has been estimated at 0.07–0.49 per 10,000 children [1–5]. The age distribution of TEE in children is bimodal, with the periods of highest risk occurring in the 1st year of life and late adolescence (Fig. 13.1). The neonatal period appears to have the highest frequency of events, with estimates of up to 14.5 per 10,000 [5]. This is likely reflective not only of the unique biology of the neonate, but also the increasing numbers of ill neonates being cared for and having more invasive medical procedures. In contrast to the adult population, spontaneous TEE occurs rarely in children, with more than 90% of pediatric thrombosis patients having contributing factors or medical conditions associated with increased risk for TEE, as is reflected in the higher incidence of TEE in hospitalized children, 5.3 per 10,000 [3, 6]. Cancer is a considerable risk factor, with 8–22% of pediatric thrombosis patients being afflicted with cancer [3, 5].

13.2.2 Incidence in the Pediatric Cancer Patients

Malignancy is one of the more common underlying conditions associated with TEE in the pediatric population. The incidence of thrombosis varies widely, dependent upon whether only symptomatic events were reported, or whether all patients were screened, regardless of the presence of symptoms. Figure 13.2 shows the anatomic distribution of symptomatic thromboembolic events in children with ALL [7]. Additionally, retrospective studies appear to

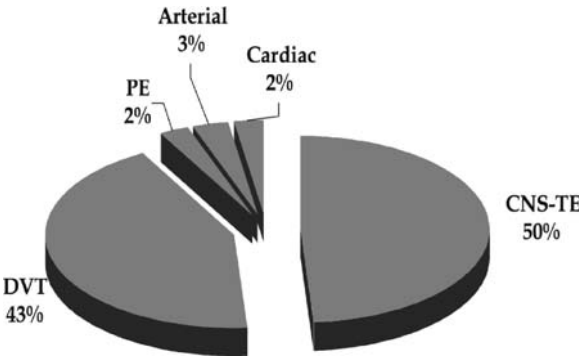

Fig. 13.2 Anatomical distribution of symptomatic TE in children with ALL; PE = pulmonary embolism; DVT= deep venous thrombosis; CNS-TE = central nervous system thromboembolism. Reprinted with permission [7]

underestimate the risk of TEE in pediatric cancer patients, compared with prospective studies [8].

As acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood, the bulk of existing data comes from this population. Mitchell et al. reported the incidence of TEE in pediatric ALL patients to be 36.7%, almost all related to the presence of indwelling central venous catheters (CVCs) [9]. In this prospective study, all patients were screened at the end of induction with bilateral venography or MRI of upper body, ultrasound of upper body, echocardiogram and MRI of the head. Only 5% of TEE in this study were symptomatic. Using spiral CT and ultrasound to screen for asymptomatic CVC-related thrombi, Farinasso observed an even higher incidence of 77% in children with ALL [10]. Only 4 of the 43 thrombotic events were symptomatic and only two were unrelated to the CVC. The clinical relevance of asymptomatic TEE in pediatric oncology patients is unknown. A similar incidence of symptomatic TEE was reported in a meta-analysis of 17 prospective studies of TEE in pediatric patients with ALL [8]. In 1,752 patients, the overall risk of symptomatic TEE was 5.2% over the entire course of therapy, a rate at least 100 times greater than that observed in the general pediatric population.

There are less data on the incidence of TEE in other pediatric cancers. A retrospective study of 75 pediatric patients with lymphoma found the overall incidence of TEE to be 12%. The presence of mediastinal lymphadenopathy was associated with a higher risk of thrombosis, while there was a non-significant trend towards a lower incidence in those patients under 10 years of age. No significant difference in incidence was noted between patients with Hodgkin's disease (HD) and those with non-Hodgkin's lymphoma (NHL), despite that treatment of NHL typically involves asparaginase, while treatment of HD does not.

Brain tumors are the most common solid tumor of childhood. The reported incidence of symptomatic TEE in children with brain tumors varied from 0.645% to 2.8% in two retrospective series [11, 12]. In contrast, in a retrospective series of 122 pediatric patients with sarcoma, 16% had either symptomatic or

incidental finding of venous thrombosis [13]. Half of the patients had thrombosis at the time of diagnosis, with only half of those being related to tumor compression. While the overall incidence of metastatic disease at the time of diagnosis was 31%, 80% of those with thrombosis at the time of diagnosis already had metastatic disease. The risk of TEE was approximately twofold higher in those patients with distant metastases.

13.3 Unique Attributes of the Pediatric Hemostatic System

Fibrinogen has been detected during the 5th week of gestation in the human fetus [14]. By the time of birth, the hemostatic system of the newborn is fully functional, as evidence by the lack of a general hemorrhagic or thrombotic state. However, significant differences are apparent when compared to adult subjects and these differences do not fully resolve until the late adolescent period (Table 13.1). Normal ranges for the components of the procoagulant, anticoagulant and fibrinolytic systems of the fetus, neonate and child ages have been published [15–19]. Newborns demonstrate the greatest disparity, much of it resolving in the first 6 months of life.

13.3.1 Primary Hemostasis

Neonatal platelets have often been described as "hypo-reactive", largely due to decreased aggregation in response to standard platelet agonists and immature

Table 13.1 Normal mean values and ranges for selected hemostatic factors at various life stages [18, 109]

	Full-term infant		Adolescent (11–16 years)		Adult	
FII (U/mL)	0.48	(0.26–0.70)	0.83	(0.61–1.04)	1.08	(0.70–1.46)
FV (U/mL)	0.72	(0.34–1.08)	0.77	(0.55–0.99)	1.06	(0.62–1.5)
FVII (U/mL)	0.66	(0.28–1.04)	0.83	(0.58–1.15)	1.05	(0.67–1.43)
vWF (U/mL)	1.53	(0.50–2.87)	1.00	(0.46–1.53)	0.92	(0.50–1.58)
FIX (U/mL)	0.53	(0.15–0.91)	0.82	(0.59–1.22)	1.09	(0.55–1.63)
FX (U/mL)	0.40	(0.12–0.68)	0.79	(0.50–1.17)	1.06	(0.70–1.52)
FXI (U/mL)	0.38	(0.10–0.66)	0.74	(0.50–0.97)	0.97	(0.67–1.27)
FXII (U/mL)	0.53	(0.13–0.93)	0.81	(0.34–1.37)	1.08	(0.52–1.64)
AT (U/mL)	0.63	(0.39–0.87)	1.05	(0.77–1.32)	1.00	(0.74–1.26)
α2-M (U/mL)	1.39	(0.95–1.83)	1.56	(0.98–2.12)	0.86	(0.52–1.2)
HCII (U/mL)	0.43	(0.10–0.93)	0.91	(0.53–1.29)	1.08	(0.66–1.26)
Protein C (U/mL)	0.35	(0.17–0.53)	0.83	(0.55–1.11)	0.96	(0.64–1.28)
Plasminogen (U/mL)	0.54	(0.35–0.74)	0.86	(0.68–1.03)	0.99	(0.77–1.22)
t-PA (ng/mL)	9.60	(5.0–18.9)	2.16	(1.0–4.0)	4.90	(1.4–8.4)
PAI-1 (U/mL)	6.40	(2.0–15.1)	6.07	(2.0–10.0)	3.60	(0.0–11.0)

signal transduction pathways [20]. Despite this attribute, the bleeding time in newborns is shorter than that in older children and adults, likely due to increased concentrations of circulating von Willebrand factor (vWF) and larger, more adhesive vWF multimers [16, 17, 21–23]. Additionally, platelets in the newborn period appear to have greater procoagulant characteristics, with greater surface phosphatidylserine (PS) expression and microparticle generation in vitro [24, 25]. However, as these differences resolve in the first few months of life, they are unlikely to have significant effect on the incidence of TEEs during most of childhood.

13.3.2 Secondary Hemostasis

In the newborn infant, levels of the vitamin K-dependent (II, VII, IX, X) and contact factors (XI, XII, prekallikrein and high molecular weight kininogen) are decreased relative to normal adult values. Conversely, the cofactors V and VIII, as well as factor XIII are present at normal adult levels, demonstrating that the lower levels of some factors is not simply due to an overall decrease in protein synthetic maturity [16, 17]. Beyond the 1st year of life and throughout childhood, the vitamin K-dependent and contact factors remain at about 80% of adult levels [18].

13.3.3 Inhibitory Proteins

Levels of the direct thrombin inhibitors antithrombin (AT) and heparin cofactor II (HCII) are present in significantly depressed levels at birth. In contrast, α_2-macroglobulin (α_2-M) levels are significantly higher than found in adult subjects and remain so throughout childhood [16–18]. Similarly, levels of the vitamin K-dependent proteins C (PC) and S (PS) are present at approximately 30% of adult levels at birth. Low PC levels persist through childhood, while PS concentrations approximate those of adults within a few months. The percentage of free PS is similar to adult levels during infancy, due to equally reduced levels of it carrier C4-binding protein [26].

13.3.4 Fibrinolysis

Similar to other components of the hemostatic system, the constituents of the fibrinolytic system are present in significantly different concentrations as compared to adult subjects. Plasminogen levels in the full-term neonate are approximately half of normal adult values [17]. Those differences that persist outside the first 6 months of life include decreased levels of tissue plasminogen activator (t-PA) and increased plasminogen activator inhibitor (PAI-1) [16–18]. Levels of

α_2-M, which also acts as a nonspecific inhibitor of both plasmin and t-PA, are elevated throughout childhood as mentioned previously. These differences would suggest a hypofibrinolytic state, as was supported by the observations of Monagle et al. using venous occlusion stress testing to compare fibrinolytic response in adolescents and adults [27].

The net effect of these characteristics in the pediatric hemostatic system that persist into adolescence is not fully known. In vitro measurements of thrombin generation would suggest that the key determinants are concentrations of prothrombin and AT [28, 29]. This is supported by the observation that thrombin generation in neonatal plasma is significantly decreased, and appears to most responsive to added prothrombin [30]. Pooled plasma from children aged 1–16 years, when compared to adult plasma, showed 27% less thrombin generation in aPTT-based assays [3]. The impact of low AT levels on thrombin generation is tempered by the elevated levels of α2-M, which contributes less thrombin inhibition as AT approaches adult levels [31, 32]. The lower concentration of PC would appear to be, if anything, prothrombotic in nature. Similarly, apparent decreased fibrinolytic activity in children would also appear to contribute to a prothrombotic state. Finally, the impact of the vascular endothelium of children on hemostasis as compared with the adult is not known and not easily measured. The influence of age, disease and toxic exposure on the vessel wall may be a significant contributor to the increased risk of TEE risk in the adult cancer patient.

13.4 Prothrombotic State in Children with Cancer

The prothrombotic state in patients with cancer is due in part to the ability of malignant cells to affect all aspects of the coagulation system, as discussed at length elsewhere in this text (Fig. 13.3). Prothrombotic factors in malignancy include direct procoagulant, antifibrinolytic and pro-inflammatory activities. Malignant cells have been demonstrated to lead to activation of both primary and secondary hemostasis. Tumor cells have been shown to express procoagulant substances on their surfaces such as tissue factor (TF), cancer procoagulant (CP), and mucin, which are capable of activating the coagulation cascade at various points, as well as platelets [33–36]. Similarly, malignant cells may have both positive and negative effects on the on the fibrinolytic system, having been demonstrated to express tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), as well as plasminogen activator inhibitor1-1 (PAI-1) [37–39]. The prothrombotic tendency of cancer patients is further enhanced by therapies such as surgery, chemotherapy and radiotherapy, interventions such as surgery and CVCs and complications such as infections and hemodynamic compromise. Finally, inherited prothrombotic defects may contribute to the overall risk factors for thrombotic events in children with cancer. As much of the data in children has been generated from studies on subjects with leukemia,

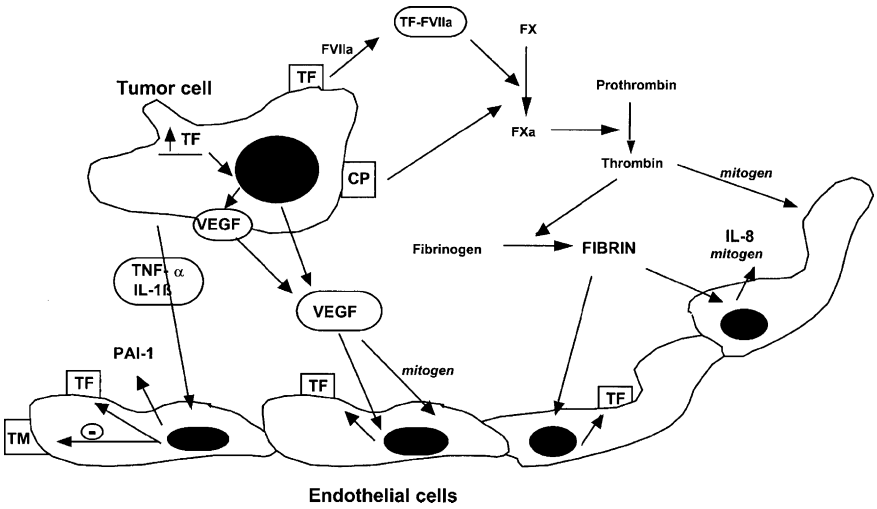

Fig. 13.3 Expression of tumor and endothelial cell procoagulant functions. Tissue factor (TF) and cancer procoagulant (CP) are expressed on the surface of the tumor cell, with enhancement of effect through local production of IL-8 by the endothelium and VEGF and inflammatory cytokines TNF-α and IL-1β from tumor cells. The cytokines convert the normal anticoagulant properties of the endothelium to procoagulant by down-regulating TM expression and increasing TF and PAI-1 expression. Subsequent fibrin production leads induces further endothelial production of TF and IL-8. Reprinted with permission [107]

it is difficult to draw conclusions from and to rank the impact of these multiple factors on the incidence of thrombotic episodes in all children with cancer.

13.4.1 Procoagulant Properties of Cancer in Children

A common finding amongst the small number of studies in children with leukemia and lymphoma is evidence of increased thrombin generation and activity at the time of diagnosis, persisting long after the start of treatment. Evidence of this was offered in the form of elevated thrombin-antithrombin complexes (TAT), prothrombin fragment 1.2 (F1.2) and fibrinopeptide A [40–43]. Interestingly, is has been observed that children with T-cell leukemia have significantly higher TAT levels than do children with pre B-cell ALL. Despite the increased thrombin activity, Mitchell reported that the thrombin potential of plasma from children with leukemia was not greater than that of controls [44]. Abshire et al. found increased tissue factor activity in the bone marrow of children with AML, while that from children with ALL was normal [45]. Similarly, Semararo found that circulating mononuclear cells from pediatric lymphoma patients had a greater capacity for tissue factor expression than did normal controls [46].

Reported alterations of circulating anticoagulant includes decreased protein C and S activity in children with ALL at diagnosis, while AT, HCII and α_2-M activity appear to be normal or elevated. Semararo found that while both tPA and PAI-1 were increased in plasma from children with lymphoma, the increase in PAI-1 levels was five times that observed in tPA, suggesting an overall hypofibrinolytic state [46]. Observed increases in plasma levels of factor VII and von Willebrand factor were felt to be reflective of an acute phase reaction, while increased levels of thrombomodulin were suggested to be a marker of ongoing endothelial damage [42, 44].

13.5 Role of Thrombophilia

Prospective studies in children with cancer have offered conflicting data regarding the impact of inherited and acquired thrombophilia on the incidence of TEEs (Table 13.2) [47–51]. Again, the majority of data concerns children with ALL. Overall the prevalence of thrombophilia in children with cancer was reported as ranging from 18% to 32%. This variation is likely related to the ethnic variation in patient population and different thrombophilias studied. The incidence of TEE in children with thrombophilia was variable and may be related to whether asymptomatic subjects were screened for TEE, different surgical techniques of CVC placement and the chemotherapy protocol used. Nowak-Gottl et al. reported significantly higher incidence of thrombosis in children with ALL with at least one inherited prothrombotic defect compared to those without any prothrombotic defect 48.5% vs 2.2%, $p<0.001$ [50]. Of the 289 subjects investigated, 58 had one established single prothrombotic risk factor. In contrast, the PAARKA study found no such effect of prothrombotic defects on the development of thrombosis, although a smaller set of thrombophilias were studied and all patients were screened for thrombosis, not only those with symptoms [9]. Wermes et al. observed that, although inherited prothrombotic defects were associated with a higher risk TE in children with ALL, the same trend was not seen in subjects with malignancies other than ALL [51]. Based on available data and no evidence for an effective thromboprophylaxis strategy, no specific recommendations regarding screening for thrombophilias and the use of this information clinically can be made.

13.6 Role of Central Venous Catheters

CVCs have assumed a critical role in the care of children with acute and chronic disease, easing the task of delivering medical therapies and nutrition, as well as performing frequent phlebotomies. However, they are not without potential morbidities, primarily bacterial infection and thrombotic events. In fact, the presence of a CVC is the major exogenous risk factor for the development of

Table 13.2 Prospective studies of the impact of thrombophilia on thromboembolic disease in pediatric cancer patients. AT = antithrombin; PC = protein C; PS = protein S; FVL = factor V Leiden mutation; PT = prothrombin gene mutation; MTHFR = homozygous methylenetetrahydrofolate reductase C677T; Lp(a) = Lipoprotein (a); APLA = antiphospholipid antibody; ACLA = anticardiolipin antibody; FXII= factor XII deficiency

Study	Disease	Prevalence of Thrombophilia	Thrombophilia Detected	Incidence of TEE	Incidence of TEE with Thrombophilia	Comments
Nowak-Göttl, et al. [50]	ALL	58/289 (20%)	AT, PC, PS,FVL,PT, MTHFR, Lp(a)	32/289 (11%)	27/58 (46.5%)	
Mauz-Körholz, et al. [48]	ALL	35/108 (32%)	PC, PS, FVL, PT, MTHFR, Lp(a)	3/108 (2.8%)	0/35 (0%)	
Mitchell, et al. [9]	ALL	11/60 (18%)	FVL, PT, APLA	22/60 (36.7%)	4/11 (36%)	Includes asymptomatic TEE
Knofler, et al. [47]	Hematologic and solid tumors	17/77 (22%)	PS, PC, FVL, PT, Lp(a), FXII	11/77 (14%)	7/17 (41%)	
Wermes, et al. [51]	ALL	18/73 (25%)	PS, FVL, PT, MTHFR	7/73 (8.2%)	4/18 (22%)	
	Other hematologic and solid tumors	12/64 (19%)	FVL, PT, MTHFR	4/64 (6.2%)	0/12 (0%)	

thromboembolism in children [3]. The placement of a CVC disturbs the hemostatic system by damaging the blood vessel and endothelium at the insertion site and along the course of the catheter and contributing to stasis, particularly in the smaller veins of children. Following placement, the surface of the CVC is rapidly coated with a biofilm, comprised of platelets, fibrin and bacteria [52, 53]. The presence of such biofilms may serve as the nidus for infection, inflammation and further fibrin clot formation. The use of special heparin-coated catheters has been demonstrated to decrease the incidence of catheter-related infection and thrombosis in children, while preliminary in vitro studies indicate that a catheter coated with an AT-heparin complex may have even less prothrombotic properties [54–56].

The reported incidence of CVC-related thrombosis in children with cancer varies widely, dependent upon study design, imaging methods used, types of malignancies studied, etc. Children with hematologic malignancy appear to be at greater risk of developing CVC-related thrombosis than those with other forms of malignancy [57, 58]. In a retrospective study of patients with ALL, McLean et al. found a 6% incidence of thrombosis. As discussed previously, with prospective screening for asymptomatic events, Mitchell, et al. reported the incidence of CVC-related TEE in pediatric ALL patients to be 35% and Farinasso et al. reported an incidence of 73% [9, 10]. In a population of children with a variety of cancers, Glaser et al. reported 50% of subjects were discovered to have evidence of CVC-related thrombosis using spiral CT and ultrasound to screen patients, with only 3 out of 12 having abnormal physical findings on exam.

A sub-study of the Prophylactic Antithrombin Replacement in Children with Acute Lymphoblastic Leukemia on Asparaginase study (PARKAA) assessed whether CVC location and insertion technique were associated with an increased incidence of thrombosis. The study found a 33% incidence of CVC-related thrombosis in children with ALL [59]. Left sided CVC, subclavian location and percutaneous technique were associated with a higher incidence of thrombosis, although none of these risk factors were confirmed in the study by Farinasso [10]. Additionally, McLean noted that external CVCs carried a significantly greater risk of thrombosis than did internal CVCs [60].

The consequences of CVC-related thrombosis in children can be significant. The Canadian Childhood Thrombophilia Registry monitored 224 children with CVC-related thrombosis and reported a 3.7% incidence of mortality, 6.5% incidence of recurrent TEE and 9.4% incidence of post-thrombotic syndrome (PTS), with a mean follow-up of 2 years [61]. While the definition and incidence of post-thrombotic syndrome in children with CVC-related thrombosis is not consistent, severe PTS appears to a rare event [57, 62, 63]. The natural history of asymptomatic CVC-related thrombosis is largely unknown and more prospective studies with larger patient population followed over longer periods of time are required.

13.7 Role of Chemotherapy

Specific chemotherapeutic agents, alone or as part of combination therapy, are known risk factors for the development of TE in patients with cancer, as addressed at length elsewhere in this text. Chemotherapy alters the hemostatic system via direct effects on the endothelium, decreased anticoagulants and fibrinolytic activity, platelet activation and aggregation and increased tissue factor activity [41, 64–68]. We will address those findings having the greatest impact on pediatric subjects in this section.

L-Asparaginase (L-asp), the substance in guinea pig serum noted to have activity against lymphoma, is an important agent in the treatment of childhood ALL, due in part to its unique mechanism of action, in addition to a relative lack of myelosuppression [69]. Its effects on levels of coagulation proteins and AT in particular were noted early in its history [70, 71]. It was somewhat later that its effect on AT was linked to thrombotic events observed during treatment with L-asp containing combination therapy for pediatric ALL, particularly sinovenous thrombosis, in patients both with and without CNS involvement [67, 70–73]. The effects of L-asp on the hemostatic system of children have usually been studied in the context of various combination chemotherapeutic regimens, with other agents that are also capable of affecting hemostasis. Land et al. noted that induction with L-asp alone appeared to have a slightly greater effect on coagulation proteins and screening tests than did L-asp containing combination therapy [71]. In a more detailed study, Mitchell et al. observed a similar trend on 21 hemostatic variables, measured after 5 days of therapy with asparaginase alone in children with ALL [44]. While a global decrease in protein synthesis was observed, statistically significant decreases were seen in HMWK, fibrinogen, vWF, factors IX and XI, AT, α_2-M, HCII, alpha$_2$ antiplasmin (α_2-AP) and plasminogen. The decrease in AT levels was of statistically greater magnitude that the other proteins. During the following phase of combination therapy *without* asparaginase, significant increases above baseline in many of the coagulation proteins were observed, with a decrease in a few others proteins, most notably α_2-M. Subsequent combination therapy *with* asparaginase demonstrated a more moderate effect on the studied proteins, with significant increases measured in factor VII and PC and decreases in factors IX, XI and XII, vWF and AT.

In addition to the effect on AT levels, other common (but not universal) findings during asparaginase-containing combination therapy for childhood ALL were decreased levels of PC, PS, AT, α_2-M, plasminogen and fibrinogen [42, 74–76]. The cause of the differential effect on coagulation protein levels is not known. It does not appear to correlate with the asparagine content of the individual proteins, nor their plasma half-lives. It has been suggested that increased consumption of AT, due to the increased thrombin generation present in childhood ALL, may contribute, in addition to decreased translation and/or secretion of AT by hepatocytes [41, 77]. Increased platelet aggregation

following combination therapy with L-asp has been also reported, while other investigators observed no effect and yet others demonstrated a direct agonist effect of L-asp on platelets [74, 75, 78].

Corticosteroids may induce a prothrombotic state through elevations in individual procoagulant factors and by reduction in fibrinolytic potential, as noted in patients with Cushing's disease and those treated with exogenous steroids for other conditions [79–89]. Consistent effects noted on procoagulant proteins were elevations in factor VIII, von Willebrand factor (vWF) and fibrinogen [8, 80–82, 88]. A decrease in fibrinolytic potential, as demonstrated with a venous occlusion test, was felt to be related primarily to an increase in PAI-1 activity [83, 84, 86].

Data in pediatric patients would suggest potential synergy with steroids and L-asp, in addition to differences in procoagulant properties of those steroids typically used to treat children with ALL. Nowak-Gottl et al. published a comparison of two ALL treatment protocols, with significant differences in episodes of symptomatic thrombosis [90]. In the BFM protocol, induction of remission (IR) therapy included concurrent administration of prednisone and L-asp, with 10.0% incidence of thrombotic events. In contrast, in patients on the COALL protocol, in which L-asp was administered post-prednisone, the rate of thrombotic episodes during IR was 0.8%. A multivariate analysis indicated that it was the combination of L-asp and prednisone and the presence of thrombophilia that significantly increased risk for thrombosis. In both the BFM and COALL protocols, re-induction therapy included L-asp and dexamethasone, with a 1.7% rate of TEE observed in both. The same group noted a decreased incidence of thrombotic events in pediatric ALL patients treated with L-asp and dexamethasone during IR (1.8%), as compared to another BFM protocol with L-asp and prednisone (10.4%) [91]. Caruso et al. also reported an increased incidence with use of prednisone as compared to dexamethasone in their meta-analysis of risk of thrombosis in children in ALL, but the difference was not found to be statistically significant [8].

One in vitro study offered data that the differences in hemostatic protein concentrations in children may exacerbate the thrombogenic effects of standard chemotherapeutic agents. Mewhort-Buist et al. found that endothelial cells treated with vincristine expressed significantly less endothelial cell protein C receptor on their surface, while the amount of TM was relatively unchanged [92]. The amount of activated protein C (APC) generated on the treated endothelial cells when incubated with in adult plasma showed only a 20% decrease as compared to the amount of APC generated on untreated cells. In contrast, at least an 80% decrease in APC generation was noted when the same experiment was repeated with newborn plasma. The authors speculate that the observed effect may be due, in part, to the decreased levels of PC and increased levels of α_2-M observed in children.

13.8 Prophylaxis and Treatment

The management of thromboembolic disease in children has been largely extrapolated from the practices used to treat adult patients, although data from pediatric studies are accumulating. A recent review of the existing data, with graded recommendations for treatment, has been published [93]. Currently, it is recommended that thromboembolic disease in children with cancer be managed in a manner similar to that in other children, adapted to the specific clinical situation.

While routine antithrombotic prophylaxis in children with cancer and CVCs is not recommended, current practice and available data regarding routine thromboprophylaxis in children with cancer is mixed. The data from three prospective trials are summarized in Table 13.3. The PARKAA trial compared the use of regular AT infusions during IR. Although there were fewer thrombotic events in the treatment group, the study was underpowered to detect statistical significance [49]. Interestingly, Farinasso et al. found a 73% rate of CVC-related thrombosis (both symptomatic and asymptomatic) in pediatric patients receiving AT infusions to maintain minimum AT levels while receiving L-asparaginase as a matter of routine practice [10].

Ruud et al. saw no effect of low dose warfarin in children with CVCs and either hematologic or solid malignancies, with screening of subjects at regular intervals over a 6-month period [94]. However, on average, the patients treated with warfarin had sub-therapeutic 25% of the study period. A remarkable finding of the study was that the majority of thromboses were transient: only 6 out of 16 thromboses diagnosed at 1 month were still present at the 6-month examination. Meister et al. combined infusions of AT and subcutaneous

Table 13.3 Prospective trials of thromboprophylaxis in pediatric cancer patients. ALL = Acute lymphoblastic leukemia; AT = antithrombin concentrate; LMWH = low molecular weight heparin (enoxaparin)

Study	Diagnosis	Treatment	Control	Patients (tx/control)	Thrombosis (tx/control)	Comments
PARKAA Mitchell, et al. [49]	ALL	AT	No Tx	20/60	28% vs 36.7%	All patients screened for thrombosis; includes asymptomatic events Underpowered to detect significant difference
Ruud, et al. [94]	Hematologic and solid tumors	Low dose warfarin	No Tx	29/33	48% vs 36%	All patients screened for thrombosis; includes asymptomatic events
Meister, et al. [95]	ALL	AT and LMWH	AT	41/71	0% vs 12.7%	Symptomatic events only Historical controls treated with different steroid regimen

enoxaparin during induction and re-induction therapy for ALL, as it had been demonstrated in vitro that the effect of low molecular weight heparin (LMWH) in children was significantly decreased during ALL therapy [6, 95]. No thrombotic events were noted in the treatment group, as compared with historical controls treated with AT alone. The study was weakened to some degree by the different treatment protocols for the controls and study group and that only symptomatic thromboses were studied.

The agents most commonly used for the treatment of thrombotic episodes in children are unfractionated heparin (UFH), LMWH (the greatest published experience with enoxaparin) and warfarin. A single attempt to compare the efficacy of LMWH to UFH/warfarin in the treatment of DVT in children was closed before achieving sufficient statistical power, due to poor enrollment [96]. However, LMWH is recommended for first line and short-term treatment of TEE in children with cancer, presumably due to the stable pharmacokinetics, lack of interaction with other medications and relative ease of adjustment periprocedurally and during periods of thrombocytopenia [93].

In dose-finding and pharmacokinetic studies of anticoagulant agents in children, a common observation was the requirement for greater doses per kilogram of body weight in younger children to achieve and maintain similar monitoring parameters (PTT, INR, anti-Xa level) as in adults. In a prospective cohort study, Andrew et al. found that infants required approximately 50% higher heparin infusion rates to maintain the same PTT as in adults, presumably due to higher clearance rates [97, 98]. It was not until adolescence that dose requirements equaled that of adult subjects. A similar increased dose requirement was found for enoxaparin, however only in children less than 2 months of age [99]. Even with weight- and age-based dose adjustment, there remains sufficient inter-individual variability in response that monitoring of anti-Xa levels in children treated with LMWH is recommended [93]. In children treated with warfarin, dose requirements to maintain an INR varied from 0.33 mg/kg in infants to 0.09 mg/kg in adolescent subjects [100].

While the use of thrombolytics in children with both arterial and venous thrombosis is increasing, adequate large, well-design prospective clinical trials have not been performed. The majority of data in the literature consists of case reports, small series and retrospective studies. Practically, tPA is the agent readily available in North America for thrombolytic therapy, most often administered systemically in children. Decreased levels of plasminogen in the first 6 months of life dampen the response to tPA, although the response in older children is similar to adults [101]. Several comprehensive retrospective reviews found a wide spectrum of dosing regimens, with infusion rates varying from 0.04 to 3.75 mg/kg/h and incidences of bleeding complications of from 6% to 68% [102–104]. Recently, the trend has been towards lower doses of systemic tPA therapy, as it appears offer similar efficacy, with less risk of bleeding [105, 106].

In summary, both the amount and the quality of data related to thrombotic disease in children with and without cancer have increased significantly.

Thrombotic events in pediatric oncology patients are common and potentially serious events. There remain many questions regarding the etiology of these events, as well as the most appropriate means of prevention and treatment. Given the continued increase in survival of pediatric oncology patients, it is imperative that well-designed and adequately-sized controlled trials be conducted to address these issues of growing importance.

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Chapter 14
Treatment of Venous Thrombosis

Agnes Y. Y. Lee

14.1 Introduction

Approximately 20% of all cases of venous thromboembolism (VTE) are related to underlying malignancy. Many of these patients are elderly, receiving anticancer therapy, and have advanced malignancy and other comorbid diseases. Consequently, managing VTE in these patients is a challenging task because they have high risks of recurrent thrombosis and anticoagulant-related bleeding. Quality of life is also an important consideration when planning therapy, especially if the patient's life expectancy is short. Of the currently available anticoagulants, monotherapy with a low-molecular-weight heparin (LMWH) is the recommended treatment of choice over unfractionated heparin and warfarin. Preliminary evidence has also suggested that LMWHs may improve survival in cancer patients. However, many aspects of treatment, such as duration of therapy, remain unstudied. This chapter will review the general approach to treating cancer patients with acute deep vein thrombosis (DVT) or pulmonary embolism (PE) and briefly discuss some controversial areas of management.

14.2 Initial Therapy of VTE

There are two major objectives in treating VTE: to relieve the acute symptoms of DVT and/or PE, and to reduce the risk of recurrent thrombosis, including fatal PE, and other long-term consequences such as post thrombotic syndrome. To achieve these goals, anticoagulant therapy has been the gold standard for decades. The traditional treatment regimen consists of an initial phase with intravenous infusion of unfractionated heparin (UFH), followed by long-term

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therapy with a vitamin K antagonist (VKA), such as warfarin. This approach is needed because VKAs have a delayed onset of action and so UFH is needed upfront to achieve an immediate anticoagulant effect. To ensure that adequate anticoagulant response is achieved with UFH, dose adjustment based on the activated partial thromboplastin time (APTT) is required. To measure the anticoagulant effect of warfarin, monitoring of the international normalized ratio (INR) is necessary. Consequently, treatment of VTE can be simplified with the use of a single anticoagulant that has a rapid onset of action, does not require laboratory monitoring, and that is safe and well tolerated with prolonged exposure.

14.2.1 Choice of Anticoagulants

The anticoagulants currently available for the initial treatment of acute VTE are UFH, LMWH and fondaparinux. All three agents augment the inhibitory action of antithrombin by binding to the enzyme via a unique pentasaccharide sequence. In contrast to UFH and LMWH, which inhibit both thrombin and activated factor X (fXa), fondaparinux is a selective inhibitor of fXa with no activity against thrombin.

In trials that included patients with and without cancer, weight-adjusted doses of LMWH given subcutaneously once or twice daily were more effective than intravenously administered UFH [1]. LMWH was also found to produce a significantly lower risk of major hemorrhage and a lower incidence of overall mortality during follow-up. Furthermore, LMWH can be given in an outpatient setting without the need for laboratory monitoring and is infrequently associated with heparin-induced thrombocytopenia [2]. A recent randomized trial showed that UFH can be given subcutaneously using weight-based dosing but relatively large volumes of injections are required twice a day [3]. In many developed countries, outpatient LMWH is considered the standard of care for the initial treatment in patients with DVT or hemodynamically stable PE. Fondaparinux has comparable efficacy and safety with the heparins and is approved in some countries for the initial therapy of VTE [4, 5].

However, whether LMWH, UFH and fondaparinux are equally effective and safe in patients with cancer has not been formally investigated. Patients with advanced disease or significant co-morbid conditions were generally excluded from participation in clinical trials that compared these anticoagulants. Based on published data extracted from trials that reported on the outcomes of the subgroup of cancer patients, it appears that weighted-adjusted, subcutaneous doses of LMWH and APTT-adjusted intravenous infusions of UFH have similar efficacy in patients with and without cancer [6–9]. Data on the bleeding risk of therapeutic doses of these anticoagulants in cancer patients have not been published. Nonetheless, it is reasonable to assume that UFH and LMWH are comparable in their efficacy in preventing recurrent VTE and in their risk of major hemorrhage in most patients with advanced disease. The experience with fondaparinux in cancer patients is very limited.

Aside from efficacy, practical aspects of therapy are important considerations for patients and their families. LMWH is more attractive than UFH because outpatient LMWH therapy eliminates the need for routine hospitalization and requires only once-daily injections. LMWH also comes in precalibrated, prefilled syringes, which reduces the risk of dosing errors compared with having to draw up UFH from multi-dose vials. Cohort studies have shown that cancer patients can be treated safely at home with LMWH [10–13]. The vast majority of patients are able to perform self-injections when they are given adequate support and appropriate education. For patients who do require hospitalization to control severe symptoms or receive additional supportive therapy, LMWH is also more attractive than UFH because it reduces nursing time, obviates the need for venepunctures to draw blood samples for monitoring of the anticoagulant effect, and may reduce drug dosing errors. Cost-minimization modeling has also shown that LMWH is less expensive than UFH for the inpatient treatment of DVT among cancer patients [14].

New oral anticoagulants in advanced stages of development have not been studied in patients with cancer. It is important that new agents are tested specifically for treatment of cancer-associated thrombosis because cancer patients tend to have more aggressive forms of VTE and they experience a higher likelihood of drug interactions and organ dysfunction. Hence, anticoagulant dosing, duration of therapy and drug-related toxicities are potentially very different between patients with and without cancer.

14.2.2 Once or Twice Daily Dosing of Low-Molecular-Weight Heparin

For initial treatment, subcutaneous LMWH may be administered either once daily or twice daily and some agents have regulatory approval for both regimens. Significant differences in efficacy and safety between these regimens have not been shown, although some studies have suggested that twice-daily regimens may be more efficacious [9, 15]. It is possible that twice-daily administration of a LMWH avoids high peak and low trough levels, thus providing a more steady state of anticoagulation, and leading to a lower incidence of recurrent VTE and bleeding. This hypothesis, however, has not been properly tested. Given the current evidence available, once-daily injection is safe and effective. It is reasonable to consider twice-daily injections in patients who develop recurrent VTE while receiving therapeutic doses of LMWH once a day.

14.3 Long-Term Therapy

To reduce the risk of recurrent thrombosis, anticoagulant therapy must be continued for a minimum of 3 months. For decades, vitamin K antagonists were the only feasible treatment option, but LMWH is now the preferred treatment for long-term anticoagulant therapy in patients with cancer.

14.3.1 Vitamin K Antagonists (VKA)

Warfarin is the most commonly used VKA worldwide [2]. When a patient is diagnosed with an acute DVT or PE, warfarin is administered along with a heparin and is continued for 3 months or longer. Due to differences in the anticoagulant response between patients and within patients over time, dose adjustments are needed based on the INR. For the treatment of VTE, the target therapeutic INR range is 2.0–3.0. When VKA therapy is used following therapeutic doses of UFH, LMWH or fondaparinux, the 3-month risk of symptomatic recurrent VTE is approximately 3–4% for patients without cancer.

However, in patients with cancer, warfarin therapy is problematic. Due to its pharmacology, unpredictable anticoagulant responses can result from drug interactions, changes in vitamin K status, liver dysfunction, and gastrointestinal disturbances such as vomiting and diarrhea. Furthermore, because vitamin K antagonists have a delayed onset of action and prolonged clearance of the anticoagulant effect, they are difficult to manage in patients who require periodic invasive procedures (e.g., therapeutic paracentesis, lumbar puncture) or experience frequent episodes of chemotherapy-induced thrombocytopenia.

Cancer patients also experience recurrent VTE and have a high risk of major bleeding while on warfarin therapy. According to prospective studies, the annual risk of recurrent VTE is 21–27% in cancer patients while on warfarin therapy (Fig. 14.1) [16, 17]. This is two- to threefold higher than in patients without cancer. Recurrent VTE can also occur when the INR is therapeutic [17]. Cancer patients on oral anticoagulant therapy also have an annual risk of major bleeding of 12–13%, vs 3–4% for patients without cancer (Table 14.1) [16, 17]. In contrast to patients without cancer, the risk of bleeding does not correlate with the INR level and it continues to increase over the course of therapy.

Lastly, the social burden of warfarin is significant in patients with cancer. Having to undergo venipuncture is painful for patients who have poor venous access after many cycles of chemotherapy, and going to a laboratory or the hospital for weekly monitoring is burdensome because many of these patients are dependent on others for transportation. The frequent dosing changes are also confusing and frustrating for elderly patients, who are often on many other medications.

14.3.2 Low-Molecular-Weight Heparin

In contrast to VKAs, LMWH does not require routine laboratory monitoring and has minimal drug interactions. The parenteral route of administration also ensures drug delivery, especially in patients who are unable to tolerate oral intake or have significant gastrointestinal problems. Finally, dose adjustments or withholding of LMWH can be made readily to accommodate thrombocytopenia or invasive procedures. However, LMWH should be avoided in most

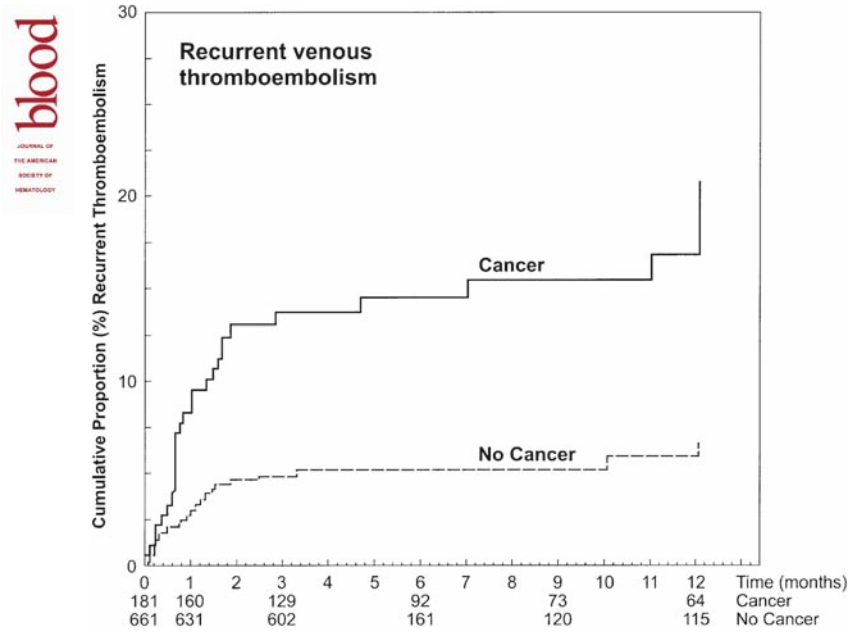

Fig. 14.1 Cumulative risks of recurrent thrombosis among patients with and without cancer while on vitamin K antagonist therapy. Modified from Prandoni et al. [16] Reproduced with permission

Table 14.1 The incidence of recurrent venous thromboembolism and major bleeding in relation to the INR [17]

	International normalized ratio range		
Recurrent thrombosis	<2.0	2.1–3.0	>3.0
Patients with cancer: no. of events (per 100 patient-years)	54.0	18.9	18.4
Patients without cancer: no. of events (per 100 patient-years)	15.9	7.2	6.4
Major bleeding			
Patients with cancer[a]: no. of events (per 100 patient-years)	30.6	11.2	0.0
Patients without cancer: no. of events (per 100 patient-years)	0.0	0.8	6.3

[a] Small number of bleeding events overall may account for paradoxical decrease in bleeding with increase in INR

patients with severe renal dysfunction. It is also associated with a low risk of heparin-induced thrombocytopenia and possibly osteoporosis [18–20].

To date, a number of open-label trials have compared different LMWHs with VKAs for long-term treatment of VTE (Table 14.2). The largest of these studies is the CLOT trial. This study randomized 676 cancer patients with acute VTE to standard treatment with dalteparin followed by VKA therapy or to

Table 14.2 Randomized controlled trials evaluating monotherapy with LMWH for long-term treatment of VTE in cancer patients

Study	N[a]	Treatment regimens	Recurrent VTE during treatment	Major bleeding during treatment	Death	P-value for primary outcome
Lee [21]	336	Initial: dalteparin (200 IU/kg × 5–7 days) Long-term: coumarin (INR 2–3 × 6 months)	17%	4%	41%	0.002[c]
	336	Dalteparin (200 IU/kg OD × 1 month) then Dalteparin (~150 IU/kg OD × 5 months)	9%	6%	39%	
Meyer [23]	71	Initial: enoxaparin (1.5 mg/kg OD × >4 days) Long-term: warfarin (INR 2–3 × 3 months)	21.1%[b]		22.7%	0.09[d]
	67	Enoxaparin (1.5 mg/kg OD × 3 months)	10.5%[b]		11.3%	
Deitcher [24]	30	Initial: enoxparin (1 mg/kg BID × 5 days) Long-term: warfarin (INR 2–3 × 180 days)	10.0%	2.9%	8.8%	
	29	Initial: enoxaparin (1 mg/kg BID × 5 days) Long-term: enoxaparin (1 mg/kg OD × 175 days)	6.9%	6.5%	6.5%	NS[e]
	32	Initial: enoxaparin (1 mg/kg BID × 5 days) Long-term: enoxaparin (1.5 mg/kg OD × 175 days)	6.3%	11.1%	19.4%	
Hull [25]	100	Initial: IV UFH (APTT adjusted × 6 days) Long-term: warfarin (INR 2–3 × 3 months)	10%	7%	19%	NS[f]
	100	Tinzaparin (175 IU/kg OD × 3 months)	6%	7%	20%	

[a] N, number of patients evaluable for the primary outcome in the respective treatment groups.
[b] Combined events of recurrent VTE and major bleeding
[c] Primary outcome of cumulative incidence of objectively, documented recurrent VTE was assessed at 6 months. Hazard ratio in the dalteparin group as compared with the coumarin group was 0.48 (95% CI 0.30 to 0.77), log-rank P = 0.002
[d] Primary outcome was a composite endpoint of objectively, documented recurrent VTE or major bleeding. There was no statistically significant difference between treatment groups with a relative risk of 2.02 (95% CI 0.88 to 4.65)
[e] Primary objective was to evaluate the feasibility of recruitment and compliance with long-term (180 days) injections of enoxaparin. No difference was observed in overall compliance with an average rate of 95% amongst all groups
[f] Primary outcome measure of objectively, documented recurrent VTE or death was assessed at 3 months. There was no statistically significant difference between treatment groups (difference –4.0; 95% CI –12.0 to 4.1)
NS, not statistically significant

experimental therapy with dalteparin alone for 6 months [21]. Patients in the dalteparin group received therapeutic doses at 200 IU/kg once daily for the 1st month and then 75–80% of the full dose for the next 5 months. Patients in the control group, received dalteparin 200 IU/kg once daily for a minimum of 5 days and a VKA at doses to target the INR value at 2.5 for 6 months. All patients were followed for symptomatic recurrent VTE, bleeding and death. These outcomes were centrally adjudicated by an expert committee masked to treatment assignment. Over the 6-month treatment period, 27 of 338 in the dalteparin group and 53 of 338 in the VKA group had symptomatic, recurrent VTE. The cumulative risk of recurrent VTE was reduced from 17% in the oral anticoagulant group to 9% in the dalteparin group, resulting in a statistically significant risk reduction of 52% (log-rank P value 0.002) (Fig. 14.2). The INR values were therapeutic or higher during 70% of the total treatment time, indicating that patients in the control group were adequately treated. As compared with VKA, one episode of recurrent VTE is prevented for every 13 patients treated with dalteparin. Overall, there were no differences in major or any bleeding; 6% of patients in the dalteparin group vs 4% of the control group experienced major bleeding. By 6 months, about 40% of the patients in each group had died; 90% of the deaths were due to progressive cancer. Thus, the CLOT study unequivocally demonstrated the superior efficacy of dalteparin over VKAs for preventing recurrent VTE. A prospective cohort study later evaluated a fixed dose of dalteparin once daily for long term treatment. The study included 203 patients with metastatic cancer who received an initial 7-day

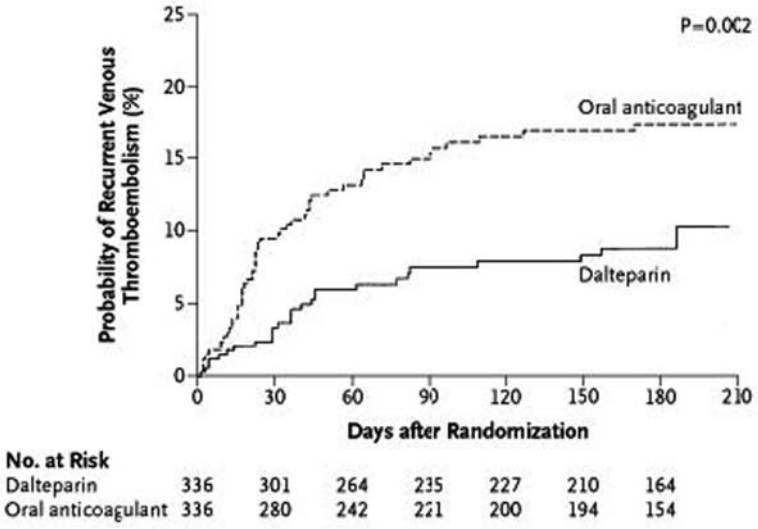

Fig. 14.2 Cumulative risk of recurrent symptomatic thromboembolism among cancer patients treated with dalteparin or vitamin K antagonist therapy [21]. Reproduced with permission

course of weight-adjusted dalteparin followed by dalteparin 10,000 IU once daily, regardless of the patients' weight. During 3 months of follow-up, 18 patients (9%) developed recurrent VTE and 11 patients (5%) had major bleeding [22].

Smaller trials have studied other LMWHs. The CANTHANOX trial compared 3 months of standard warfarin therapy with enoxaparin in cancer patients with proximal DVT, PE or both [23]. The study was closed prematurely because of slow recruitment and only 147 patients were randomized. After 3 months of treatment, 15 of 71 patients in the warfarin group had recurrent VTE or major bleeding, as compared with 7 of 67 patients assigned to receive enoxaparin ($P = 0.09$). There were six fatal bleeding events in patients receiving warfarin and none in the enoxaparin group. Another study evaluating enoxaparin for long-term treatment included 101 cancer patients with VTE. Two different doses of enoxaparin were tested, but the study was underpowered to demonstrate any difference between warfarin and enoxaparin [24].

Lastly, a smaller randomized trial compared tinzaparin with warfarin for long-term use in cancer patients [25]. In this study, 100 patients were randomized to receive 3 months of tinzaparin 175 units/kg once daily and 100 were assigned to receive usual care with intravenous UFH then warfarin. At the end of the 3-month treatment period, 6% of patients in the tinzaparin group vs 10% of the usual care group had developed symptomatic recurrent VTE. This difference was not statistically significant (–4.0%; 95% confidence interval –12.0% to 4.1%). Both groups had similar incidences of major bleeding and overall mortality.

Based on the above evidence, LMWH is recommended for extended treatment in cancer patients by the 2008 American College of Chest Physicians Consensus Guidelines, the British Society of Haematology, the National Comprehensive Cancer Network, and the American Society of Clinical Oncology guideline as the treatment of choice for cancer patients with VTE [2, 26–28]. Currently, dalteparin is the only LMWH to receive regulatory approval for the extended treatment of VTE in patients with cancer. The major obstacle to the use of LMWHs is the cost of the drugs. Because of the substantial pharmacy costs of extended LMWH prophylaxis in the US, this treatment is relatively expensive compared with warfarin [29]. Many patients find it prohibitive to use LMWH long-term because of limited insurance coverage and reimbursement policies.

14.3.3 Pentasaccharides

Idraparinux is a long acting pentasaccharide derivative of fondaparinux and has been evaluated as an alternative to VKA therapy. With a long plasma half-life of about 80 h, idraparinux is given only once weekly via subcutaneous injection. In a multicenter study, 1,215 patients were randomized to receive idraparinux 2.5 mg or placebo subcutaneous injections after completing 6 months of therapy with an anticoagulant [30]. After 6 months of study

treatment, 1.0% of patients in the idraparinux group vs 3.7% in the placebo group had recurrent VTE ($P = 0.002$), while 1.9% vs none had major bleeding, respectively ($P < 0.001$). There were 120 patients with cancer in the study but their results were not reported separately. Given the increased risk of bleeding, the lack of a specific antidote, and the prolonged duration of anticoagulant effects, idraparinux is not be suitable for use in cancer patients.

14.4 Duration of Therapy

The length of treatment with an anticoagulant for the secondary prevention of recurrent VTE has not been studied in patients with cancer or advanced disease. These patients are usually excluded from clinical trials studying optimal duration and they frequently have or develop medical complications that make continuation of anticoagulation difficult. Therefore, it may not be appropriate to extrapolate the results from clinical trials of largely patients without cancer and apply them to patients with malignancy.

However, based on the consensus that patients with ongoing or irreversible risk factors for VTE need an extended duration of anticoagulant therapy, most cancer patients receive anticoagulation for longer than 6 months. Beyond this period, "indefinite" therapy is traditionally recommended in patients with metastatic disease because their risk of recurrent VTE is high. Indeed, the risk of recurrent VTE is 9–17% while on anticoagulant therapy. In patients without metastases, anticoagulant treatment is continued if cancer is clinically detectable or while the patient is receiving antitumor therapy. To-date, biochemical markers or imaging assessment have not been evaluated for guiding duration of anticoagulation in this population.

Patients should be re-evaluated frequently to assess the risk-benefit ratio of continuing anticoagulant therapy. Besides the risks of recurrence and bleeding, the patient's quality of life, life expectancy and preference should be taken into consideration. Without evidence from randomized trials or validated methods of identifying patients who would benefit from extended secondary prevention, anticoagulant therapy beyond the usual 6-month period must be carefully assessed and tailored for each individual patient.

14.5 Challenges in the Treatment of Cancer-Associated Thrombosis

14.5.1 Treatment of VTE in Patients with Bleeding

Treatment of VTE in patients with active bleeding or a high risk of bleeding is particularly challenging and there are no guidelines for management. Clearly, treatment must be individualized and it is important to consider the overall

status of the patient. It is essential to discuss the risks and benefits of the available options with the patient and choose the most acceptable management.

Anticoagulant therapy is generally contraindicated in patients with bleeding. However, minor or nuisance bleeding in patients with cancer should not prevent the use of anticoagulants, especially in patients with symptomatic proximal DVT or PE involving segmental or more central pulmonary arteries. In cases where the bleeding can be easily monitored and is arising from a source that is not likely to be life-threatening (e.g., nosebleeds), LMWH therapy can be started (or continued) and the patient followed closely. If the bleeding is from a mucosal surface due to tumor invasion (e.g., bladder carcinoma), more caution is required. In such situations, anticoagulant therapy can be started using prophylactic or subtherapeutic doses. If bleeding is not aggravated and the hemoglobin remains stable, the dose of LMWH can be slowly increased towards therapeutic levels. Of course, measures should be taken to stop and treat the source of bleeding, if possible.

When active bleeding is potentially life threatening (e.g., intracranial bleed, upper gastrointestinal bleeding) anticoagulant therapy is not recommended. Under these circumstances, inserting an inferior vena cava (IVC) filter can be considered if the patient has an acute proximal DVT. Permanent or retrievable filters are available but they have not been compared directly for efficacy, safety and durability. In situations where the risk of thrombus embolization is high and temporary cessation of anticoagulant therapy is needed (e.g., surgery), retrievable filters may be preferred over a permanent filter. This will allow removal of the filter when the bleeding challenge is over. However, there is no evidence that filter placement will improve the clinical outcome or prognosis of in such situations. Indeed, this approach does not suppress the thrombotic process or relieve the symptoms of VTE. Also, fatal PE can occur from thrombus formation proximal to the filter and this complication has been reported in cancer patients. In the end-of-life setting, insertion of a filter should be avoided as benefit to the patient has yet to be demonstrated.

14.5.2 Treatment of Recurrent VTE

LMWH has been shown in small case series to be effective in treating patients who develop recurrent VTE while on warfarin therapy [31] but published data are not available for managing patients who develop recurrence while on LMWH. Feasible approaches include increasing the dose of LMWH, changing to twice-daily injections, or switching to intravenous or subcutaneous UFH. From a convenience point of view, increasing the dose of LMWH is the most attractive and logical option. In patients who develop recurrence while receiving a subtherapeutic dose of LMWH, the dose should be increased back to weight-based therapeutic levels. This is often sufficient to provide symptomatic relief and prevent further thrombus extension. In patients who develop

recurrence while receiving a therapeutic dose of LMWH, an empiric dose increase of 25% is reasonable. Also, the anti-factor Xa level may be useful to guide dose increases in such cases. The therapeutic level for twice-daily dosing is 0.5–1.2 anti-Xa U/mL. It has been observed that patients who have recurrent VTE despite receiving standard weight-adjusted doses of LMWH tend to have subtherapeutic anti-factor Xa levels and often tolerate dose escalation without experiencing bleeding. The reason for the subtherapeutic anti-Xa level is unknown but it may be related to greater nonspecific binding of LMWH to acute phase reactants in sick patients or more rapid plasma clearance of the drug.

Heparin-induced thrombocytopenia (HIT) should be considered in all patients who develop recurrent thrombosis and an unexplained fall in the platelet count while receiving LMWH. Investigations for HIT should be done if the timing and the fall in platelet count are consistent with HIT, and a direct thrombin inhibitor or heparinoid should be used in place of the LMWH until the diagnosis of HIT is excluded.

Another option that is often recommended in patients with recurrent VTE is insertion of an IVC filter. However, the indications, efficacy and safety of this therapeutic modality are poorly studied [32]. In the only randomized trial of patients with proximal DVT, it was shown that an IVC filter in combination with therapeutic anticoagulant therapy can reduce the short-term risk of PE compared with anticoagulation alone, but patients with filters also had a higher incidence of recurrent DVT at 2 years of follow-up, such that the total number of patients with recurrent thrombotic events are the same between the two treatment groups [33]. Furthermore, there were no differences in symptomatic PE at 3 months, 1 year and 2 years. At 8 years of follow-up, there were fewer patients with symptomatic PE in the filter group but there was no difference in overall survival between the filter and nonfilter groups [34]. A large population-based study also failed to find a significant reduction in the incidence of rehospitalization for PE in patients following IVC filter insertion [35]. Clearly, the randomized trial established that an IVC filter does not provide additional benefit to anticoagulation to patients with proximal DVT, but it remains unknown whether filters are effective in preventing clinically significant or fatal PE when anticoagulants cannot be used.

Evidence in the oncology population is even weaker. In a retrospective study, 32% of cancer patients with VTE and inferior vena cava filters developed recurrent DVT [36]. It is possible that filters enhance the risk of recurrent DVT in cancer patients because of the hypercoagulable state in these patients, but patient selection for filter insertion can bias these results. In a retrospective study of 116 patients with cancer and filter placement, survival rates were 69% at 30 days, 49% at 3 months and 27% at 1 year [37].

Overall, given that there is no evidence that these costly, invasive devices reduce symptomatic PE, alleviate symptoms of VTE or improve overall survival, the use of vena caval filters should be avoided in patients who can tolerate anticoagulant therapy. In particular, in patients with advanced disease, poor

life expectancy and absolute contraindications to anticoagulation, comfort measures only is a reasonable course.

14.6 Quality of Life

Few studies have addressed the quality of life in patients receiving long-term anticoagulation [38, 39]. In one report, 40 palliative care patients who were receiving treatment for VTE preferred LMWH over warfarin, which was felt to have a negative impact on quality of life [38]. In contrast to expectations, patients were not distressed by daily injections and did not perceive this as an added burden. Instead, patients reported that LMWH was simpler to administer, provided them freedom from laboratory testing, hospitals, and even worry. A substudy in a randomized trial found that post-phlebitic syndrome and ulcer formation occur less frequently in patients treated with LMWH than with warfarin [40].

In patients receiving end of life care, it is imperative that the patient and family members be informed of the risks and benefits of starting or continuing anticoagulant therapy. In such patients, the main goal of treatment is to provide symptomatic relief. Hence, it is of questionable benefit to administer anticoagulants if such patients are asymptomatic from their DVT or PE. Daily injection of an LMWH is relatively straight forward but can exacerbate bleeding, while administration of warfarin with laboratory monitoring is burdensome and should be avoided. More aggressive interventions, such as insertion of an IVC filter, are strongly discouraged in this setting.

Frank and thorough discussions with the family and patient are needed to understand their needs and formulate a care plan that maximizes quality of life.

14.7 Antineoplastic Potential of LMWHs

In the palliative setting, whether LMWH or other anticoagulants provide a survival benefit is of minimal relevance. However, it is worth noting that experimental studies and clinical trials have shown that LMWH use is associated with a reduction in mortality, particularly in patients with limited or early stage malignancy [41–45] (see Chapter 15). The most compelling evidence comes from two clinical trials. In the MALT study, 302 patients with non-curable solid tumors were randomized to receive nadroparin or placebo for 6 weeks [44]. A statistically significant improvement in median survival was associated with nadroparin. In a Turkish study, 84 patients with newly diagnosed small cell lung cancer were randomized to receive standard chemotherapy with or without dalteparin [42]. Progression-free survival and overall survival were better in patients who received dalteparin. Although these results need to be confirmed in larger studies and in different tumor types, they do support the concept that activation of coagulation is intrinsically involved in tumor growth

and that LMWHs is able to interrupt these critical processes. Of note, the survival difference observed in the clinical trials have occurred after the discontinuation of the LMWH, indicating that the improvement in survival is not due to a reduction in fatal PE. Multiple non-anticoagulant related mechanisms have been proposed, but the exact mechanisms have not been elucidated.

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Chapter 15
Effects of Anticoagulants on Cancer: Heparins

Graham F. Pineo and Russell D. Hull

15.1 Introduction

The association of venous thrombosis and cancer has been recognized since the description by Armand Trousseau in 1865 [1]. In fact, Buller and colleagues have discovered an earlier report by Bouillaud in which he described three patients with cancer and deep vein thrombosis in 1823, some 42 years before the publication by Trousseau [2]. In the past two to three decades there has been increasing interest in the diagnosis, prevention, and treatment of venous thromboembolism associated with cancer [3]. The heparins [unfractionated heparin (UFH) and low molecular weight heparin (LMWH)] have proven efficacy and safety for the prevention of venous thromboembolism (VTE) in a wide variety of cancers including both solid tumors and hematologic malignancies. Thus either UFH or LMWH are recommended for the prevention of VTE in hospitalized cancer patients, particularly in association with other risk factors for VTE [4, 5] and for cancer patients undergoing surgical procedures where UFH and more particularly LMWH are recommended for the initial and extended prophylaxis of VTE [6]. Both UFH and more particularly LMWH are widely used for the initial treatment of VTE (deep vein thrombosis (DVT) and/or pulmonary embolism (PE), including those patients with the VTE associated with cancer [7]. More recently LMWH has been recommended for the long-term treatment of VTE in cancer patients in place of the oral anticoagulants with proven efficacy in reducing the incidence of recurrent VTE with no increased risk for major bleeding [7–9].

However, what has attracted more interest recently, is the evidence that the heparins, and in particular LMWH, can have a positive effect on the survival of patients with a wide variety of malignancies, particularly if they are nonmetastatic or are associated with a more favourable prognosis at the time of initiation of treatment [10–13]. This chapter will review the evidence supporting the

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beneficial effect of the heparins in clinical trials in cancer patients as well as data derived from in vitro and in vivo laboratory studies and will review the available evidence supporting this beneficial effect.

15.2 Anticoagulants as Chemotherapeutic Agents

The heparins are a heterogeneous mixture of glycosaminoglycans with UFH and LMWH being the products which have been used therapeutically [14]. Additional members of the glycosaminoglycans family include heparan sulfate, chondroitin sulfate, dermatin sulfate and hyaluronic acid. The glycosaminoglycans are linear carbohydrate polymers composed of alternating uronic acid and hexosamine saccharides linked by glycocidic linkages. UFH has a molecular weight ranging from 3,000 to 30,000 with approximately 45 monosaccharide chains. LMWH is produced by either enzymatic or chemical depolymerization of UFH and has less than 18 saccharide units and a mean molecular weight of approximately 5,000 Da; see Table 15.1. The anticoagulant effect of UFH depends on its ability to bind antithrombin and thereby inactivate a number of proteases in the coagulation pathway including thrombin and activated factors X, IX, II, and XII [14]. Heparin also inactivates heparin cofactor 2 through a second mechanism dependent upon electrostatic charge [14]. In addition to its antithrombotic effect, UFH binds to a wide variety of other proteins and cells based on electrostatic interactions and these interactions help explain the multiple nonanticoagulant properties of UFH [15–17].

LMWH with less than 18 saccharide unit side chains is less able to inactivate thrombin because the smaller fragments cannot bind simultaneously to antithrombin and thrombin [14, 15]. They thus have a more potent inhibitory effect on activated factor X, with the ratio being between 2:1 and 4:1 for inhibition of activated factor X vs thrombin. LMWHs are less likely to bind to the multiple proteins and cells as compared to UFH. Zacharski and Ornstein have summarized the various differences between UFH and LMWH which may have an impact on their differential capabilities as anticancer agents [17]. As an example, it has been shown that angiogenesis is inhibited by LMWH in experimental

Table 15.1 Commonly used low-molecular weight heparins

Agent	Molecular weight (mean and range)	Method of preparation
Dalteparin (fragmin)	5,000 (2,000–9,000)	Nitrous acid depolymerization
Nadroparin (fraxiparin)	4,500 (2,000–8,000)	Nitrous acid depolymerization
Enoxaparin (lovenox)	4,200 (3,800–5,000)	Benzylation followed by alkaline Depolymerization
Tinzaparin (innohep) depolymerization	4,500 (2,000–8,000)	Enzymatic with heparinase
Certoparin (mono-embolex)	6,000 (1,500–11,000)	Isoamylnitrite depolymerization

animals whereas heparins with molecular weight greater than 22 kDa actually stimulate angiogenesis [17]. These differential effects may explain the different outcomes in experiments where outcomes are determined by tumor metastases or increased markers of cell proliferation and migration.

Low molecular weight heparin can be rendered nonanticoagulant by various chemical treatments and these nonanticoagulant LMWH fractions have been demonstrated to retain their ability to suppress experimental tumor metastases in mice similar to the activity of LMWH (enoxaparin) [18]. Numerous other procedures have been used to produce modified heparins with little or no anticoagulant activity as well as synthetic compounds which mimic heparan sulfate and which consist of sulfated oligosaccharides [19–22]. All of these compounds have been shown to inhibit tumor cell growth and metastasis. In one such study, it was demonstrated that the inhibition of P-selectin correlated very well with tumor cell metastasis in the presence of UFH or the LMWH (nadroparin) whereas suppression with another LMWH (enoxaparin) or the synthetic pentasaccharide fondaparinux showed little suppression compared to controls [23].

It is not surprising that suppression of cancer cell proliferation and dissemination differs in different experimental models as well as in clinical trials with different antithrombotic agents. There is a complex interrelationship between various constituents of the hemostatic system including coagulation and fibrinolytic proteins and platelets and the growth and proliferation of cancer cells [3]. Therefore, as in other aspects of cancer pathology, it is unlikely that any single mechanism can explain the favourable effects of the heparins and related agents in the treatment of cancer. The most convincing evidence of their efficacy will have to come from well designed randomized clinical trials in human subjects with carefully defined tumor histology, extent of tumor burden and measurement of performance status.

15.3 Beneficial Effects of the Heparins on Survival in Cancer Patients

The suggestion that LMWH may be superior to UFH in prolonging survival in cancer patients with proximal DVT came from two randomized clinical trials comparing initial treatment with either UFH or LMWH followed by long term oral anticoagulant therapy in this population of patients. In the study by Prandoni et al. it was noted that there was a reduction in total mortality in the subgroup of VTE patients who had cancer at the time of enrolment if they received LMWH (nadroparin) instead of UFH [24]. At 3 months, 44% (8/18) of cancer patients died in the UFH group vs 7% (1/15) in the LMWH group $P = 0.021$. In the other clinical trial published by Hull et al. comparing LMWH (tinzaparin) with UFH for the initial treatment of proximal DVT, it was noted that 10 patients who received LMWH died during the 3-month treatment compared with 21 patients who received UFH (9.6%), a risk reduction of 51% ($P = 0.49$) [25]. The most striking difference was in the abrupt deaths in patients with metastatic

carcinoma. The majority of these deaths occurred within the first 3 months. When the cancer deaths were combined from these two studies there were 21/67 (31%) for UFH and 7/62 (11%) for LMWH; P = 0.005; 95% confidence intervals for the odds ratio 1.4–9.2 [26]. The difference in survival could not be attributed to thrombotic or bleeding events which happened with the same frequency in both groups. A number of meta-analyses of studies comparing LMWH with UFH for the initial treatment of VTE confirmed a significant decrease in mortality in the sub-group of patients with cancer [27].

Further evidence of a positive effect of LMWH on survival in cancer patients was seen in the study by von Tempelhoff [28]. In that study, LMWH (certoparin) 3000 units daily plus 2 placebo injections daily was compared with UFH 5000 units 3 times daily both for 7 days in patients undergoing surgery for breast cancer or pelvic cancer. There was no significance in mortality at 650 days or 1,050 days in the breast cancer group whereas there was a significant decrease in mortality favouring LMWH in the patients with pelvic cancer. At 650 days, the mortality rate was 8.7% in the LMWH group vs 28.6% in the UFH group, P = 0.01. However, there was no significant difference in mortality rates at 1,050 days.

The most convincing evidence supporting a beneficial effect of the heparins in improving survival in cancer patients comes from the randomized clinical trials specifically designed to compare UFH or LMWH with placebo in patients with local or metastatic cancer. Two studies included only patients with small cell lung cancer. In the study by Lebeau, the patients with limited or extensive small cell lung cancer were randomized to receive one of two chemotherapy regimens with either prophylactic doses of UFH or no additional treatment [29]. The patients who received UFH in addition to their chemotherapy had better median survival rates (317 days vs 261 days, P = 0.01) and better survival at 1, 2, and 3 years (40% vs 30%, 11% vs 9%, and 9% vs 6%, respectively). Sub-group analysis demonstrated that the survival benefit was confined to patients with limited disease but not for those with extensive disease.

The other study in patients with small cell lung cancer was carried out by Altinbas et al. and included patients with both limited and extensive disease [30]. In this study, LMWH (dalteparin 5000 units daily) was compared with placebo for 18 weeks or less if disease progressed and these were given in combination with chemotherapy. There was a significant increase in the 1 year survival (51.3% in the LMWH group vs 29.5% in the controlled group, P = 0.01) and 2 year overall survival (17.2% in the LMWH group vs 0% in the control group, P = 0.01).

The other three studies were carried out in patients with either local or metastatic malignant disease and included a variety of tumor groups. In the FAMOUS Study, Kakkar et al. compared LMWH (dalteparin 5000 units daily) with placebo for 12 months with no restriction on concomitant chemotherapy or radiotherapy [31]. A wide variety of advanced local or metastatic tumors were included and the outcomes were mortality at 12, 24, and 36 months. At 12 months there was a nonsignificant 5% improvement in survival in the patients receiving LMWH. However, a post hoc analysis of patients with a good prognosis who survived beyond 17 months revealed that

the Kaplan-Meier survivals at 24 and 36 months were 78% vs 55% and 60% vs 36%, respectively; P = 0.03.

In the study by Klerk, nadroparin given in treatment doses (9500 units twice daily for 14 days and once daily for another 4 weeks) was compared with placebo in a variety of locally advanced or metastatic solid tumors [32]. Attending physicians determined whether the patients had a favourable or non-favourable prognosis at the outset of the study. The main outcome was mortality at 6, 12, and 24 months. The median survival favouring LMWH was 8.0 months vs 6.6 months in the placebo group, P = 0.02. In the sub-group of patients with an expected life expectancy of more than 6 months at randomization, the median survival was 15.4 months in the LMWH group vs 9.4 months in the placebo group, P = 0.01.

Sideras et al. compared the effect of dalteparin 5000 units daily with placebo in patients receiving chemotherapy for breast, colon, lung, or prostate cancer for an indefinite duration [33]. Part way through the study the control injections were discontinued so that LMWH was compared with standard care. The outcome was mortality at 12, 24, and 36 months. In this study, there was no difference in the median survival for the blinded or unblinded LMWH groups compared with placebo or no treatment. In the three studies which designated patients as having limited disease or favourable prognosis vs all others, there was a significant difference favouring the UFH or LMWH groups (risk reduction 0.47; 95% CI 0.30–0.75); see also Table 15.2 [10].

In all of the studies comparing UFH or LMWH with placebo there was an increase incidence of major bleeding, but this increase was not significant with either UFH or LMWH.

The results of the randomized clinical trials in cancer patients are supported by data from clinical trials designed to compare the efficacy of long term LMWH with initial UFH or LMWH followed by oral anticoagulants in patients with cancer and VTE. In the study by Lee et al., patients with VTE and cancer were randomized to receive dalteparin 200 units/kg daily for 1 month followed by 150 units/kg for 5 months or LMWH initially followed by oral anticoagulants for 6 months [34]. The main outcome was recurrent VTE. There was no benefit demonstrated in terms of survival in the overall population at 1 year. However, in a sub-group analysis of patients without metastatic disease at randomization, the probability of death at 12 months was 20% in the dalteparin group compared with 36% in the oral anticoagulant group (hazard ratio 0.50; 95% CI 0.27–0.95; P = 0.03). In those with metastatic cancer there was no difference in the mortality rates. In a smaller study, Meyer et al. compared the effects of LMWH (enoxaparin 1.5 mg/kg once daily) with initial UFH followed by warfarin for 3 months in patients with VTE and cancer [35]. Although the differences were not significant, 15 of 75 patients receiving enoxaparin died compared with 26 of 75 patients who received oral anticoagulation.

Although the following two studies were less rigorous than the randomized clinical trial, they were noteworthy in that they were carried out in patients with advanced pancreatic cancer which carries a poor prognosis. In the study by Icli et al., consecutive patients with advanced pancreatic adenocarcinoma were treated

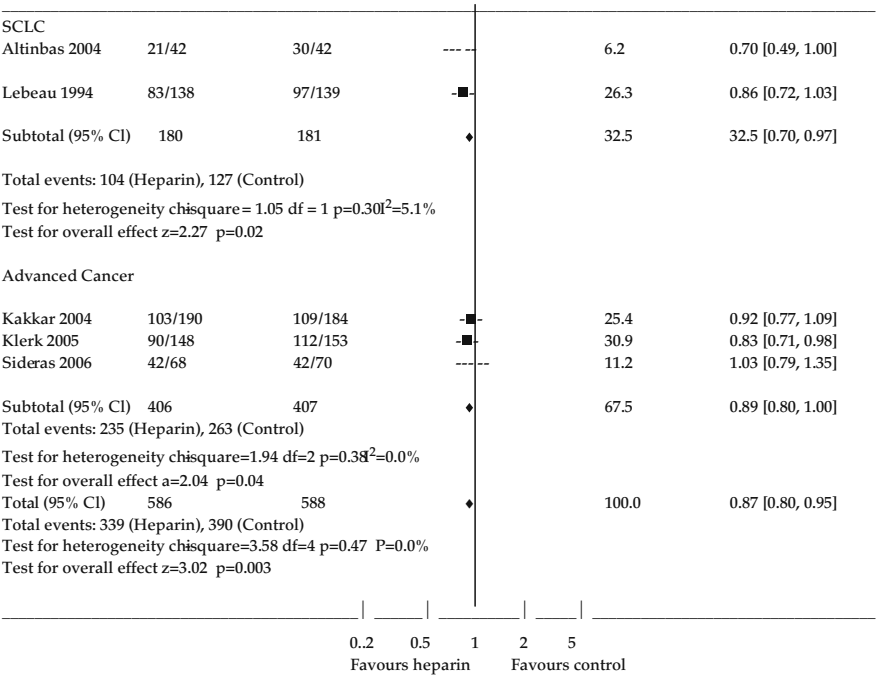
Table 15.2 Comparison heparin vs placebo: outcome of mortality at 12 months

Review: Parenteral anticoagulation for prolonging survival in patients with cancer who have no other indication for anticoagulation

Comparison: Heparin vs placebo

Outcome: Mortality at 12 months

Study	Heparin n/N	Control n/N	Relative Risk(Random) 95% CI	Weight (%)	Relative Risk (Random) 95% CI
SCLC					
Altinbas 2004	21/42	30/42		6.2	0.70 [0.49, 1.00]
Lebeau 1994	83/138	97/139		26.3	0.86 [0.72, 1.03]
Subtotal (95% Cl)	180	181		32.5	32.5 [0.70, 0.97]
Total events: 104 (Heparin), 127 (Control)					
Test for heterogeneity chi-square = 1.05 df = 1 p=0.30 I^2=5.1%					
Test for overall effect z=2.27 p=0.02					
Advanced Cancer					
Kakkar 2004	103/190	109/184		25.4	0.92 [0.77, 1.09]
Klerk 2005	90/148	112/153		30.9	0.83 [0.71, 0.98]
Sideras 2006	42/68	42/70		11.2	1.03 [0.79, 1.35]
Subtotal (95% Cl)	406	407		67.5	0.89 [0.80, 1.00]
Total events: 235 (Heparin), 263 (Control)					
Test for heterogeneity chi-square=1.94 df=2 p=0.38 I^2=0.0%					
Test for overall effect a=2.04 p=0.04					
Total (95% Cl)	586	588		100.0	0.87 [0.80, 0.95]
Total events: 339 (Heparin), 390 (Control)					
Test for heterogeneity chi-square=3.58 df=4 p=0.47 P=0.0%					
Test for overall effect z=3.02 p=0.003					



Outcome for mortality at 12 months for SCLC and advanced cancer separately and combined. For details see AKI [10]

With permission from AKI [10]

Square and lines = mean and 95% CI, Diamonds = aggregate of means.

with gemcitabine and cisplatin in standard doses with either LMWH (nadroparin 2850 IU per day until disease progression) or no additional treatment [36]. The median time to progression was 7.3 months in the LMWH group vs 4.0 months in the no LMWH group, P = 0.0001 and the overall survival was 13.0 vs 5.5 months in the chemotherapy plus LMWH vs the non-LMWH group, P = 0.0001. There was no difference in toxicity between the two groups. In the report by von Delius et al., 243 patients who received the chemotherapy for advanced pancreatic carcinoma were identified from a prospectively maintained database [37]. Attending physicians ordering various protocols for chemotherapy plus or minus prophylactic doses of LMWH (dalteparin or certoparin) or therapeutic doses of nadroparin

at the discretion of their attending physicians. This is obviously not a randomized clinical trial and the patients did receive different chemotherapy regimens but otherwise the clinical characteristics were similar. There was no difference in the overall survival in the two groups (7.1 months in the LMWH group vs 5.9 months in the non-LMWH group). Unlike previous reports, there was the survival benefit in those patients with metastatic disease vs those without metastatic disease (6.6 months vs 3.8 months, $P = 0.005$). These two studies suggest that LMWH in addition to chemotherapy may be beneficial in patients with advanced pancreatic cancer and warrant further studies in properly designed randomized clinical trials.

15.4 Experimental Models of Tumor Growth and Metastasis

Numerous laboratory models for cancer cell proliferation and metastasis have been developed to study the effects of the heparins and related compounds. The oldest and most commonly used model is the injection of tumor cells into experimental animals (usually mice) with a subsequent measurement of lung and liver metastasis. Cells can be infused intravenously or implanted either subcutaneously, into foot pads or into the spleen [23, 38–41]. The cell lines most commonly used are carcinoma or melanoma tumor lines. In some settings assessing the immunologic effects of the drug under investigation, SCID mice have been used [42].

The other common laboratory model is the study of various cancer cells in cultures usually in a collagen or Matrigel matrix [19, 43]. Using such cell cultures, capillary tube formation and proliferation can be assessed as a measure of angiogenesis [44]. Studies using the chorioallontoic membrane model have also been useful [20, 45]. Cell adhesion and migration and the effects of various chemotaxins can also be assessed using the cell culture model [46].

15.5 The Role of Heparins on Cancer Progression and Metastasis in Experimental Studies

Various experimental models have been used to assess the effect of UFH and LMWH as well as heparan sulfate-glycoaminoglycans (HS-GAGs) or heparan sulfate-proteoglycans (HSPGs) [15]. Using these experimental models the multiple steps in cancer progression can be assessed in the presence of the heparins or similar agents including cancer cell proliferation, angiogenesis, migration and invasion of cancer cells and endothelial cells and the adhesion of cancer cells to vascular endothelium [15, 46]. As Cosgrove et al. pointed out, the first report of inhibition of tumor growth in experimental animals by heparin was published by Goerner in 1930 [47]. Since that time, numerous similar studies have been carried out starting in the 1960s. Studies carried out between 1960 and 1999 were reported in a comprehensive review by Smorenburg and Van Noorden [15]. These studies all involved the use of

UFH in a variety of tumor cell lines given either intravenously or subcutaneously, and metastases to the lung – and in some cases liver – were measured. The studies assessed the effects of heparin on cancer cell proliferation, migration and invasion, the adherence of cancer cells to vascular endothelium, and their effects on angiogenesis. Most of the studies demonstrated that heparin had a positive effect on the inhibition of cancer cells but others demonstrated the opposite effect. Thus the effect of heparin may differ depending on the tumor types being assessed and the complex interactions between cancer cells and the heparins.

The low molecular weight heparins have differing effects from UFH in some studies [38, 44] but not in others [23, 39]. The LMWH (nadroparin) was shown to inhibit tumor metastases in experimental mice whereas UFH did not [38]. Some LMWH compounds were effective in suppression of tumor cell growth, metastasis (nadroparin [23], tinzaparin [39], enoxaparin [40]), and angiogenesis (tinzaparin [45], dalteparin and enoxaparin [44]), whereas fondaparinux (a selective factor Xa inhibitor) was not [23, 39]. In studies correlating selectin inhibition with experimental metastasis UFH [23, 39], tinzaparin [39] and nadroparin [23] suppressed both tumor metastasis and selectin whereas enoxaparin [23] and fondaparinux did not [23, 39]. The question has been raised whether or not the specific inhibitors of thrombin or activated factor X may have the same inhibitory effect on tumor cell suppression as UFH or LMWH [47] but the fact that hirudin, a specific antithrombin, was able to suppress melanoma cell metastases suggests that the interactions are more complex [48].

An exciting area of research in this regard is the use of nonanticoagulant LMWH and other heparin or heparan sulfate mimetics which possess the ability to inhibit angiogenesis in experimental models, thus suggesting that such agents may be capable of suppressing cancer cell proliferation, migration, and metastasis without any increase in bleeding risks [21].

15.6 Potential Mechanisms to Explain the Suppression of Cancer Cell Growth and Proliferation by the Heparins

15.6.1 Inhibition of Thrombin Generation

The inhibition of thrombin by anticoagulants has the potential to decrease tumor cell growth by both coagulation related and noncoagulation related mechanisms [49]. Thrombin generation leads to fibrin deposition which can be deposited around tumor cells, thus providing scaffolding for tumor cell adhesion and angiogenesis [50–53]. Fibrin can be found on vascular endothelium of new vessels in tumors and found bound to inflammatory cells or tumor cells [49, 50]. Increased fibrinogen turnover and deposition of cross linked fibrin has been demonstrated in a number of tumors [54, 55]. This fibrin matrix has been shown to sequester a number of growth factors including vascular

endothelial growth factor (VEGF) and similar compounds which have a prominent role in angiogenesis [49].

Thrombin also activates platelets which can release a number of growth factors from their alpha granules as well as participating in cancer cell migration via their P-selectin function [49, 56].

As discussed in previous chapters in this book, thrombin has a number of other noncoagulation functions including triggering the release of numerous growth factors and chemokines, mobilizing adhesion molecules, and up-regulating the principle thrombin receptor PAR-1 (protease-activated receptors) [49]. PAR are detected on a number of cells including cancer cells and correlate with the metastatic potential of these cancer cells [49, 57, 58].

15.6.2 Tissue Factor

In addition to interacting with factor VIIa to induce coagulation, tissue factor is also a potent regulator of vascular endothelial growth factor (VEGF), thus contributing to the development of tumor angiogenesis [49, 59, 60]. Studies have shown that tissue factor contributes to tumor metastasis and angiogenesis by both coagulation dependent and coagulation independent mechanisms [40, 61–65]. Tissue factor pathway inhibitor (TFPI) is a natural inhibitor of the tissue factor – factor VIIa pathway [48, 66]. TFPI can be released by the heparins, and in particular LMWH; this has been suggested as one mechanism whereby LMWH may have an impact on survival in cancer patients [40, 48, 66].

15.6.3 Heparanase

Heparan sulfate is a complex glycosaminoglycan consisting of up to 400 modified sugars. It appears ubiquitously throughout the body and is expressed on cell surfaces and in the extracellular matrices (ECM) of most tissues [67–70]. The ECM binds basic fibroblast growth factor (bFGF) which, amongst other enzymes, can be released by thrombin and heparanase [70]. The degradation of heparan sulfate by heparanase may thus release bFGF, which has a central role in angiogenesis and tumor invasion [15, 70, 71]. Destruction of the ECM by heparanase may also promote invasion and metastases of cancer cells [68, 70–72]. Heparanase has been shown to be expressed on a number of tumor types, including breast cancer, colon cancer, gastric cancer, and this has been correlated with metastatic potential of these cancers [68, 73–75]. UFH, LMWH, and synthetic molecules have been demonstrated to suppress heparanase activity and may thus interfere with cell invasion, angiogenesis, and metastasis [70, 76–78].

15.7 Adhesion Molecules – P-Selectin and L-Selectin

Selectins are vascular cell adhesion molecules involved in mediating interactions of leukocytes, platelets, and endothelial cells [79, 80]. In addition to their role in hemostasis, they can interact with cancer cells and promote metastases by their presence in platelets and on endothelial cells and leukocytes [79–81]. These selectins (and in particular P-selectin) are suppressed by heparin and LMWH [79, 82]. In experimental studies so far, not all LMWHs have the same activity. Thus in one study the LMWH nadroparin was shown to inhibit selectin activity as well as decreasing lung metastases in an experimental model whereas enoxaparin and fondaparinux did not [23]. Similarly, the pentasaccharide fondaparinux demonstrated no inhibitory effect on the selectins [23, 39]. Studies have also been done with nonanticoagulant heparins as well as O-sulfated bacterial polysaccharides and these were also demonstrated to suppress both P- and E-selectin mediated interactions and to attenuate tumor metastases and progression [18, 19, 23, 41, 83, 84]. These studies therefore provide further evidence that suppression of tumor growth and metastases is not related to the anticoagulant potential of the treatment agents in many cases. Certain breast cancer cells express the chemokin receptor CXCR 4 which allows breast cancer cells to migrate towards their specific metastatic target sites which express CXCL 12 within normal tissues [42, 46]. The presentation of chemokins to their receptors is dependent on glycosaminoglycan components on the cell surface or ECM. Short length oligosaccharides of heparin which have decreased anticoagulant effect were shown to reduce the metastatic spread of human breast cancer cells in a murine model; similarly UFH and the LMWH (tinzaparin) suppressed metastases [42, 46].

15.7.1 Angiogenesis

All of the interactions between the hemostatic system and tumor cells are involved in the promotion of angiogenesis [41, 48, 59, 63, 85–89]. Furthermore, several of the steps in the development of angiogenesis can be suppressed by UFH, LMWH, and nonanticoagulant molecules [20, 22, 41, 45, 47, 48, 90–92]. The complex interactions of these various mechanisms was recently reviewed by Ruf [89]. Thus prominent roles in angiogenesis involve tissue factor, particularly its cytoplasmic tail, the tissue factor VIIA complex leading to the production of thrombin, the up regulation and activation of protease-activated receptors (PARs), the release of growth factors such as VEGF and beta fibroblast growth factor (beta FGF) and the role of the extracellular matrix and heparanase [89]. A number of the steps in the promotion of angiogenesis can be inhibited by tissue factor pathway inhibitor (TFPI) [45, 48]. The suppression of angiogenesis has been promoted as one of the mechanisms whereby anticoagulants such as UFH and LMWH may suppress tumor growth and metastasis. In experimental models assessing endothelial capillary tube formation, cell proliferation in cultures and in a chorioallantoic membrane system studies have shown that UFH and various LMWHs can

suppress angiogenesis, although not all to the same extent [44, 45, 47, 90]. Also, as seen in other studies, angiogenesis can be suppressed by nonanticoagulant heparins or synthetic compounds [20, 22, 91, 92]. Finally, the association of thrombosis with antiangiogenic therapy further highlights the complex interaction of angiogenesis with the hemostatic system [93]. The various sites where the heparins (UFH, LMWH and non-anticoagulant heparin-like compounds) may inhibit or block cancer cell growth, migration, and metastasis are shown in Fig. 15.1.

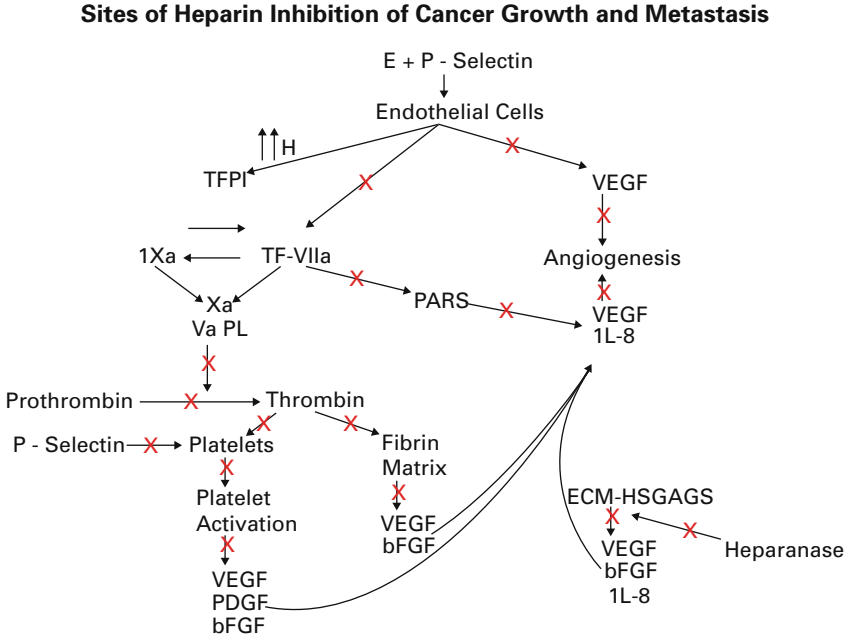


Fig. 15.1 Sites of heparin inhibition of cancer growth and metastasis. Sites where the heparins (UFH, LMWH and non-anticoagulant heparin-like compounds) inhibit or block cancer cell growth,Sites of heparin inhibition of cancer growth and metastasis. Sites where the heparins (UFH, LMWH and non-anticoagulant heparin-like compounds) inhibit or block cancer cell growth, migration and metastasis are designated by the *red boxes*. TF-VIIa (tissue factor-factor VIIa) activates Xa and also IXa via the tissue factor pathway. The heparins increase release of TFPI (tissue factor pathway inhibitor) from endothelial cells and other storage sites and thus increase the effects of TFPI on angiogenesis as well as its other activities. The selectins (E-endothelial; P-platelet and L-leukocyte) which interact with cancer cells and promote metastasis are blocked by the heparins. The release of VEGF (vascular endothelial growth factor), PDGF (platelet derived growth factor), bFGF (beta fibroblast growth factor) and IL-8 (interleukin-8) which promote angiogenesis are all blocked by the heparins. PARS (protease activated receptors) are upregulated and activated by thrombin and the TF-VIIa complex and are located on various cancer cells. Activation of PARs leads to the release of various growth factors which promote angiogenesis. Suppression of thrombin generation and TF-VIIa by the heparins decreases PARs activity. ECM-HSGAGs (extracellular matrix, heparan sulfate-like glycosaminoglycans) store and release VEGF, bFGF and IL-8 by the action of heparanase. The activity of heparanase is blocked by the heparins

In their review of preclinical studies published between 1960 and 2005, Niers and colleagues concluded that the anticancer activity of heparins was more dependent upon inhibition of metastases than on the effect on primary tumor growth [94]. Furthermore, chemically modified heparins with little or no anticoagulant activity showed antimetastatic properties as well. As with other comprehensive reviews, they concluded that the anticancer effect of the heparins was related both to coagulation and noncoagulation dependent factors.

15.8 Conclusion

The hemostatic system and cancer are integrally related with many forms of cancer inducing a hypercoagulable state resulting in venous thromboembolism, disseminated intervascular coagulation, and marantic endocarditis. The interaction between cancer cells and the hemostatic system are extremely complex and help explain why no single line of attack can be successful in all cases. Over the years it has become increasingly evident that the thrombotic process plays a vital role in cancer cell development, proliferation, migration and metastasis leading to the hope that suppression of the coagulation cascade could have a beneficial effect on the cancer. Data from clinical trials initially aimed at treatment of venous thromboembolism in cancer patients and later directly in cancer patients who did not have thrombosis provided evidence that LMWH could improve survival in these patients who had a wide variety of primary tumor sites. Current trials are ongoing in patients with specific tumor sites, with better quantitation of tumor burden, the effect of the tumor on the patient being determined using different LMWHs compared with placebo, with the primary outcome event being survival at 12, 18, and 24 months.

In recent years there has been a determined effort to understand how the heparins may suppress cancer cell development, proliferation, and metastasis in a variety of experimental models. Many of these studies have yielded contradictory results with heparin producing an inhibitory effect on cancer cells in most studies but in others cancer cell growth and angiogenesis was actually stimulated. The use of LMWH and other lower molecular weight compounds has a similar but more potent effect than UFH in most experimental models. An exciting result of these studies is that a number of nonanticoagulant sulfated oligosaccharides and similar compounds have a potent anticancer effect without any anticoagulant effect, suggesting that these could have a prominent role as adjuvant agents in the treatment of a variety of cancers in humans without any potential for increasing bleeding complications.

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Part III
Bleeding Disorders Associated with Cancer

Chapter 16
Thrombocytopenia and Cancer

Elizabeth A. Eklund

16.1 Introduction

Thrombocytopenia is frequently encountered in patients with solid tumors or hematologic malignancies. In these patients, it may be related to the underlying disease or it may be a consequence of treatment. In some cases, thrombocytopenia may be an isolated hematologic defect. However, thrombocytopenia in cancer patients will often be accompanied by variable amounts of anemia and/or neutropenia. In the majority of cases, thrombocytopenia in cancer patients is not clinically significant, but is discovered during routine blood counts. However, some of the causes of thrombocytopenia indicate the development of serious disorders which should be addressed promptly. Since the etiology of thrombocytopenia in cancer patients may be multifactorial, diagnosis is often complex. The main causes of thrombocytopenia are increased platelet destruction and decreased platelet production. Less frequent are splenic sequestration and dilutional thrombocytopenia.

16.2 Thrombocytopenia Due to Peripheral Destruction

16.2.1 Disseminated Intravascular Coagulation

Consumptive coagulopathy is common in patients with cancer and one of the most common causes is disseminated intravascular coagulation (DIC). In one study, DIC was found in 50% of all patients with cancer and in 90% of patients with disseminated malignancy [1, 2]. In the majority of such cases, DIC is subclinical and only detected with specific laboratory testing. In a cancer patient

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with thrombocytopenia, DIC should be an early consideration. However, since it is generally asymptomatic in this setting, specific therapy is rarely required and treatments are directed to the underlying malignancy.

Analysis of the peripheral blood smear is the most important initial test in the evaluation of thrombocytopenia in a patient with a known malignancy. The presence of a consumptive coagulopathy is suggested by the identification of schistocytes and red cell fragments on the peripheral smear. These may also be observed in thrombotic thrombocytopenic purpura (TTP), which is also an important cause of consumptive coagulopathy in cancer patients. A diagnosis of DIC is indicated by an increase in the PT, aPTT and D-dimer, and a decreased fibrinogen level. In contrast, TTP would be suggested by increased lactic dehydrogenase (LDH) and serum creatinine, increased ultra large von Willebrand multimers, minor abnormalities in PT or PTT, and no decrease in fibrinogen or increase in D-dimer.

Distinguishing DIC from TTP or other causes of thrombocytopenia is important because the therapeutic approaches are very different. However, since many of these disorders have common characteristics, this distinction can be difficult. To assist with this dilemma, a scoring system was developed by the International Society of Thrombosis and Haemostasis [3]. This scoring system is applicable to patients with an underlying cause for DIC, such as a disseminated malignancy. A numerical score is assigned to each of four factors: platelet count ($>100{,}000 = 0$, $<100{,}000 = 1$, $<50{,}000 = 2$), elevated fibrin related marker (no increase = 0, moderate increase = 2, strong increase = 3), prolonged PT ($<3\,s = 0$, $>3\,s$ but $<6\,s = 1$, $>6\,s = 2$) and fibrinogen level ($>100 = 1$, $<100 = 1$). In this system, a score of >5 is consistent with DIC.

A number of factors are involved in the pathogenesis of DIC in cancer patients. In some cases, cancer related abnormalities in coagulation factors may contribute to a procoagulant state in these patients. For example, a variety of tumor cells exhibit both surface expression and shedding of tissue factor (TF). Cancers for which this mechanism may result in DIC include adenocarcinomas, leukemias, lymphomas, and osteogenic sarcomas [4–7]. Release of TF into the circulation with resultant DIC has also been described after placement of peritoneal-venous shunts to manage malignant ascites in patients with advanced cancer [8]. Cancer cells may also produce cancer procoagulant (CP), a serine protease which activates factor X [9, 10]. Cancers for which increased CP production may contribute to DIC include carcinomas of the lung, breast, colon and kidney. Increased production of CP is also found in sarcomas, neuroblastoma, melanoma, leukemia and lymphoma [11–14]. In addition, patients with carcinoma have may have decreased urokinase plasminogen activator (uPA) and tissue type plasminogen activator (tPA), and increased plasminogen activator inhibitor type 1 (PAI-1), leading to a procoagulant state [15–18].

Elaboration of inflammatory mediators may also contribute to DIC in cancer patients. These mediators may be produced by either cells of the immune system which are activated in response to the tumor, or by the tumor itself.

Inflammatory mediators which exert a procoagulant effect include tumor necrosis factor alpha (TNFα), IL-1, and vascular endothelial growth factor (VEGF) [19, 20]. Additionally, abnormal vasculature in tumors may provide a site of denuded epithelium which activates the coagulation cascade, resulting in DIC. Kaposi's sarcoma has been associated with DIC via this mechanism.

It is also important to consider the possibility of underlying infection in a cancer patient with DIC. Immuno-suppression may be present in such patients related to treatment with radiation or chemotherapy. In the case of hematologic malignancies, immuno-suppression may be a consequence of the malignancy itself. This is an important consideration in the differential diagnosis, because treatment of the underlying infection would be the preferred approach to addressing DIC in this case. Another important diagnosis to exclude is TTP. Treatment for this disorder is dramatically different than treatment of DIC, as is discussed below. In general, treatment of DIC in a patient with cancer will be directed to the underlying malignancy. As discussed above, DIC in this setting is often sub-clinical and does not require specific treatment.

16.2.2 Thrombotic Thrombocytopenic Purpura (TTP)

In patients with malignancies, TTP may cause a consumptive coagulopathy. TTP may be due to either the underlying cancer or drugs used for treatment. In some patients, TTP may be the first sign of the presence of an occult malignancy. A recent study found that 10 out of 351 consecutive, newly diagnosed TTP patients had an underlying malignancy [21]. In only one of these cases had the malignancy been previously diagnosed. TTP in patients with malignancies is often more chronic in onset than in other patients, and is more likely to be asymptomatic [21]. TTP should be considered early in the diagnostic evaluation of a patient with cancer and thrombocytopenia.

As discussed above, the presence of schistocytes and/or red cell fragments in the peripheral smear of a cancer patient with thrombocytopenia suggests the possibility of a consumptive coagulopathy. In TTP, the LDH is generally greatly elevated and serum creatinine may also be increased. The presence of ultra-large von Willebrand multimers confirms the diagnosis; however treatment should not be delayed while awaiting this test if the other evidence is consistent with TTP. In contrast to DIC, abnormalities in PT or aPTT are minor or absent, fibrinogen is not decreased and D-dimer titers are not elevated in TTP [22]. Since the treatment of TTP and DIC are very different, it is important to distinguish between these two entities. Use of the scoring system for DIC (discussed above) is useful in making this distinction [3].

In TTP patients without cancer, antibodies to ADAMTS-13 (A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13, also known as Von Willebrand factor cleaving protease) are often found in the circulation [23]. Deficiency in ADAMTS-13 impairs processing of ultra large

von Willebrand multimers, leading to accumulation of platelet micro-aggregates in the circulation [24]. Cancer patients with TTP rarely have antibodies to ADAMTS-13, suggesting a different pathogenesis than in the de novo disease [25]. Data regarding circulating ADAMTS-13 levels in cancer patients is variable in different studies. One study found that ADAMTS-13 levels were less than 5% of normal in patients with disseminated cancer, but were normal in patients with localized tumors [25]. In another study, patients with either localized or metastatic cancer had decreased ADAMTS-13 levels [26, 27]. Other studies have found no correlation between ADAMTS-13 levels and TTP in cancer patients [28].

Additional mechanisms for TTP in cancer patients have been hypothesized. One such hypothesis is that endothelial disruption in tumor vasculature results in excess release of unprocessed ultra large von Willebrand factor multimers. Since TTP is relatively more frequent in cancer patients with bone marrow involvement and fibrosis, it has also been hypothesized that vascular damage as a result of this process contributes to TTP [29]. Pro-coagulant factors released by mucin producing tumors have also been hypothesized to contribute to the pathogenesis of TTP in patients with various types of mucinous adenocarcinomas [30].

The most common cancers associated with thrombotic microangiopathy (TMA) are carcinomas of the breast and stomach [30]. TMA is also found in patients with cancer of the colon, prostate, lung, ovary, endometrium and pancreas [22, 31, 32]. Additionally, some of the chemotherapeutic agents used in the treatment of cancer are specifically associated with TTP. These agents include gemcitibine, mitomycin C and anti-thymocyte globulin [33–35]. The differential diagnosis for TTP in a cancer patient includes other consumptive coagulopathies, such as DIC. Treatment of TTP in the cancer patient is similar to the treatment approach to this disease in a patient without malignancy. Specifically, several studies have shown the benefit of plasma exchange or plasmaphoresis in TTP patients with cancer, even when the underlying malignant process is not controlled [22, 36].

16.2.3 Immune Mediated Thrombocytopenia (ITP)

Thrombocytopenia in cancer patients may be due to the development of autoantibodies to platelet associated antigens. Such auto-immune causes of thrombocytopenia are more common in patients with hematologic malignancies than in patients with solid tumors. Therefore, the index of suspicion for this diagnosis should be greatest in patients with chronic lymphoid leukemia (CLL), indolent B cell lymphomas, Hodgkin's disease or myelodysplastic syndromes [37–40]. In these patients, ITP or auto-immune thrombocytopenia may occur in isolation, or may be associated with auto-immune hemolytic anemia.

The most important initial diagnostic test to distinguish ITP from a consumptive coagulopathy is the peripheral blood smear. ITP should be suspected

if the peripheral blood smear indicates thrombocytopenia with normal red cell morphology. Bone marrow biopsy is generally not required to make the diagnosis of ITP in a patient without a diagnosed malignancy. However, the potential differential diagnosis of thrombocytopenia in a patient with a known diagnosis of hematologic malignancy or solid tumor makes bone marrow evaluation more important. In such a patient, a normocellular bone marrow with increased numbers of normal appearing megakaryocytes would be consistent with a diagnosis of ITP. The bone marrow specimen should be closely examined for the presence of tumor cells or fibrosis, which may provide the first clue to an underlying malignancy.

ITP in patients with hematologic malignancies is hypothesized to be due to dysregulation of the immune system. As with the "primary" disorder, ITP in patients with hematologic malignancy involves production of antibodies to platelet glycoproteins IIb/IIIa or Ib/IX with subsequent clearance of antibody coated platelets by the reticuloendothelial system. However, testing for such antibodies is not generally recommended. Studies of platelet associated IgG have low specificity and detection of anti-IIb/IIIa or Ib/IX in the serum has low sensitivity [41, 42]. As for primary ITP patients, the diagnosis of ITP in a patient with a diagnosis of malignancy is one of exclusion.

Although ITP is generally associated with hematologic malignancies, there have been some reports of ITP in patients with breast or ovarian cancer [43–45]. The conclusion of the studies was that ITP in patients with these cancers may represent coincidence. ITP is more common in women and there is significant overlap in the age groups in which ITP and breast or ovarian cancer are most prevalent. A larger accumulation of cases will be required to determine if the incidence of ITP in breast or ovarian cancer patients is greater than might be expected for age and gender matched controls. In patients with breast or ovarian cancer and thrombocytopenia, it would be especially important to exclude other causes, such as consumptive coagulopathy or bone marrow involvement.

16.2.4 Heparin Induced Thrombocytopenia (HIT)

Another potential cause of thrombocytopenia in a cancer patient is HIT. Patients at risk for this complication include those with vascular access devices who are receiving heparin flushes, patients receiving unfractionated heparin for venous thromboembolism (VTE) prophylaxis, and patients undergoing anticoagulation with heparin for VTE. This complication generally occurs within 5–15 days of initiating heparin therapy and platelet counts generally decrease to less than 50% of pre-treatment levels [46–48]. Because HIT can lead to arterial or venous thrombosis and even death, it is important to identify this complication quickly and initiate specific therapy.

The HIT syndrome is caused by development of antibodies to epitopes created by interaction of heparin with platelet factor 4 (PF4) [49, 50]. This

was previously referred to as HIT type II. Binding of the antibody-heparin-PF4 complex to the platelet surface results in de-granulation and release of pro-thrombotic microparticles. These microparticles activate monocytes, resulting in release of inflammatory mediators, and also cause endothelial damage. Interaction between the antibody-heparin-PF4 complex and PF4 receptors on other platelets leads to formation of platelet aggregates in the circulation, further facilitating the consumptive coagulopathy.

A number of studies have investigated the frequency with which patients receiving heparin develop anti-heparin-PF4 antibodies. In these studies, anti-heparin-PF4 antibodies develop in approximately 17% of patients receiving heparin for any indication [46, 47]. One study also determined the incidence of platelet activation in patients who developed anti-heparin-PF4 antibodies and were treated for longer than 2 weeks with heparin. This study determined that less than 20% of these patients developed platelet activation and were therefore at risk for arterial or venous thrombosis [46]. However, another study found that the incidence of HIT syndrome is increased in cancer patients receiving heparin, relative to similarly treated patients without cancer [51].

Examination of the peripheral smear is important to the diagnosis of HIT. Although schistocytes may be present in patients with this disorder, they are less prominent than in DIC or TTP. Paradoxically, the numbers of schistocytes may be inversely correlated with thrombotic complications in this disorder. In contrast to DIC, the PT and aPTT will not generally be abnormal in HIT, except as might be expected related to administration of heparin. Also, fibrinogen will not be decreased in HIT as they are in DIC. In contrast to TTP, there will be no marked elevation in LDH or creatinine. The most commonly available tests for the diagnosis of HIT are immunological studies to detect anti-heparin-PF4 antibodies in the serum. These tests are very sensitive for detecting circulating antibodies, but not very specific for determining which patients have HIT syndrome [52]. Another diagnostic test which may be performed is the serotonin release assay, which evaluates the ability of patient's serum to activate platelets. This test is very specific for the presence of functionally significant anti-heparin-PF4 antibodies, but it is not very sensitive [53].

However, in a patient for whom the clinical suspicion for HIT is high, treatment should not await the results of specific testing for anti-heparin-PF4 antibodies. In a patient in whom the HIT syndrome is suspected, any heparin administration should be discontinued immediately. Even patients without clinically evident thrombosis at the time of diagnosis have a significantly increased risk for subsequent development of thrombosis [51]. Therefore, anticoagulation with a direct thrombin inhibitor such as lepirudin, argatroban or bivalirudin (all FDA approved for this indication) should be initiated in any patient with suspected HIT syndrome [54]. For patients without clinically evident thrombosis, therapeutic levels of these agents should be continued until the platelet count recovers, then for an additional 2–4 weeks [55]. In a patient with HIT syndrome and documented thrombosis, anticoagulation with

warfarin should commence once the platelet count is greater than 150,000/μL and continue for 3–6 months [55].

The differential diagnosis for HIT includes DIC and TTP, as discussed above. The differential also includes a non-immune mediated form of thrombocytopenia due to adhesion of heparin to platelets with subsequent clearance by the reticuloendothelial system. This entity was previously referred to as type I HIT, but it is not associated with the thrombotic complications that accompany the immune-mediated disorder [48].

16.3 Thrombocytopenia Due to Decreased Production

16.3.1 Bone Marrow Suppression Due to Tumor Involvement

Bone marrow involvement may result in leukoerythroblastic anemia in patients with solid tumors or hematologic malignancies. In these cases, the peripheral blood smear is characterized by thrombocytopenia, mild anemia and the presence of immature myeloid and red cells in the circulation. In patients with solid tumors, this disorder is generally associated with greater than 25% bone marrow involvement with tumor, or with extensive fibrosis [56]. Bone marrow fibrosis may be induced by elaboration of cytokines by cells of the immune system as part of the inflammatory reaction to the tumor. Alternatively, the tumor cells themselves may produce cytokines which result in fibrosis [57]. In hematologic malignancies, the bone marrow space may be replaced with malignant cells, physically limiting hematopoiesis. Either of these mechanisms would be anticipated to interfere with the development of all the blood cell lineages. However, increased platelet consumption in cancer patients (discussed above) may cause thrombocytopenia to be the first peripheral manifestation of bone marrow involvement.

Leukemias and lymphomas are the most common malignancies to be diagnosed by detecting asymptomatic cytopenias on routine blood testing. For non-hematologic malignancies, cancers of the prostate or breast are the cancers most commonly associated with bone marrow involvement and suppression of hematopoiesis. This is related to both the relatively high incidence of these cancers in the population and bone marrow tropism of the tumor cells. Small cell cancers are also commonly found in the bone marrow and may be associated with thrombocytopenia or leukoerythroblastic anemia. However, bone marrow involvement and cytopenias are not a common enough manifestation of any of these solid tumors to warrant bone marrow examination as part of the staging process [58].

The differential diagnosis of thrombocytopenia in cancer patients, with or without other cytopenias, is extensive. It is important to determine whether thrombocytopenia and/or anemia are due to peripheral consumption early in the diagnostic process. It is also important to consider the possible effects of

recent treatments with chemotherapy or radiation in the pathogenesis of thrombocytopenia in the cancer patient. This issue is discussed further below.

16.3.2 Treatment-Related Bone Marrow Suppression

Treatment with chemotherapy is a common cause of bone marrow suppression and pancytopenia in cancer patients. However, some chemotherapeutic agents mainly cause thrombocytopenia. Progressive thrombocytopenia which does not recover between treatment cycles may be seen with cis-platinum or carboplatin. Treatment with 2 chloro-deoxy-adenosine is associated with prolonged thrombocytopenia which may persist for months after discontinuing treatment. Specific thrombocytopenia has been associated with thalidomide treatment for multiple myeloma [59]. In each of these cases, the dominant mechanism is suppression of platelet production.

Treatment with alkylating agents or topoisomerase inhibitors, especially in combination with radiation, can damage the hematopoietic stem cell, leading to development of myelodysplastic syndrome (MDS) as a late complication. Therapy related MDS (tMDS) generally presents as progressive thrombocytopenia or anemia years after termination of therapy [60, 61]. Bone marrow examination in such patients reveals hypoplasia with varying degrees of dysplasia. Approximately half of patients with tMDS have an unfavorable karyotype at diagnosis as compared to 20% of patients with de novo MDS [60, 61]. Consistent with this, tMDS has a poor prognosis and is often associated with rapid progression to acute myeloid leukemia (AML). Because treatment regimes for Hodgkin's and non-Hodgkin's lymphoma often involve alkylating agents and radiation, tMDS/AML is most common after treatment of these disorders. Patients with solid tumors who are at the greatest risk for tMDS/AML are those with breast, ovary and prostate cancers. This diagnosis should also be considered in cancer survivors who demonstrate a trend of decreasing platelet counts during long term follow up. Both the patients themselves and their primary care physicians should be made aware of this potential long term complication of cancer.

Pancytopenia is a well recognized complication of external beam radiation therapy. The increased use of therapeutic radio-isotopes is also associated with an increase in cytopenias during and post treatment. Strontium-89 is increasingly commonly used in palliative treatment of boney metastasis in prostate and breast cancers [62]. Such treatments may be associated with development of thrombocytopenia out of proportion to anemia or neutropenia. Ongoing, low grade platelet consumption may contribute to this effect, especially in patients with prostate cancer (as is discussed above).

The time course for the development of thrombocytopenia relative to treatment with radiation or chemotherapy is important in determining whether other causes should be sought. If there is a question of the role of current

treatment vs other etiologies for bone marrow suppression, the diagnosis requires bone marrow evaluation. The relevance of recent or remote treatments to thrombocytopenia is generally suggested by the clinical course of the patient, and the presence of other symptoms. For example, unexplained fever may suggest underlying infection as a cause of bone marrow suppression. Especially in patients with hematologic malignancies, opportunistic infections which involve the bone marrow should be a consideration. Infections with fungal pathogens or acid fast organisms may sometimes be detected on bone marrow biopsy, and should be considered in the appropriate clinical setting. In a patient who is actively undergoing treatment, the diagnosis of thrombocytopenia may require a combination of patience and intervention.

16.3.3 Treatment of Thrombocytopenia Related to Decreased Production

Guidelines were developed by the American Society of Clinical Oncology for the use of platelet transfusion in patients with malignancies and thrombocytopenia due to decreased platelet production [63]. Therapeutic platelet transfusion is obviously indicated to treat bleeding in a patient with hematologic malignancy or a solid tumor and thrombocytopenia. In addition, the use of prophylactic platelet transfusions for patients with malignancy and thrombocytopenia has become routine (see Chap. 10). For patients with hematologic malignancies, the use of prophylactic platelet transfusion is supported by studies which indicate improved morbidity with this approach [64, 65]. The threshold for initiation of prophylactic platelet transfusion in patients with hematologic malignancies was addressed in a number of randomized clinical trials. Based on these studies, ASCO guidelines recommend a threshold platelet count of 10,000/μL for prophylactic transfusion in patients with hematologic malignancies undergoing chemotherapy [66–68]. This recommendation excepts situations where bleeding risk is increased, such as patients undergoing procedures. In these cases, transfusion to achieve a platelet count between 30,000 and 50,000/μL may be indicated, depending on the procedure.

In contrast, there are less specific data regarding the use of prophylactic platelet transfusion for patients with solid tumors and thrombocytopenia [63]. No prospective, randomized trials of the utility of this approach have been performed for patients undergoing chemotherapy for solid tumors. However, several retrospective studies found little correlation between platelet count and frequency of clinically severe bleeding episodes in patient with solid tumors undergoing chemotherapy. It was noted that bleeding often occurs at sites of necrotic tumors in such patients and can be observed with platelet counts >50,000/μL [69]. Based on the available data, the recommendations of the ASCO panel for the threshold for prophylactic platelet transfusion in patients with solid tumors undergoing chemotherapy was 10,000/μL [63].

In considering platelet transfusion, it is important to be certain that thrombocytopenia is due to decreased production rather than increased destruction. For a patient with DIC or ITP, platelet transfusion is unlikely to result in clinical benefit because of ongoing destruction. However, platelets should be transfused in the case of clinically important bleeding in either of these disorders. This should be accompanied with more specific treatments to address these disorders. For patients with HIT, the major complication is thrombosis, not bleeding. Platelet transfusion in these patients is generally not indicated and the therapeutic approach should concentrate on discontinuing all exposure to heparin. In the case of TTP, the transfusion of platelets has been shown to exacerbate the thrombotic complications of the consumptive coagulopathy [70]. However, transfusion should not be withheld in the case of life-threatening hemorrhage. Also, there is anecdotal evidence that judicious platelet transfusion following plasmapheresis can be safely used to prepare patients for urgent procedures [71].

16.4 Summary: Diagnostic Evaluation

16.4.1 Thrombocytopenia with Abnormal Peripheral Smear

The most important initial consideration in approaching a cancer patient with thrombocytopenia is to determine whether a consumptive coagulopathy is present. Therefore, review of the peripheral blood smear for schistocytes or other abnormal red blood cell forms is the most important initial diagnostic test. The presence of such red cell forms would suggest a consumptive coagulopathy and dictate the initial diagnostic evaluation. In this case, the differential diagnosis would include DIC, TTP and HIT (see Fig. 16.1). Additional testing that should be performed at this point include PT, aPTT, fibrinogen, D-dimer titer, LDH and creatinine.

A diagnosis of DIC would be suggested by increased PT, aPTT and D-dimer with decreased fibrinogen, but without an elevated LDH or creatinine. For TTP, the major expected abnormality would be an elevated LDH, which is often greater than 1,000 U/L. The creatinine may be increased, but the diagnosis of TTP can be made by the presence of elevated LDH and schistocytes alone. In contrast, only minor abnormalities of PT or PTT would be expected in TTP, and the fibrinogen and D-dimer should be normal.

The diagnosis of HIT syndrome in a patient with thrombocytosis and schistocytes in the peripheral smear requires clinical correlation. The index of suspicion should be high in a patient who has been treated with heparin for 1–2 weeks and experiences a relentless drop in the platelet count over several days. In such a patient, the development of new arterial or venous thrombosis would be an ominous sign. The presence of schistocytes is variable in HIT and they are generally less prominent than in TTP or DIC. As discussed above, a decrease in schistocytes may portend development of thrombosis in HIT.

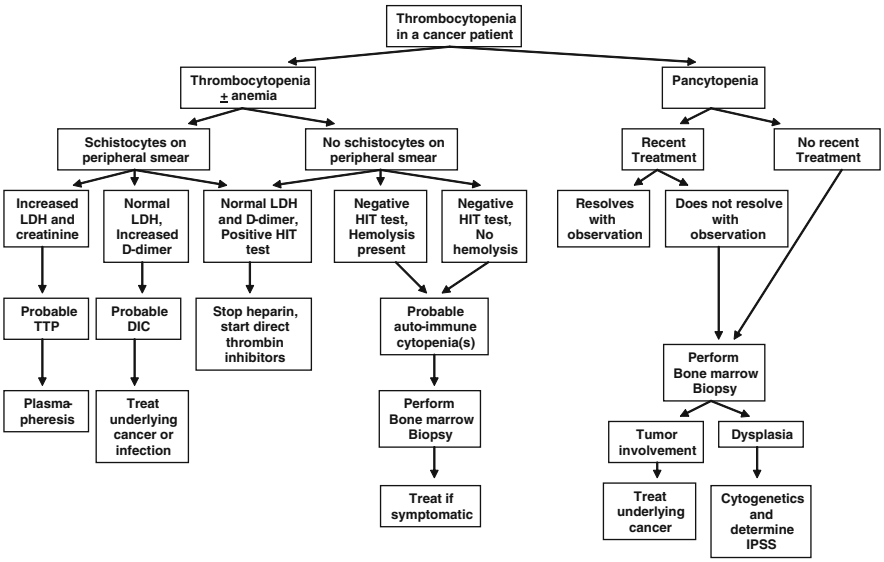

Fig. 16.1 Diagnostic approach to a cancer patient with thrombocytopenia. Differential diagnosis of the cause of thrombocytopenia in a cancer patient involves initial determination of the presence of a consumptive coagulopathy. Subsequent testing will be dictated by this result and the clinical picture
IPSS=International Prognostic Scoring System; LDH=Lactic Dehydrogenase; HIT=Heparin Induced Thrombocytopenia; TTP=Thrombotic Thrombocytopenic Purpura; DIC=Disseminated Intravascular Coagulation

Although an anti-heparin-PF4 antibody test should be ordered, treatment for HIT syndrome should not await the results of this test.

16.4.2 Thrombocytopenia with Normal Peripheral Smear

The absence of schistocytes or abnormal red cell forms in a cancer patient with thrombocytopenia suggests a different line of investigation (Fig. 16.1). In the appropriate clinical setting, HIT should still be high on the differential, especially in the presence of thrombosis. The approach to treatment and diagnosis of HIT is discussed in the section above. In the absence of clinical suspicion for HIT, isolated thrombocytopenia in a cancer patient could be due to ITP. As was discussed above, the most efficient way to make this diagnosis would be by bone marrow analysis. Especially in patients with CLL or lymphoma, patients may have both ITP and auto-immune-hemolytic anemia (AIHA). Laboratory results that would be consistent with AIHA include an increased reticulocyte count and bilirubin, a positive Coombs test, and decreased serum haptoglobin.

Thrombocytopenia, with or without other cytopenias, may be either an early or a late consequence of chemotherapy or radiation therapy. The role of cancer treatment in thrombocytopenia is usually suggested by the timing of the disorder relative to chemotherapy or radiation therapy. If thrombocytopenia fails to resolve within the usual time frame after treatment, bone marrow evaluation is indicated to identify possible other causes. These might include infection or tumor involvement of the bone marrow, resulting in suppression of blood cell production. Thrombocytopenia, with or without anemia, may also be the first sign of development of tMDS years after treatment with chemotherapy and/or radiation therapy. Such events would warrant early bone marrow evaluation to determine prognosis and the timing of aggressive treatments such as stem cell transplantation.

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Chapter 17
Bleeding Disorders Associated with Cancer Dysproteinemias

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17.1 Introduction

Acquired coagulation abnormalities are not infrequently detected in the laboratory evaluation of patients with paraproteinemias; however, clinically evident manifestations are observed in only 10% of such patients [1]. The hemorrhages observed with these abnormalities are rarely spontaneous, but often associated with surgical procedures. Hemostatic abnormalities have been reported in patients with primary AL amyloidosis, Waldenström macroglobulinemia, multiple myeloma and monoclonal gammopathy of undetermined significance (MGUS).

Overt bleeding is a relatively uncommon presenting symptom of MGUS and multiple myeloma <1% [2], but evident in 17% of patients with Waldenstrom's macroglobulinemia [3] and most frequently (40%) observed at presentation in patients with amyloidosis [4]. Rather than a direct consequence of the paraprotein, bleeding complications are common causes of morbidity and mortality during chemotherapy treatment, in patients with renal insufficiency, infections, or after invasive procedures. Many publications, generally restricted to small series of subjects, have described abnormal laboratory findings in coagulation tests. However, these abnormalities are not always associated with clinical symptoms; for example, low factor VII and VIII activity, abnormal platelet function tests, prothrombin time (PT) and thromboplastin generation assays correlate with bleeding, whereas thrombocytopenia and prolonged thrombin time (TT) do not seem to predict hemorrhagic complications [5, 6].

In a study of 62 patients with plasma cell disorders, platelet count and thrombin time were more frequently observed in IgG and IgA paraproteinemias, whereas platelet aggregation tests, factor VIII levels, PT and partial thromboplastin time (PTT) were associated with IgA and IgM paraproteinemias. Mixing studies with normal plasma indicated the presence of an inhibitor

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responsible for the reduced levels of coagulation factors and for prolonged TT, PT and PTT times [5].

17.2 Bleeding Diathesis in Amyloidosis and Waldenström Macroglobulinemia

At diagnosis, 15–40% of patients with amyloidosis show hemorrhagic manifestations, often purpura in periorbital and facial areas [4, 7]. Waldenström macroglobulinemia is associated with symptomatic bleeding (in particular oronasal and retinal) in 17% of cases at presentation [8]. Disruption of vascular integrity by deposition of insoluble fiber can impair vessel vasoconstriction; other causes of bleeding are acquired coagulation factor deficiencies, in particular factor X, the presence of plasma or heparin-like inhibitors; hyperfibrinolysis; and acquired von Willebrand disease.

Amyloid angiopathy is most common in patients with cerebral amyloid angiopathies (CAAs). Approximately 12–15% of all cerebral hemorrhages in elderly persons are related to the presence of CAA [9]. Increased fragility of blood vessels and impaired vasoconstriction are present not only in primary amyloidosis but also in multiple myeloma. Biopsies demonstrate deposit of amyloid substance in the vascular wall [9, 10].

Acquired coagulation factor deficiency, most commonly factor X, is a unique feature of AL amyloidosis. In a recently reported large test series of patients with primary amyloidosis, 8.9% showed factor X deficiency (defined as factor X activity $<50\%$); about half of these patients experienced significant bleeding episodes. The frequency and severity of bleeding was most pronounced with the lowest factor X levels [11]. Absorption of the coagulation factor by AL fibrils, primarily in the liver and spleen, is the generally accepted pathogenetic mechanism [12]. This hypothesis is supported by the rapid clearance from the circulation of I^{131}-labeled factor X and its accumulation in areas involved by amyloid deposits, and by quantitative affinity chromatography studies which show factor X bound to amyloid fibrils [12, 13]. Interestingly, deficiency of factor X associated with amyloid involvement of the spleen [14] was in some cases corrected by splenectomy with resolution of the bleeding diathesis [15, 16]. The rapid clearance of infused factor X after makes it difficult to control bleeding episodes [4]. Few amyloid patients achieved normalisation of factor X levels after oral melphalan chemotherapy. Resolution of the bleeding diathesis was observed in 5 out of 10 patients treated with high dose melphalan followed by autologous stem cell transplantation; however, bleeding complications in the peritransplant period were fatal in two patients [11]. In a retrospective clinical analysis of 337 individuals with systemic (AL)-amyloidosis, abnormal bleeding was reported in 28% of cases, and abnormalities in coagulation screening were observed in half of the patients [7]. The most common abnormalities were prolongation of the TT and PT (32% and 24% respectively). In multi-variate analysis, a prolonged PT was the only coagulation abnormality associated with abnormal bleeding

($P = 0.0012$), but the degree of prolongation was independent of the whole-body amyloid content. Prolongation of the TT was correlated with hepatic amyloid infiltration ($P < 0.00001$), with proteinuria ($P < 0.001$) and with low serum albumin ($P < 0.00001$). In 154 patients who were studied further [7], impaired factor X activity (FX:C) was found in 22 cases (14%). In patients with subnormal FX:C, the corresponding factor X antigen (FX:Ag) measurements were consistently higher than in those patients with normal FX:C, suggesting a dysfunctional FX. No evidence was found of an FX inhibitor. The reptilase time (RT) was prolonged in three of four patients with a prolonged TT.

In a series of 36 patients with amylodosis and monoclonal gammopathy [6], laboratory clotting anomalies were present in the majority of individuals and hemorrhagic manifestations occurred in 10 patients (9 mild-moderate episodes of purpura or ecchymoses but in 1 patient a fatal mucosal hemorrhage). The laboratory abnormalities included prolongation of TT, RT, and Russell's viper venom time (RVVT). Low levels of factor X did not correlate with the RVVT. The presence of a plasma inhibitor was hypothesized to be the cause of coagulation abnormalities, but the serum monoclonal paraprotein was probably not responsible for the alterations of these tests, as they were found to be abnormal even in sera with undetectable paraprotein levels [6]. The possibility that paraproteins are able to inhibit fibrin polymerization, resulting in prolonged TT and abnormal clot formation has also been postulated [17]. Although prolongation of TT and RT is a peculiar feature of amyloidosis, it does not predict bleeding manifestations. Other mechanisms such as factor X deficiency, enhanced fibrinolysis, and amyloid angiopathy seem to correlate better with clinical symptoms [6].

Deficiencies in specific coagulation factors in AL-amyloidosis have long been recognized [18] and, although factor X deficiency has been most studied, there are also anecdotal reports of acquired deficiencies in factor IX [18–20], factor II [19, 20], and factor VII [20]. Hypofibrinogenemia has been described in this setting in association with disseminated intravascular coagulation and increased fibrinolysis [5, 21].

A patient with primary amyloidosis, life-threatening bleeding and acquired factor V deficiency has recently been described [22]. Assays to detect a factor V inhibitor were repeatedly performed but such an inhibitor could not be demonstrated. Although a transient response was observed after the intravenous administration of fresh frozen plasma, the patient exsanguinated from a massive retroperitoneal hemorrhage. Autopsy revealed hepatosplenomegaly with massive AL amyloid deposition [22].

Hemorrhagic episodes are also characteristic of the hyperviscosity syndrome, frequently observed in patients with Waldenström macroglobulinemia and less commonly in multiple myeloma. The syndrome is caused by accumulation of large positively charged IgM molecules with abnormal polymerization and abnormal shape, which bind electrostatically to red cells and increase the blood viscosity. Retinal hemorrhages, epistaxis and gingival bleeding are the most common clinical manifestations [23]. Plasma exchange is an effective treatment to reduce viscosity and control associated bleeding.

17.3 Abnormalities of Primary Hemostasis

Thrombocytopenia (platelet count <100,000/μL) has been reported in 5% of patients with advanced multiple myeloma [2] and in 2.4% of those with Waldenström macroglobulinemia [3]. Overall, thrombocytopenia does not seem to play a major role in the bleeding manifestations of these patients [5]. Abnormalities in platelet function associated with hemorrhagic symptoms have also been described [5, 24]. Defects in primary hemostasis include decreased platelet aggregation, reduced platelet factor 3 availability, and acquired von Willebrand syndrome. Platelet function can be affected by the presence of high concentration of immunoglobulins: addition of purified paraprotein to platelet-rich plasma impairs platelet aggregation and release of platelet factor 3 [24]. Abnormal platelet adhesion to fibrin with associated impaired aggregation is observed in myeloma patients [24, 25].

Non-specific coating of platelets by immunoglobulin has been proposed as the major pathogenetic mechanism. This hypothesis is supported by the beneficial effect of plasmapheresis in correcting the bleeding time and controlling bleeding symptoms [1]. A specific interaction of the paraprotein with the glycoprotein IIIa on the platelet surface was demonstrated in a patient with IgG/kappa multiple myeloma and bleeding complications; the immunoglobulin coated platelets showed reduced adhesion and aggregation in response to ADP or epinephrine and clot retraction was almost completely suppressed [26]. Oxidative stress can also affect platelet function. Zima et al. [27] have described increased levels of plasma malondialdehyde (a marker of oxidative stress) in multiple myeloma and MGUS. Diminished platelet aggregation in response to collagen or ADP was associated with an elevated malondialdehyde concentration and a parallel increase in vitamins A and E [28].

Circulating paraproteins that inhibit VWF and factor VIII activity have been observed in patients with MGUS [29], multiple myeloma, Waldenström macroglobulinemia, lymphoma, chronic lymphocytic leukemia and amyloidosis [30, 31]. In a large international registry, 38% of patients with acquired von Willebrand syndrome (AVWS) had lymphoproliferative diseases, associated with the presence of an IgG or IgM paraproteins [32]. New onset of mucocutaneous bleeding in elderly patients without a family or personal history of bleeding diathesis and the coexistence of a lymphoproliferative disorder should suggest acquired, paraprotein-associated, von Willebrand disease. Similar to type 2A von Willebrand disease, these patients usually have a prolonged bleeding time, reduced ristocetin cofactor activity, a decreased VWF activity-antigen ratio, and absence of large multimers [33]. The presence of antibodies to VWF has been demonstrated in many cases; however, mixing studies frequently fail to show inhibition of VWF function [34]. A recent report has reviewed the sensitivity of several laboratory tests (factor VIII, von Willebrand factor, ristocetin cofactor, collagen-binding factor, PFA-100) for the diagnosis of AVWS in patients with a history of bleeding, negative family history of VW disease, and

abnormal plasma VWF multimers. This single institution study demonstrated the lack of sensitivity of routine tests to rule out AVWS suggesting that VWF multimers should always be included in the diagnostic workup [35]. The decreased in vivo survival of VWF levels after replacement (with cryoprecipitate, FVIII/VWF concentrate infusions or induced by desmopressin) supports the hypothesis that there is accelerated clearance of VWF-autoantibody immunocomplexes from the circulation [36]. It is unclear if the monoclonal immunoglobulin directly neutralizes VWF. In a patient with IgD/lambda myeloma who rapidly developed a large subcutaneous hematoma after puncture of the inguinal artery, a lambda dimeric protein was purified that prolonged PTT and RVVT and inhibited ristocetin-induced platelet aggregation [37]. The lambda dimer showed specific inhibition of VWF binding to glycoprotein Ibα on platelet surface. Inhibition of platelet–VWF interaction was mediated in this case by the negative ionic charge of the dimer. Specific paraprotein interference of VWF binding to collagen has also been demonstrated in a patient with low-grade non-Hodgkin lymphoma and associated to IgG/lambda paraprotein [38]. Mixing experiments suggested the presence of an inhibitor directed against the VWF; immunoprecipitation experiments using recombinant fragments of VWF showed that the inhibitor reacted with both the glycoprotein Ib binding domain and the A3 domain of VWF, but not with the A2 or D4 domains. Increased VWF clearance by immunoabsorption on the surface of malignant lymphocytes or plasma cells has been reported. In a patient with Waldenström macroglobulinemia, binding of VWF to the monomeric IgM on the surface of the malignant cells induced the acquired von Willebrand disease [39].

17.4 Abnormalities of Secondary Hemostasis and Fibrinolysis

Decreased plasma concentration or activity of coagulation proteins, and abnormal fibrin clot formation, have been observed in some patients with paraproteinemias [1]. The paraprotein can directly affect the activity of coagulation factor by inhibition of specific steps in the clotting cascade. In some cases, the hemostatic abnormalities do not depend on the presence of inhibitory paraproteins, but they are associated with accelerated clearance of coagulation factor and paraprotein complexes, or with interference with the natural anticoagulant mechanisms. Although prolonged thrombin times (TT) and reptilase-times are frequently observed in patients with multiple myeloma, this abnormality rarely translates into clinical bleeding symptoms [5].

Inhibition of fibrin monomer aggregation by the paraprotein was suspected in a patient with multiple myeloma who presented with a prolonged TT and RT; mixing studies of the patient's plasma with normal plasma demonstrated correction of TT but not RT [40]. Prolongation of TT, APTT and reptilase time have been observed in a patient with a long-standing IgG kappa monoclonal gammopathy who developed severe haemorrhagic complications.

Plasmapheresis resulted in improvement in the thrombin time and resolution of bleeding. Addition of patient's purified IgG to normal pooled plasma prolonged the TT and APTT, but the reptilase time was prolonged only at high IgG concentrations. This finding suggested that at low concentration, the IgG produced a specific antithrombin effect, but at higher concentrations it also affected fibrin polymerization; the combination of these effects resulted in clinical bleeding [41]. The inhibitory activity of paraproteins on fibrin polymerization has been extensively investigated. Paraproteins of all subtypes can produce inhibition of fibrin polymerization and abnormal clot formation [42]. These clots appear gelatin-like and show poor retraction because of a poorly branched fibrin structure [25, 43]. Studies with different immunoglobulin fragments were able to localize the immunoglobulin inhibitory activity to the antigen binding (Fab) portion of the molecule. However, the interactions between immunoglobulins and fibrin are of low affinity and are not typical antigen-antibody interactions [42, 44].

When high concentrations of pathologic immunoglobulins are required to inhibit clotting reactions, plasma exchange or large-volume plasmapheresis is an effective therapy, particularly in those patients with Waldenström macroglobulinemia [45, 46].

In patients with multiple myeloma, heparin-like anticoagulants have been described: in such patients, TT prolongation was corrected by the addition of heparinase or protamine sulfate; the latter was also able to control bleeding in one patient [47–50]. Paraproteins with anti-factor VIII activity have been reported: the low affinity of the inhibitory immunoglobulin for factor VIII resembled the type II antibody inhibitors in hemophiliacs [31, 51, 52].

Increased fibrinolytic activity has been observed in multiple myeloma and amyloidosis [21, 53]; these patients manifest a clinical bleeding diathesis associated with a shortened clot lysis time and elevated fibrin/fibrinogen degradation products. The pathogenesis of hyperfibrinolysis appear to be due to a reduced levels of α2-antiplasmin, which can also be secondary to complex formation with plasmin. Increased urokinase plasminogen activator activity also has been observed a patient with primary amyloidosis. Immunoprecipitation studies showed that single-chain urokinase plasminogen activator was the main fibrinolytic agonist in the patient's plasma [54]. Treatment with ε-aminocaproic acid was effective in controlling bleeding symptoms in some patients [55], even though accelerated fibrinolysis is not demonstrable.

17.5 Treatment-Associated Coagulation Abnormalities

The treatment of multiple myeloma, amyloidosis and Waldenström macroglobulinemia can affect the hemostatic balance in either a thrombotic or hemorrhagic direction. Standard chemotherapy and new agents can induce bleeding complications by multiple mechanisms.

Thrombocytopenia is a common toxicity associated with some of the new agents that have been added to the armamentarium of anti-myeloma and lymphoma therapy. The proteasome inhibitor bortezomib and the thalidomide derivative lenalidomide, are clear examples. In addition to the reduced platelet count, bortzomib may impair platelet function. Preclinical and clinical reports first suggested a possible anti-thrombotic effect of bortezomib [56, 57]. In vitro studies showed a dose-dependent inhibitory effect of bortezomib on ADP-induced platelet aggregation. The ATP-release reaction induced by collagen was also inhibited in a dose- and time-dependent fashion, even though collagen-induced platelet aggregation was apparently not affected [58]. We have prospectively evaluated platelet function in 10 patients with multiple myeloma treated with bortezomib. Comparing platelet aggregation before and shortly after the proteasome inhibitor infusion, we observed statistically impaired platelet activity triggered by ADP, epinephrine, or ristocetin ($P = 0.033$, 0.034, and 0.0077, respectively) [59]. PSI (Z-Ile-Glu (Ot-Bu) Ala-Leucinal) is a highly specific proteasome inhibitor, which can prevent arterial thrombus formation in renovascular hypertensive rats; this compound has been shown to inhibit platelet aggregation and to increase the initial blood flow rate after arterial thrombosis induction [56].

In the solid tumor arena, the use of anti-angiogenic compounds like bevacizumab has been associated with an increased risk of bleeding complications (mainly mild mucosal hemorrhages). With the introduction of new drugs with anti-angiogenic activity in the treatment of multiple myeloma and amyloidosis, vascular complications may be anticipated. Patients should be closely monitored as they could have pre-existing hemostatic abnormalities associated with their paraproteins.

17.6 Bleeding Management (see Chap. 18)

Treatment of bleeding episodes should be directed to the primary disease; plasmapheresis has been an effective option in selected cases. In some patients, desmopressin may be efficacious; infusions are given at 12-h intervals with monitoring of VWF activity to identify responding patients. Desmopressin may also be given pre- or post-operatively in patients in whom a response to this agent has been identified. Refractory patients may require replacement therapy with FVIII/VWF-containing concentrates. Another option is high-dose intravenous Ig infusions (1 g/kg/day for 2 days), especially in those with an IgG monoclonal component. In patients with IgG autoantibodies to VWF and active bleeding, desmopressin or factor VIII concentrates enriched in VWF, should be administered shortly after intravenous immunoglobulins infusion [60, 61]. Recently, recombinant factor VIIa has been used in sporadic cases to control severe bleeding episodes [62].

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Chapter 18
Control of Bleeding in Cancer

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Clinically significant bleeding occurs in approximately 10% of the patients with advanced cancer [1]. Massive bleeding from the nose, pharynx, lung, or gastrointestinal tract constitutes a major challenge; however, it is difficult to predict who will have a serious bleeding event requiring special management and interventions. Bleeding in patients with cancer may present as a localized bleeding diathesis, more often as a result of local injury by tumor invasion or as a generalized hemorrhagic diathesis caused by thrombocytopenia, platelet dysfunction, coagulation factor deficiencies, presence of inhibitors or increased fibrinolysis. Mild persistent bleeding can be distressing for the patient, family members and caregivers; however, catastrophic massive bleeding may also occur, demanding urgent therapeutic intervention. In consequence, advanced planning is advised especially in patients with the potential for massive bleeding. The planned interventions must take into account the prognosis, patient performance status, and previous and current treatments. This chapter will review the available interventions for managing bleeding in cancer patients.

18.1 Causes of Bleeding in Patients with Cancer

18.1.1 Cancer Invasion

Bleeding may result from local tumor infiltration of blood vessels and lymphatics. Angiogenic factors produced by some tumors promote vascularity of the tumors predisposing the patient to bleeding (Table 18.1). Tumor erosion can involve small and large vessels. In head and neck cancer, there is always the potential for catastrophic bleeding, since exposure to the environment, saliva or prior irradiation weakens the wall of large vessels [2, 3]. This complication has a mortality rate of around 40% and a high incidence of neurologic sequelae among survivors.

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Table 18.1 Causes of bleeding in patients with cancer

Local
Tumor invasion
Systemic
Thrombocytopenia
Platelet dysfunction
Coagulation factor abnormalities
Disseminated intravascular coagulation
Liver disease
Vitamin K deficiency
Acquired anticoagulants
Use of drugs
Oral anticoagulants
Aspirin, nonsteroidal antiinflammatory agents
Dysproteinemias

Patients with lung cancer may present with hemoptysis in approximately 20% of the cases during their clinical course and 3% of patients with bronchogenic carcinoma will have terminal massive hemoptysis with a mortality as high as 60–100% [4]. Hemoptysis is more likely in malignant lesions involving the large airways than in cancers located in the peripheral lung parenchyma.

Gastrointestinal tract malignancies frequently present with slow chronic bleeding. Occasionally, hematemesis may be the presenting symptom of gastric cancer. Hepatocellular carcinoma presents with bleeding in approximately 5–15%, most often among non-encapsulated tumors. Bleeding in liver cancer indicates a very poor prognosis [5].

Bleeding is also a complication of metastatic disease, especially in tumors with vascular components. Metastatic choriocarcinoma may present with hemorrhagic complications due to the hypervascular nature of the tumor.

Among patients with acute myelogenous leukaemia bleeding is a major manifestation. In one study, bleeding was noted in 47 of 106 patients (44%) and 10 of them died from cerebral hemorrhage [6].

18.1.2 Thrombocytopenia

Thrombocytopenia is the most frequent hemostatic defect found among patients with cancer, observed in approximately 10% of cases, even before chemotherapy [7]. In the acute setting, thrombocytopenia is the consequence of decreased platelet production (chemotherapy, radiation therapy or bone marrow infiltration), platelet sequestration, or increased peripheral destruction due to sepsis, disseminated intravascular coagulation, and thrombotic microangiopathy (see Chap. 8). The most common clinical manifestation of thrombocytopenia in patients with cancer is mucocutaneous bleeding. Spontaneous bleeding does not occur unless the platelet count is less than 20,000/μL.

Additional risk factors such as infection, fever, uremia, or use of drugs interfering platelet function, may increase this threshold [8, 9].

18.1.3 Coagulation Abnormalities

Coagulation abnormalities may be the result of disseminated intravascular coagulation (DIC), primary fibrinolysis, acquired anticoagulants, use of drugs, and liver disease. It is well established that DIC is the most common cause of serious bleeding among patients with cancer [10]. In DIC, increased thrombin generation and deposit, coagulation factor consumption, platelet consumption and secondary fibrinolysis contribute to the ischemic and bleeding manifestations of the syndrome [11]. It occurs more frequently with mucin-producing carcinomas of the prostate, pancreas, GI tract, lung, breast and ovary (see Chap. 7). Acute leukemias may develop DIC, but acute promyelocytic leukaemia is more often associated with secondary fibrinolysis.

Acquired anticoagulants are a relatively rare but a serious cause of bleeding in cancer. The inhibitors can be autoantibodies against factor VIII or other coagulation factors (factor V, von Willebrand factor) or heparin-like substances released by cancer cells.

Liver disease has a profound impact on hemostasis. The coagulation abnormalities associated with liver insufficiency are the result of decreased production of coagulation factors, dysfibrinogenemia, enhanced fibrinolysis and thrombocytopenia. Vitamin K deficiency produces alterations in the synthesis of many coagulation factors (factors II, VII, IX, X, protein C and protein S).

18.1.4 Use of Drugs

The use of oral anticoagulants is associated with excessive bleeding, a complication that is particularly frequent among patients with advanced cancer [12]. The risks of prophylactic anticoagulation may outweigh the benefits in some patients with advanced cancer. Despite the use of appropriate therapeutic doses of anticoagulants, the incidence of bleeding complications is much higher among patients with advanced disease when compared with those with earlier-stage cancer [13]. Antiplatelet agents such as aspirin and nonsteroidal anti-inflammatory drugs may induce or exacerbate bleeding, especially in patients with thrombocytopenia.

18.1.5 Treatment-Associated Bleeding

Mucositis is one of the most common adverse effects in patients receiving high-dose radiotherapy and/or chemotherapy for cancer [14]. Bleeding is a frequent complication in patients with advanced mucositis. Extensive mucositis of the GI tract is commonly experienced by patients undergoing conditioning for hematopoietic progenitor cell transplantation (HPCT). On the other hand,

bone marrow transplantation is associated with bleeding in the absence of mucositis [15]. The existence of graft-versus-host disease (GVHD) increases the risk of bleeding following HPCT and it has been found that the incidence of bleeding episodes is correlated with the severity of the GVHD [15].

Mucocutaneous bleeding and hemorrhagic cystitis are common manifestations of hemorrhagic diathesis in patients receiving transplantation [15].

18.1.6 Dysproteinemias

Bleeding events are common complications during treatment and progression of plasma cell dyscrasias (see Chap. 17). Hemostatic abnormalities that are characteristic of these disorders are inhibition of fibrin polymerization, qualitative platelet dysfunction, acquired von Willebrand factor disease and heparin-like circulating anticoagulants. Although major bleeding is uncommon among patients with plasma cell dyscrasias despite frequent abnormal laboratory findings, occasionally serious hemorrhagic complications may occur.

18.2 Management of Bleeding Diathesis

18.2.1 General Measures

The approach to bleeding control in cancer patients must be individualized depending on the underlying causes, the likelihood of reversing the etiology, and the cost-to-benefit ratio of the treatment, as viewed in the context of disease extension, life expectancy and goals of care. In this sense, a patient newly diagnosed with an excellent performance status will be treated differently from a patient with a poor performance status, poor prognosis and a life-expectancy of days.

Patients at risk for bleeding should be identified as a first step in order to implement preventive interventions. Table 18.2 summarizes the patients at risk for bleeding.

Table 18.2 Patients at risk for bleeding

Severe thrombocytopenia
Disseminated intravascular coagulation
Large head and neck carcinomas
Refractory acute leukemias
Severe liver disease
Metastatic tumors (choriocarcinoma, melanoma, renal cell carcinoma)
Patients on oral anticoagulants
Hematopoietic progenitor cell transplantation and GVHD
Treatment with high-dose radiation therapy

From reference [2]

Table 18.3 Control of bleeding in patients with cancer

Local measures
Packing
Compression dressings
Topical hemostatics
Radiation therapy
Endoscopy
Palliative embolization
Systemic interventions
Transfusion therapy
Platelet
Plasma products (fresh frozen plasma, cryoprecipitates)
Administration of vitamin K
Desmopressin
Antifibrinolytic agents
Recombinant factor VIIa
Prothrombin complex concentrate

Despite the lack of clinical trials to guide therapy in patients with advanced cancer, there are some general approaches that can be used to control bleeding (Table 18.3).

18.2.2 Local Measures

Local measures are mainly used in accessible primary or metastatic tumors and to control bleeding from hollow organs such as the nose, vagina, bladder and rectum. Local measures most frequently include packing, topical hemostatics, radiation therapy, endoluminal therapies, and embolization.

18.2.3 Packing

Packing is useful when the bleeding originates from a hollow organ; it can be used with or without compression. Surgical swabs of varying sizes can be coated with different chemicals to facilitate hemostasis. Acetone in vaginal packs may be useful to control vaginal bleeding, especially in patients who have undergone radiotherapy and are not eligible for other interventions [16].

Vasoconstrictors such as epinephrine and silver nitrate have been used for nose bleeding. Cocaine in 4% solution applied to cotton swabs may also control epistaxis [17].

Topical formalin is an effective agent to control vaginal bleeding, malignant cutaneous ulcers and radiation proctitis. Formalin controls bleeding by cross-linkage of proteins [18, 19].

Sucralfate has shown some benefit in controlling bleeding associated with radiation proctitis [19].

A variety of other agents, mostly used in surgical procedures, have been suggested to be of benefit in controlling bleeding in cancer patients; however, the evidence to support their use is mainly anecdotal. Prostaglandins E2 and F2 have been used for severe bladder hemorrhage [20]. Aluminum astringents, such as 1% alum, can be delivered by continuous bladder irrigation [20].

18.2.4 Topical Hemostatics

Fibrin sealants consist of plasma derivatives that simulate part of the normal coagulation cascade to produce an insoluble fibrin matrix [21, 22]. Fibrin sealants are derived from plasma and most of the commercially available products contain human fibrinogen in high concentration, human thrombin, aprotinin and calcium chloride [23]. Their content of factor XIII seems to be crucial to ensure an appropriate clot formation and for reducing the risk of rebleeding [24].

Fibrin sealants and glues are suitable for the treatment of bleeding from malignant wounds. The sealant may be applied with a needle, as spray, or using other devices. In addition to the treatment of wounds, sealants have proved to be effective and safe for the management of fistulas, such as vesicovaginal fistulas following radiation therapy [25].

18.2.5 Radiation Therapy

External-beam radiotherapy is a useful intervention for bleeding control in cancer patients. Gynecologic and lung malignancies have been effectively treated by radiation. In lung cancer, hemoptysis was controlled in up to 80% of the patients treated with radiotherapy [26, 27]. This type of treatment has also been useful in the management of cancer of the vagina, skin, rectum and bladder [28–31]. Hypofractionated regimens of short duration have been successful in managing pelvic bleeding from vaginal [32] or bladder cancer [33].

Palliative radiation therapy is frequently used to control bleeding in rectal cancer although the minimal dose has yet to be determined [34].

18.2.6 Endoscopy

The use of endoluminal therapies is a very good alternative for managing bleeding in patients who are not able to undergo aggressive treatment. The GI tract and the bronchial tree are the most common sites for use of this type of treatment. Endoscopic intervention uses ethanol, epinephrine injection, microwaves, heat, laser and argon plasma coagulation [35, 36].

Malignancy accounts for 1% of severe upper gastrointestinal bleeding. Malignancies that bleed are usually large, ulcerated masses in the esophagus, stomach or duodenum. Endoscopic hemostasis can be achieved by bipolar electrocautery, laser, or injection therapy to allow medical stabilization while determining a long-term strategy [35].

With respect to tumors of the lower GI tract, the Nd:YAG laser is used most often. It generates infrared light which is easily transmitted via the endoscope, is readily dispersed by biological tissue, and has low absorption [37].

Argon plasma coagulation is a thermoablative technique increasingly being used in endoscopy. Surface coagulation for bleeding can be accomplished very effectively, resulting in control of hemorrhage in the majority of patients.

18.2.7 Palliative Embolization

Transcutaneous arterial embolization (TAE) is a radiological technique used to reduce blood flow in selected vessels by insertion of a hemostatic agent. The procedure is performed via an axillary or femoral approach requiring only mild sedation [38]. Embolization is restricted to territories where blood vessels are readily accessible to catheters and the interruption of blood flow will not result in ischemia of a vital organ. The main contraindication to the procedure is a bleeding diathesis and lack of operator expertise.

TAE has been used to manage bleeding in many types of cancer such as head and neck, bladder, prostate, cervix, lung, hepatocellular, renal cell and also for metastatic disease [39]. The choice of material used for embolization depends on the size of the vessel and the desired duration of the occlusion. For larger vessels, wire coils are preferred since they cause an inflammatory response followed by clot formation.

In advanced cervical carcinoma, embolization seems to be as effective as ligation of major vessels in controlling massive bleeding. However, as in other treatment options in patients with advanced cancer, evidence of effectiveness relies on case reports [39] in the absence of randomized clinical trials.

18.2.8 Systemic Interventions (Table 18.3)

The main objective of systemic interventions in cancer patients with bleeding is to correct overall hemostatic defects. These measures include the transfusion of blood products, the administration of hemostatic agents such as desmopressin, vitamin K, recombinant FVIIa, prothrombin complex concentrates (PCC), and antifibrinolytics.

18.2.9 Platelet Transfusions

Thrombocytopenia is a common complication of intensive therapies in patients with hematologic malignancies and solid tumors. Platelet transfusions were

shown to reduce mortality from hemorrhage in patients with acute leukaemia in the 1950s and this procedure has become an essential part of the treatment of cancer [40]. Platelet transfusions are expensive and they can trigger serious side effects. Due to the wide variation in platelet transfusion practices, evidence-based guidelines have been developed to be used in different clinical situations [41–43].

18.2.9.1 Platelet Products

Platelets for transfusion can be prepared either from whole blood and pooled before administration, or by apheresis from individual donors. Comparative studies have shown that the post-transfusion increments, hemostatic benefit, and side effects are similar with either product. Platelet concentrates (PCs) from whole blood, often referred to as random-donor platelets, are prepared by centrifugation of standard units of whole blood. There are two different methods of separation, the platelet rich plasma (PRP) method , used in the US, and the buffycoat (BC) method that is mainly used in Europe. No differences have been shown in the quality of these PCs, although the BC-PCs contain significantly fewer white cells compared with PCs prepared with the PRP method [44].

The major advantage of apheresis platelets, usually called single-donor platelets, is that enough platelets can be collected from a single donor to constitute a transfusion dose. In contrast, to obtain an equivalent number of platelets requires 4–6 whole blood derived PCs. There is a substantial increase in costs for single-donor compared with pooled random donor PCs. As the quality seems to be similar, they can be used interchangeably depending on availability and cost considerations [45].

18.2.9.2 Indications for Transfusion

Traditionally, platelets were administered prophylactically when the platelet count of a patient with chronic thrombocytopenia fell below 20,000 platelets/μL. However, four randomized prospective transfusion trials comparing prophylactic platelet transfusion triggers of 20,000 platelets/μL vs 10,000 platelets/μL showed no differences in hemorrhagic risks [46–49]. Since fewer platelet transfusions were used in the lower trigger arm, a cost saving of 22–33% was obtained compared with the higher trigger arm. Accordingly, the Expert Panel of the ASCO Health Service Research Committee (ASCO-Panel) recommends a threshold of 10,000/μL for prophylactic platelet transfusion in adult patients receiving therapy for acute leukemia. A similar trigger is recommended for patients with solid tumors and chemotherapy-induced thrombocytopenia [41]. Transfusion at higher platelet counts are indicated in newborns or in patients with fever, hyperleukocytosis, rapid fall of platelet count or coagulation abnormalities [41, 50].

Thrombocytopenic patients with malignancies frequently require invasive diagnostic or therapeutic procedures, such as placement of central venous

catheters, transbronchial and esophageal biopsies, paranasal sinus aspiration, bone marrow biopsy and occasionally, major surgery. Based on clinical experience and consensus conference statements it is considered that a platelet count of 40,000/μL to 50,000/μL is sufficient to perform major invasive procedures safely [51, 52]. For lumbar puncture, the platelet count should be >20,000/μL, but bone marrow aspiration and biopsy can be performed at platelet counts of less than 20,000/μL. Before any invasive procedures, it is important to determine the platelet count to ensure that there are sufficient platelets to provide adequate hemostasis.

Platelet transfusions are considered therapeutic if they are given to control active bleeding whether due to thrombocytopenia or platelet dysfunction. Therapeutic platelet transfusions are usually indicated when bleeding is WHO Grade 2 in severity. WHO bleeding Grades 1 and 2 are usually closely correlated with the platelet count [53]. More severe bleeding (WHO Grades 3 and 4) are probably related to associated conditions such as uremia, drugs interfering with platelet function, coagulation factor abnormalities, or vessel damage (tumor invasion). The presence of these factors may account for the failure of platelet transfusions to prevent or control bleeding in thrombocytopenic patients [54].

18.2.9.3 Platelet Dose

The optimum platelet dose has not yet been defined and is still a matter of debate. It is well established that therapeutic platelet transfusion should increase the platelet count of the patient to a level that ensures adequate hemostasis. Based mainly on clinical practice, most centers use a standard platelet dose of about $5–10\times10^{10}$ per 10 kg of recipient body weight [43, 55]. Since the intravascular lifespan of platelets is shortened at low platelet counts [56], transfusion of higher number of platelets is usually indicated. In fact, comparisons of high dose with standard dose transfusions showed that high dose transfusion resulted in greater post-transfusion platelet increments and longer intervals between transfusion without an increase in the total number of platelets transfused [57–59].

18.2.9.4 Expected Response to Platelet Transfusion

Several parameters have been used to assess the effectiveness of platelet transfusions; most of them use platelet counts performed at 60 min and 18–24 h after the transfusion [60–62]. The corrected count increment (CCI) and the percent platelet count increment include adjustments for the dose of platelets transfused and an estimate of the patient's blood volume:

$$\text{CCI}: \frac{(\text{Platelet Increment} / \mu\text{ L}) \times (\text{Body Surface Area in Square Meters})}{\text{Number of platelets Transfused } (\times 10^{11})}$$

The usual dose of pooled platelets or an apheresis collection will produce a CCI of 10,000–20,000/μL (median 15,000/μL). When the platelet count increments are low (<5,000 at 60 min) in two consecutive transfusions, the patient is considered refractory to platelet transfusion [63].

18.2.9.5 Refractoriness to Platelet Transfusions

Failure to increase the platelet count after platelet transfusions is secondary to immune and non-immune factors (Table 18.4). In patients with cancer or haematological diseases, non-immune factors are responsible in 72–88% [64–66] and HLA antibodies (immune refractoriness) in 25–39% [67–69]. Patients with alloimmune refractoriness to platelet transfusions as defined above are best managed with platelet transfusions from donors who are HLA-A and HLA-B compatible. Platelet cross-matching technique may be used to identify compatible platelets in selected patients in whom HLA-matched platelets are not available.

Platelet transfusion therapy in patients with end-stage disease raises a number of concerns; the decision should be made on a case-by-case basis with the objective of controlling severe hemorrhage. Guidelines for transfusing patients with advanced cancer have been established and include the following conditions: continuous bleeding of mouth and gums, overt hemorrhage (GI tract, gynecologic, urinary), extensive and painful hematomas, recent disturbed vision or headache in the presence of thrombocytopenia [70].

18.2.10 Plasma Products

The use of plasma products is indicated to correct hemostatic defects due to consumption or deficiency of plasma coagulation factors. In patients with cancer the most common indications for plasma products include single or multiple coagulation factor deficiencies, reversal of oral anticoagulant effect, urgent invasive interventions, and treatment of DIC.

Table 18.4 Factors associated with refractoriness to platelet transfusions

Non-immune
Fever
Infections
Splenomegaly
Bleeding
DIC
Drugs (Amphotericin, Vancomycin)
Immune
HLA antibodies
Platelet-specific antibodies

For transfusion therapy there are two plasma products available: fresh frozen plasma (FFP) and cryoprecipitate (CP). The choice of the plasma product will depend on the specific deficiency, the clinical situation, and volume considerations.

FFP is separated from whole blood and frozen at –18 °C or colder within 8 h of collection. The volume varies between 180 and 300 mL and contains approximately 1 IU of each coagulation factor per mL. Adequate hemostasis occurs at factor concentrations of 0.2–0.3 μg/mL, depending on the half-life of each clotting factor infused, the absence of inhibitors and the extent of injury or surgery [71]. Assuming an average clotting factor potency of 1 U/mL in FFP, 1 mL of plasma per kg of body weight should increase the factor level by about 1–1.5 U/100 mL if the patient is in a steady state. Accordingly, a rapid infusion of at least 10 mL/kg of body weight is recommended to increase plasma factor concentrations to hemostatic levels [72, 73]. However, due to the dilution factor and the concentrations of clotting factors in plasma, the administration of 10–15 mL/kg will result in only a modest increase in the final concentration of the deficient factor and there will be a risk of producing pulmonary edema. If there is active bleeding, volume overload may not be an issue; however in patients with liver disease volume overload is a limiting factor in how much plasma can be given. Other problems associated with the use of FFP are the variable concentrations of clotting factors and large volume of distribution of factor IX [74, 75]. Administration of FFP is not without risk, and it may have the highest risk of any blood component. The most immediate serious complication is transfusion-related acute lung injury (TRALI), although estimation of the frequency of this complication is uncertain [76]. Allergic reactions to FFP are relatively common, with a frequency of about 1–3% of all transfusions and they can be life-threatening. Other risks are transfusion-transmitted infections; solvent detergent-treated plasma is effective in lessening the risk of viral infections but this plasma is relatively deficient in FVIII, protein C, protein S and α_2-antiplasmin.

Cryoprecipitate (CP) is prepared by thawing FFP at 1–6 °C and recovering the precipitable material which may be stored at –18 °C or colder for up to 1 year. It usually contains at least 150 mg of fibrinogen, and more than 80 U of factor VIII, von Willebrand factor and factor XIII in a volume of about 15 mL. However, CP is not a source of all coagulation factors and therefore it should not be used for replacement therapy in patients with global coagulation factor deficiencies, as frequently encountered in cancer patients. CP use is generally reserved for patients with documented isolated hypofibrinogenemia. The dose to increase the level of fibrinogen depends on the nature of the hemorrhagic episode and the basal level, although the following formulas may be used to calculate the amount of CP to be administered:

1. mg of fibrinogen required: (desired level [mg/dL] – basal level [mg/dl] × plasma volume (mL)/100 mL/dL.
2. N° of CP units: mg of fibrinogen required/150.
   If a large volume of CP is required, ABO compatibility should be taken into account, since the material may contain ABO antibodies.

18.2.11 Vitamin K and Reversal of Warfarin Effect

Vitamin K is necessary for the hepatic synthesis of several clotting factors and inhibitors, including factors II, VII, IX, X and proteins C and S. In patients with cancer many conditions associated with vitamin K deficiency are present, such as liver disease, poor nutrient intake, small bowel disease, biliary obstruction and antibiotic therapy. Coagulation tests will show a prolonged prothrombin time or high International Normalized Ratio (INR). Treatment with vitamin K is indicated to correct clotting factor deficiencies due to deficiency of the vitamin or to reverse excessive prolongation of the INR by oral anticoagulants.

The dose of vitamin K to be selected should take into account the route of administration, the current INR level, and the target INR level for the next few days. Intravenous administration of 0.5 mg of vitamin K is sufficient to decrease the INR to the therapeutic range in most patients with oral anticoagulant overdosing. The vitamin K should be diluted in at least 50 mL of saline and infused slowly, watching for signs of anaphylaxis or other untoward reactions. In case of life-threatening bleeding, the main objective is to completely correct the INR, and 2.5 mg will be adequate in most patients, but for a very high INR or liver impairment, doses of up to 10 mg may be necessary [77]. Administration of oral vitamin K allows patients to be treated at home and reduces the risk of anaphylaxis associated with intravenous vitamin K. The oral administration of 1–2 mg of vitamin K is adequate to treat patients with INR levels between 4.5 and 9, but larger doses (i.e., 5 mg) are required to correct INRs greater than 9 [78].

Patients with serious bleeding due to excessive warfarin should receive FFP or prothrombin complex concentrates (PCC) although the American College of Chest Physicians recommends PCC only for life-threatening situations [78]. The volume of FFP needed to bring a high INR down to 1.5 is approximately 2 L. This amount is difficult to infuse rapidly, especially in elderly patients. The concentration of vitamin K-dependent factors is about 25 times higher in PCC than in FFP, and therefore the volume required is substantially less. The British Committee for Standards in Haematology states that "reversal of anticoagulation in patients with major bleeding requires administration of a factor concentrate in preference to FFP, when available (grade B, level III evidence)" [79]. There is controversy regarding the optimal dose of PCC. For each IU of PCC injected per kilogram body weight, the plasma concentration increases 1%. For life-threatening bleeding it is important to achieve an INR of 1.0, that is, increase the plasma concentration of clotting factors to 100%.

18.2.12 Desmopressin

The vasopressin analog, 1-desamino-8-d-arginine vasopressin (desmopressin, DDAVP), is the treatment of choice for many patients with von Willebrand's disease (VWD) and mild hemophilia A [80]. The mechanism of action of

desmopressin is not completely understood. It induces an increase in the plasma levels of von Willebrand factor (vWF), FVIII, and tissue plasminogen activator (t-PA) and also produces vasodilation. Desmopressin shortens a prolonged activated partial thromboplastin time (APTT) and the bleeding time, enhances platelet adhesion to the vessel wall, lowers platelet count in Type 2B vWD and corrects ristocetin-induced platelet aggregation in Type 1 vWD [81].

Desmopressin is usually administered intravenously at a dose of 0.3 μg/kg diluted in 50 mL saline and infused over 30 min. This treatment increases plasma FVIII and vWF two to five times above the basal levels within 30 min, and high concentrations of FVIII and vWF usually persist for 8–12 h [82]. Most patients treated repeatedly with desmopressin over a short period of time become less responsive, a phenomenon called tachyphylaxis. The drug is available in concentrated formulations for subcutaneous injection (at the same dose as for intravenous use) and nasal inhalation (1.5 mg/mL) which is used at a fixed dose of 300 μg in adults and 150 μg in children [83].

In addition to its use in VWD, desmopressin has proved useful for the treatment of patients with other inherited or acquired bleeding disorders, due to its capacity to shorten the bleeding time, and the in vivo evidence of enhancement of platelet–vessel wall interactions [84]. In patients with cancer, desmopressin has been used in two other conditions, acquired vWD and bleeding in hematologic malignancies.

Acquired vWD is characterized by a prolonged bleeding time and variably low plasma levels of vWF and FVIII. Lymphoproliferative and myeloproliferative disorders appear to be most frequently associated with this condition, accounting for 48–63% of cases [85]. The pathophysiology is not fully understood but patients with lymphoproliferative and myeloproliferative syndromes usually have antibodies directed against functional domains of vWF. Federici et al. reported that treatment with desmopressin in patients with acquired vWD controlled the bleeding and increased vWF levels in 44% and 21% of patients with lymphoproliferative and myloproliferative disorders, respectively [86]. Interestingly, three out of four patients with acquired vWD associated with neoplasia, responded to desmopressin [86].

Patients with hematologic malignancies bleed for a variety of reasons; however, thrombocytopenia seems to be an important factor in most of these patients. In a pilot study, Castaman et al. used desmopressin to treat 15 patients with acute leukemias or blast phase of chronic myeloid leukemia. A favorable response was observed in all the patients after a single infusion of desmopressin (0.4 μg/kg) and the bleeding time shortened significantly in three out of four patients with myelodysplastic syndrome [87].

Desmopressin is safer than blood products as it carries no risk of transmitting infectious diseases. The side effects are transient and mild. Tachycardia, headache and facial flushing are common. Fluid overload and hyponatremia may occur, especially among very young patients who receive repeated infusions. Therefore, it is recommended that desmopressin is used cautiously in small children and patients with congestive heart failure, and free water intake should be curtailed.

18.2.13 Antifibrinolytic Agents

Binding to fibrin facilitates the activation of plasminogen to plasmin, and protects the molecule from inactivation by its natural inhibitors. The synthetic lysine analogs, ϵ-aminocaproic acid (EACA) and tranexamic acid, inhibit fibrinolysis by competing for lysine-binding sites on the plasminogen molecule. The clinical efficacy of these drugs is based on the inhibition of tissue fibrinolysis and the consequent stabilization of clots [88]. Tranexamic acid is approximately 10 times more potent than EACA in vitro.

Fibrinolytic inhibitors have been used extensively in a variety of clinical settings associated with systemic hyperfibrinolysis such as cardiac surgery, liver transplant, antiplasmin deficiency (congenital or acquired), and excessive therapeutic thrombolysis [88]. Conditions where local enhancement of fibrinolysis has been demonstrated or suspected are also successfully managed with antifibrinolytics, including urinary tract bleeding, dysfunctional uterine bleeding, subarachnoid hemorrhage, gastrointestinal bleeding, and mucocutaneous hemorrhages (epistaxis). Antifibrinolytic agents have played an important role in controlling bleeding episodes in clinical situations in which there is no evidence of systemic hyperfibrinolyisis but impaired hemostasis, such as hemophilia or quantitative/qualitative platelet defects. In these disorders, hyperfibrinolysis is probably secondary to decreased thrombin generation and impaired activation of thrombin activatable fibrinolysis inhibitor [89].

In cancer patients, only case reports and few studies, mainly in patients with acute leukemias, have demonstrated a beneficial effect of fibrinolytic inhibitors in the management of bleeding complications [90–93]. However, in the oncology setting, the use of antifibrinolytic agents may contribute to the control of bleeding, especially when other measures have failed or are not suitable. One pilot study of 16 patients evaluated the use of EACA and tranexamic acid for the management of hemorrhage in patients with solid tumors. In 14 out of 16 patients the bleeding stopped at day 4 after the initiation of therapy, suggesting that these agents are potentially useful in this setting [90].

Tranexamic acid and EACA may be given orally or intravenously. The suggested oral dose of EACA is 5 g every 6 h and the intravenous dose is 4–5 g in 250 mL over the 1st hour, followed by infusion at a rate of 1 g/h. The recommended intravenous dose of tranexamic acid is 10 mg/kg three to four times a day, infused over 1 h. Renal function should be monitored regularly and the dose adjusted accordingly because these agents are excreted exclusively by the kidney.

The most common adverse effects of antifibrinolytic drugs are hypotension and bradycardia associated with the intravenous infusion of EACA. Other adverse reactions are nausea, vomiting and diarrhea; these occur in about 25% of the cases and are usually dose-dependent [88]. Thromboembolism is an uncommon but serious adverse effect, most likely to occur when antifibrinolytic agents are used in patients with DIC or concurrently with other drugs

such as all-trans retinoic acid [94–96]. Antifibrinolytic agents are contraindicated in renal failure and caution should be used when prescribing for patients with genito-urinary bleeding, as the resultant clots may obstruct the ureters and/or urethra, resulting in hydronephrosis.

18.2.14 Recombinant Factor VIIa

Recombinant factor VIIa (rFVIIa) was developed as an agent that could bypass inhibitors to factors VIII and IX. Subsequently, rFVIIa was found to improve hemostasis in other hereditary and acquired bleeding disorders. Therefore, the product has been used as a general hemostatic agent to control bleeding in an amazing number of surgical and medical conditions ranging from severe trauma to diffuse alveolar hemorrhage [97].

The mechanism of action of rFVIIa is somewhat controversial. It is generally agreed that about 1% of circulating factor VII in healthy individuals is in the activated form and that the amount of the factor VIIa required for "bypassing activity" is much larger than this. Factor VIIa has a high affinity to tissue factor (TF), but whether rFVIIa has an effect independent of tissue factor has not been completely resolved [98].

rFVIIa binds to TF and/or activated platelets exposed at the site of injury and generates, through factor X activation on TF-bearing cells or the platelet membrane, enough thrombin to activate factors VIII, V, and XI, as well as platelets. The thrombin-activated platelets provide a surface for binding of activated factors VIII, IX, and V, further activate factor X, and generate more thrombin. Because high concentrations of rFVIIa are capable of activating factor X on the platelet surface independent of the presence of factor VIII or factor IX, the drug is capable of initiating hemostasis in severe hemophilia patients with inhibitors [99–101]. Since rFVIIa enhances thrombin generation and provides the formation of tight, stable fibrin hemostatic plugs resistant to premature lysis, it has also been used as a general hemostatic agent in other situations characterized by impaired thrombin generation. Control of bleeding has been reported in patients with various platelet disorders [102–104] and factor XI deficiency [105]. Moreover, a hemostatic effect of rFVIIa has been reported in patients with trauma and extensive surgery who experienced profuse and excessive bleeding [106] It has also been used successfully in patients with acquired hemophilia associated with hematologic malignancies [107].

Initially, doses of rFVIIa were chosen on the basis of subjective evaluation of the response of patients with hemophilia and inhibitors of FVIII [108]. Subsequently prospective randomized trials were performed comparing 35 and 90 μg/kg in such patients undergoing elective surgery. It was found that the higher dose was more effective than the lower dose [109]. More recently, doses of 120 μg per kg body weight have been used routinely [110]. Furthermore, because of the short T1/2 of factor VIIa, doses have been administered

intravenously every 2 h for the first 48 h and every 2–6 h for the next 72 h in nonsurgical cases. Achievement of hemostasis has varied, but in later studies up to 90% of patients have responded to 90–120 μg/kg doses during the first 48 h. Even though the recommended dose is 90 μg/kg, it is clear that the optimal dose and dosing intervals of factor VIIa have not been established with certainty. Recent developments suggest that doses higher than those recommended may be more efficacious in patients with hemophilia and may require dosing at less frequent intervals [111]. Note that all the above studies refer only to patients with hemophilia or acquired hemophilia; dosing intensity and frequency have not been determined in other patients, and most of the use in such patients is off-label.

There is major concern that factor VIIa may cause thromboembolic side effects largely because it is an activated factor given in doses to raise FVIIa levels more than 1000-fold. The adverse event (AE) reports to the FDA record thromboembolic complications occurring in patients who received rFVIIa in a 5-year period after licensure of product. A total of 431 AE reports for rFVIIa were found, of which 168 reports described 185 thromboembolic events. A total of 17 events occurred in patients with hemophilia and 59 occurred in patients enrolled in postlicensure trials [112]. Reported AEs were thromboembolic cerebrovascular accident, acute myocardial infarction, other arterial thromboses, pulmonary embolism, other venous thromboses (including deep vein thrombosis), and clotted devices. In 36 (72%) of 50 reported deaths, the probable cause of death was the thromboembolic event. However, the global frequency of thromboembolic complications has been highly variable. In hemophilic patients it has been reported that less than 1% developed thrombosis associated with the use of rFVIIa [113], whereas in non-hemophilics with intracranial hemorrhage the frequency of thromboembolic events is close to 7% [114]. The incidence of thrombotic complications seems to be dependent on the age and concurrent morbidities of the patients as well as the dose of rFVIIa. Therefore, in patients susceptible of being at risk of thrombosis, it is recommended that lower doses, perhaps of the order of 10–20 μg/kg, should be initiated and the dose increased as necessary [115]. In patients with active bleeding not controlled by rVIIa, consideration should be given to switching to an activated PCC.

18.2.15 Activated Prothrombin Complex Concentrate

FEIBA is a hemostatic bypassing agent that activates FX directly without requiring factor VIII [116]. It contains a mixture of prothrombin complex zymogens, prothrombin, FVII, FIX, FX and their activation products, including FVIIa. The target site of action of FEIBA is the prothrombinase complex in which prothrombin is converted into thrombin by FXa on a phospholipid surface only if FV or FVa is present [116]. This requirement for platelet-associated

FV may reduce the risk of systemic thrombosis by localizing the coagulation process to the bleeding site.

FEIBA has been used mainly to control bleeding in hemophilic patients with inhibitory antibodies against factor VIII or IX, but there are anecdotal reports on its use in liver failure and reversal of anticoagulant excess.

Recommended FEIBA dosage depends on the type and severity of the hemorrhagic episode, with single doses ranging between 50 and 100 IU/kg every 12 h for minor and major bleedings, respectively [117]. The increased thrombotic risks associated with higher FEIBA dosages should be taken into account, avoiding doses exceeding 100 IU/kg as a single dose and daily doses of more than 200 IU/kg [118].

A recent randomized trial compared the efficacy and safety of FEIBA and rFVIIa in hemophilic patients demonstrated that the products are equivalent with respect to their effect on joint bleeds [118, 119].

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