Pathogenesis of Increased Risk of Thrombosis in Cancer

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ABSTRACT

Since the observations of Trousseau, not only has the association of cancer and thrombosis been widely recognized but its pathogenesis is now better understood. Attention to the tumor cell as an important source of procoagulants has also contributed to our knowledge of this problem. Tumor cells express tissue factor (TF) and a cancer procoagulant (CP). TF is dormant in the living cell. However, it is activated during apoptosis of the cell, initiating the coagulation cascade and leading to thrombin generation. Because increased apoptosis occurs during treatment with chemotherapeutic agents, hormones, radiation, and hematopoietic growth factors, as well as when there is rapid tumor proliferation, the thrombosis risk is heightened accordingly. These developments have obvious basic and clinical implications.

KEYWORDS: Thrombosis, cancer, apoptosis, tissue factor, fibrinolysis

Objectives: Upon completion of the article the reader should be able to (1) list procoagulant forces that cause the increased risk of thromboembolism in cancer patients and (2) describe some of the mechanisms that these procoagulants set in motion.
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Trousseau is generally credited with clinically recognizing the connection between phlegmasia alba dolens and cancer.[1] However, the first to present sound histological evidence for the presence of fibrin in tumors was Billroth.[2] Since then, much knowledge has been gained as to the clinical picture, laboratory findings, and pathogenesis of thrombosis in malignant diseases. Abnormalities in the blood of cancer patients have been observed by many investigators, especially in the levels of coagulation factors and in the fibrinolytic system.[3] This subject was recently reviewed by Rickles et al[4] and Falanga et al[5] and will not be discussed in this article.

As to the pathogenesis of thrombosis, the principles put forth by Virchow[6] apply to the cancer patient as well. Abnormalities in the blood flow and changes in the blood vessels and in the contents of blood are all present in malignant diseases. There are additional factors as a result of the contribution of tumor cells. Most of these changes are extensively reviewed elsewhere.[7] This article will be devoted to updating the role of tumor cells in the hypercoagulable state in cancer.

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PROCOAGULANTS PRODUCED BY TUMOR CELLS

Most tumor cells are thrombogenic. O'Meara and Thornes[8] and O'Meara[9] observed that the "thromboplastin" level in malignant tissues is much higher than that in benign tissues. Others attributed these to procoagulants being released from "vesicles" shed from tumor cells.[10] More recently, two procoagulant factors expressed by a variety of tumor cells—TF and CP—have been isolated and characterized. These will be discussed in detail later.

Tissue Factor

CHARACTERISTICS

TF is a membrane glycoprotein that functions as a receptor for factor (F) VII and as the site of activation of F VII to F VIIa. F VIIa then forms a complex with TF and membrane phospholipids to further activate F X. This is the major starting point of the coagulation cascade when tissue injury or cell necrosis is involved and is an important cellular activator of the coagulation system in the cancer patient. The expression of TF is upregulated by tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), and other cytokines.[11] The activity of the F VIIa–TF complex can be inhibited by tissue factor pathway inhibitor (TFPI) in conjunction with F Xa. The procoagulant activity of TF on the cell surface is largely dormant (TF encryption) until alterations of the plasma membrane occur. Thus, disrupted cells generate more procoagulant activity than intact cells.One explanation is that TF encryption is the result of sequestration of phosphatidylserine (PS), which acts as a cofactor for TF procoagulant activity. Membrane alterations during cell injury enhance TF procoagulant activity (TF decryption).[12]

PRESENCE IN TUMOR CELLS

TF has been found in most cancer tissues.[13,14] The high expression of TF may be a characteristic of the malignancy of a cell, as illustrated in the case of acute promyelocytic leukemia (APL). The upregulated messenger RNA (mRNA) of TF in the APL cell returns rapidly to normal levels upon the differentiation of the cell to normal neutrophils after treatment with all-*trans* retinoic acid (ATRA).[15]

ACTIVATION OF TISSUE FACTOR DURING APOPTOSIS

Despite the rich content of TF in tumor cells, it is dormant. Activation will occur when the tumor undergoes apoptosis. In the nonapoptotic cell, the distribution of phospholipids is asymmetrical, and, as such, almost the entire PS molecule is sequestered in the inner leaflet of the plasma membrane. During apoptosis, PS exposure ("flip-flop" action) is an early event preceding DNA fragmentation, membrane blebbing, and loss of membrane integrity. This process is believed to enable the activation of TF and brings about its potent procoagulant activity. This phenomenon of increased TF activity in apoptosis is illustrated in Figure 1. A human APL cell line, NB4, was induced by camptothecin, a topoisomerase inhibitor. Increasing doses of camptothecin resulted in progressive degrees of apoptosis. The TF activ-

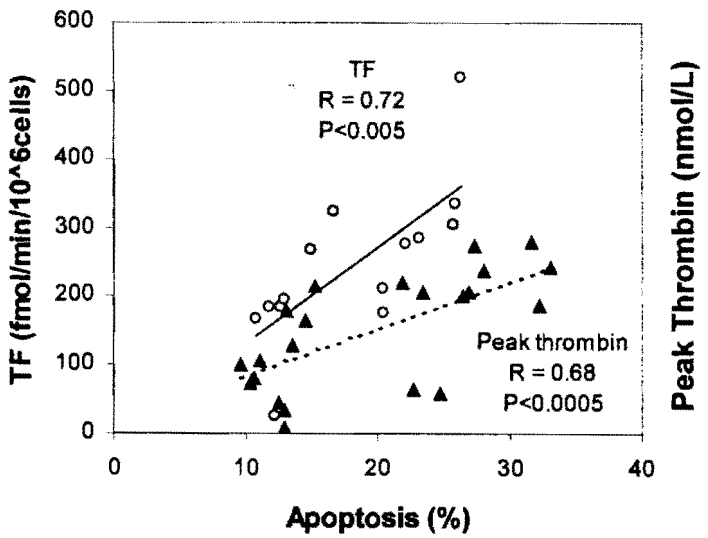

Figure 1 Regression analysis and correlation between TF activity, thrombin generation, and apoptosis index in NB4 cells. Thrombin generation and TF activity assay were carried out with the cell suspensions. The values of TF activity and peak thrombin of thrombin generation curves were plotted against the percentage of apoptosis. The apoptosis index was measured by counting the number of apoptotic cells expressed as percentage of the total cells. The apoptotic cells were recognized by staining with fluorescein isothiocyanate (FITC)–labeled annexin V.

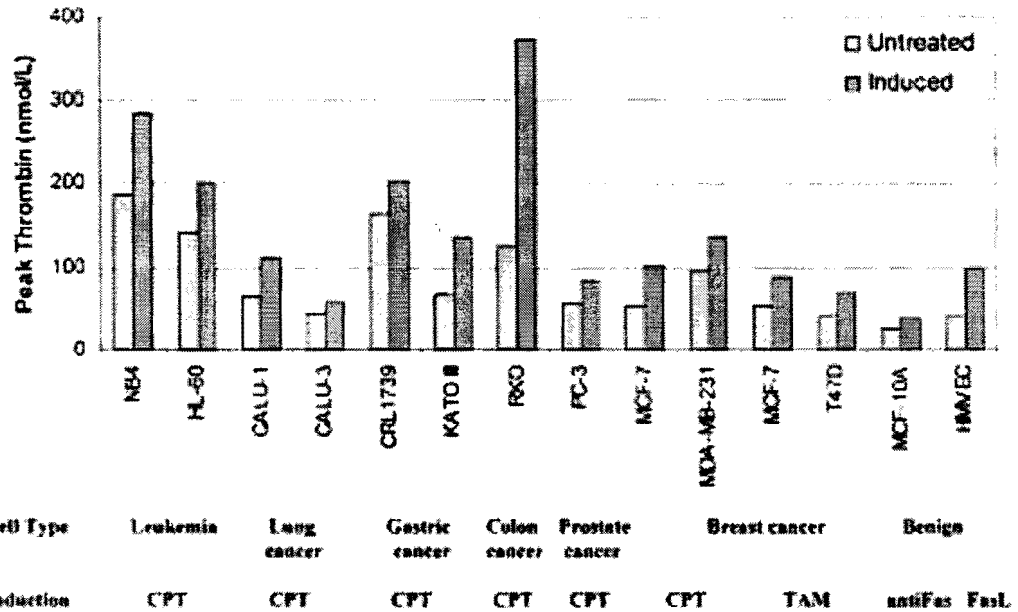

Figure 2 Thrombin generation in various cell lines. The cell lines include acute promyelocytic leukemia (NB4), human acute myeloid leukemia (HL60), gastric adenocarcinoma (CRL-1739, KATO-III), colorectal carcinoma (RKO), adenocarcinoma of lung (CALU-3), epidermoid carcinoma of lung (CALU-1), adenocarcinoma of prostate (PC-3), adenocarcinoma of breast (estrogen receptor–negative MDA-MB-231, estrogen receptor–positive MCF-7 and T47D), benign breast epithelial cells (MCF-10), and human microvascular endothelial cells of dermal origin (HMVEC). Malignant cells were treated with 0.15 μmol/L of camptothecin (CPT) for 4 hours or with 10 nmol/L tamoxifen (TAM) for 48 hours to induce apoptosis. The benign MCF-10A cells were treated with 0.5 μg/mL anti-Fas antibody (antiFas) for 48 hours to induce apoptosis. The human microvascular endothelial cells of dermal origin were treated with 0.1 μg/mL Fas ligand (FasL) for 24 hours to induce apoptosis. The peak values of thrombin generation curves (peak thrombin) were calculated to provide the measurement of thrombin generation.

ity and the thrombin generated by these apoptotic cells were then measured. It can be seen that the amount of TF activity and the thrombin generated by these cells was directly proportional to the degree of apoptosis ($p < 0.005$ and < 0.0005, respectively).[16] Both TF activation and thrombin generation were also studied in a variety of apoptotic cells, both benign and malignant, including APL (NB4), human acute myeloid leukemia (HL60), gastric adenocarcinoma (CRL-1739, KATO-III), colorectal carcinoma (RKO), adenocarcinoma of lung (CALU-3), epidermoid carcinoma of lung (CALU-1), adenocarcinoma of prostate (PC-3), adenocarcinoma of breast (estrogen receptor–negative MDA-MB-231, estrogen receptor–positive MCF-7 and T47D), benign breast epithelial cells (MCF-10), and benign human microvascular endothelial cells of dermal origin (HMVEC). The results are shown in Figure 2. In all cases, a higher degree of apoptosis is accompanied by increasing TF activation and greater amounts of thrombin generated. These data support the concept that during apoptosis, PS localized normally in the inner plasma membrane is exteriorized, resulting in the activation of TF. Annexin V is a plasma protein with a high affinity for PS. It binds and neutralizes the action of PS. When added to apoptotic cells, annexin V was able to prevent the activation of TF and of thrombin generation, supporting the role of PS in the thrombogenicity of apoptotic cells. Because apoptosis can be induced when the tumor cell is subjected to anoxia, chemotherapeutic agents, radiation, hormones, or hematopoietic growth factors, this finding is of obvious clinical significance.

Cancer Procoagulant

CP is a cysteine proteinase that can directly activate F X in the absence of F VII.[17] It is expressed in embryonic (amniotic and chorionic cells) tissues and in many malignant cells and tissues including APL blasts. Because it is not found in normal, differentiated cells, its presence has been used to differentiate normal from malignant tissues. Using enzyme-linked immunosorbent assay (ELISA), CP has been used as a tumor marker.[18,19] CP antigen is present in the blood of cancer patients. However, when the CP activity was investigated in the blood of breast cancer patients, it was found in the blood of patients with stages I and II but not in those with stages III and IV disease. The investigators believed that antibodies might have formed in those with advanced disease blocking the CP activity.[20] If this finding of a lack of correlation between the tumor burden and the CP activity were to be verified, then the significance of CP as a clinically relevant diagnostic test could be significantly attenuated. A compilation of both TF and CP activities in human and animal tumors was

undertaken by the International Society of Haemostasis and Thrombosis.[13,14] It is also notable that in APL the leukemic cells express CP. As the leukemic cells undergo differentiation into normal neutrophils under the influence of ATRA, however, their expression of CP decreases.[21] Interestingly, this loss of CP expression was found only in those APL cells that respond to ATRA but not in ATRA-resistant cells. This is in contrast to TF, which is also expressed by APL cells and whose expression was reduced by ATRA irrespective of their response in differentiation. In the APL cell line NB4, both TF activity and CP activity are present.[22-24]

Downstream Effects of Tumor-Derived Procoagulants

THROMBIN

Thrombin is generated as a result of the release of procoagulant from tumor cells and the activation of the coagulation cascade. In addition to causing thrombosis, thrombin has other effects on cancer (Table 1). Thrombin receptor is present in tumor cells.[25] Upon binding to tumor cells, thrombin has been shown to upregulate the expression of urokinase (u-PA)[26] and its receptor, u-PAR.[27] Because u-PA plays a role in tumor growth and metastasis, this may be one of the pathways for the tumorogenicity of thrombin. In addition, thrombin has been shown to be mitogenic to cells in culture.[28,29] Phosphorylation of tyrosine kinase can be induced by thrombin, leading to second messenger events involved in cell proliferation. Thrombin also increases the adhesiveness of tumor cells to platelets, leading to platelet activation.[30-32]

TISSUE FACTOR PATHWAY INHIBITOR

TFPI is a Kunitz-type serine proteinase inhibitor comprised of 276 amino acids. It has three domains.[33] The first domain binds and inhibits F VIIa. The second domain binds and inhibits F Xa. The third domain may be related to lipoprotein-binding property.[34] TFPI may inhibit the activity of F VIIa–TF by forming a quaternary complex composed of F Xa, TFPI, F VIIa, and TF.[35] Increased TFPI levels in plasma have been found in patients with various types of cancer, including colon and pancreatic cancer.[36-39] TFPI is, however, not expressed by tumor cells in cancer tissues but is mainly produced by the vascular endothelial cells in the tumor. The expression of TFPI may be upregulated by inflammatory cytokines, such as IL-1β and TNFα, produced by cancer cells.[40,41] In a cancer patient, its inhibitory effect on thrombosis is believed to be overwhelmed when there is excessive apoptosis with TF activation.

Table 1 Effect of Thrombin on Tumor Cells

Thrombin receptors are present on tumor cells[32]
Thrombin upregulates u-PA expression in tumor cells[27]
Thrombin upregulates u-PA receptor expression in tumor cells[28]
Thrombin increases cell proliferation[32]
Thrombin increases adhesiveness of tumor cells[32,33]
Thrombin initiates the activation of platelets and adhesion of platelets to tumor cells[31]

Changes in the Fibrinolytic System

The presence of inhibitors of fibrinolysis was observed in cancer patients long before the plasminogen activator inhibitor type 1 (PAI-1) was identified. Many of the early studies centered on the clinical observations of increased thrombosis in carcinoma of the pancreas and were based on global measurements of fibrinolysis, such as the euglobulin lysis time and fibrin plate lysis of euglobulin fractions of patients' blood. Subsequent studies employing more elaborate assays of plasminogen activators, α_2-antiplasmin, and PAI-1 revealed an overall picture of impaired fibrinolytic activity. The contribution of the tumor tissues in the inhibition of plasma fibrinolytic activity was also documented. Many of the studies were also confounded by the presence of disseminated intravascular coagulation (DIC) in the patients, resulting in the activation of secondary fibrinolysis. Thus, the presence of decreased fibrinolysis in cancer may not necessarily add to the thrombophilic state of this condition, and its role in thrombogenesis is overshadowed by more recent findings that this system is involved in tumor growth and metastasis.[42,43]

CLINICAL IMPLICATIONS

Thromboembolic Event as First Manifestation of a Malignant Disease

If there is a strong procoagulant activity of tumor cells, under the appropriate conditions, even with a small tumor load, the effects may be that of a causative factor for a thromboembolic event. In Kwaan and coworkers'[3] early observation in hepatocellular carcinoma, the plasma fibrinolytic activity was inhibited even when the tumor size was as small as 2 × 2 cm. This was explained on the basis of a high content of inhibitors of fibrinolysis in the tumor. A parallel example may be seen when procoagulants are released by tumor cells that express high levels of TF, as in the case of APL. A DIC picture is a common presentation even in early disease. Thus, this concept may be relevant in another clinical situation. In a patient presenting with "idiopathic" thrombosis, without an apparent precipitating cause, the underlying etiology of the thrombosis may be an occult cancer. In a prospective study by Heittiarachchi et al,[44] 13 new malignancies were diagnosed among 326 patients presenting with acute

deep vein thrombosis. Two recent large Scandinavian studies[45,46] revealed that the incidence of newly diagnosed cancer was twofold to fourfold higher than the expected incidence in a comparable population of the same age and gender but without venous thromboembolism (VTE). Increased risk of cancer is present, especially in the first 6 to 12 months after an episode of thromboembolism. When considered overall, the pooled odds ratio for newly diagnosed malignancy in patients with VTE as compared with that in subjects without VTE is 2.09 (95% confidence interval [CI] 1.72 to 2.54).[47] Malignancies commonly associated with presentation of venous thrombosis as the first presentation were cancer of the prostate, lung, colon, pancreas, stomach, ovary, liver, leukemia, breast, kidney, brain, bladder, and rectum, in decreasing order.[48]

The Combination of Multiple Risk Factors

In an individual cancer patient, susceptibility to thrombosis can be enhanced by the concurrence of other factors, including immobilization, dehydration, advanced age, smoking, diabetes, hypertension, nephrotic syndrome, inflammatory bowel disease, and prior to thromboembolism. In a given form of cancer, multiple factors may be present, each contributing to the added risk of thrombosis. This is well-exemplified in the case of APL and in carcinoma of the breast, both of which have been extensively studied.

ACUTE PROMYELOCYTIC LEUKEMIA

APL corresponds to the M3 subtype of acute myelogenous leukemia according to the French-American-British (FAB) classification. It typically presents with a life-threatening hemorrhagic diathesis compatible with DIC. This is particularly common in the microgranular variant (M3v) characterized by marked hyperleukocytosis.[49,50] One laboratory feature distinguishing the coagulopathy of APL from DIC of other clinical conditions is that the level of two coagulation inhibitors, antithrombin (AT) and protein C (PC), is often not decreased. The level of these proteins also correlates with the amount of hepatic dysfunction. Recently, some investigators have described increased level of annexin II–dependent fibrinolytic activity in APL myeloblasts.[51] In the APL leukemic NB4 cell line, this activity is responsive to treatment with ATRA.

Both CP and TF have been identified in the NB4 cell line. The ATRA-induced APL differentiation in vitro is associated with the loss of capacity to express either CP or TF.[21–24] APL subtype expresses the greatest CP activity.[52] In addition, the CP levels in the patients' bone marrow samples are related to the phase of disease, which is measurable at the onset but virtually absent during complete remission.[53]

Major determinants of coagulopathy of APL are factors associated with leukemic cells, including expression of procoagulant activities (PCA), the expression of fibrinolytic and proteolytic properties, and the secretion of inflammatory cytokines, in other words, IL-1β and TNFα. Leukemic blasts from patients with DIC secrete more of these factors. TNFα and IL-1β induce expression of procoagulant TF, fibrinolysis inhibitor PAI-1, and surface adhesion molecules by endothelial cells. These cytokines also downregulate the expression of endothelial cell–derived thrombomodulin (TM). The TM-thrombin complex activates the PC system, which functions as a potent anticoagulant. TF upregulation and TM downregulation lead to a prothrombotic condition of the vascular wall. Leukemic promyelocytes contain both u-PA and tissue plasminogen activator (t-PA).[54–57] In addition, granulocytic proteases such as elastase and chymotrypsin are found in the granules of myeloid blasts and are released into the bloodstream. These enzymes can interfere in several ways with the hemostatic system, that is, by degrading clotting factors in vitro and proteolysing fibrinolysis inhibitors. Elastase can also produce degradation of fibrinogen, producing a pattern of fibrin degradation products (FDP) different from that of plasmin.

Breast Cancer

Thromboembolism in cancer patients has been well-studied in breast cancer. Ten studies concerning the incidence and prevention of thrombotic disorders were listed by Levine[58] and involved 6844 patients with stage I to IV breast cancer undergoing chemotherapy. The incidence of thrombosis increased from 2.1% in stages I/II to 17.6 % in stage IV. Postmenopausal patients were found to have an elevated risk of developing thrombosis. The anticancer drugs used in these studies were adriamycin, cyclophosphamide, epirubicin, flourouracil, methotrexate, prednisone, and vincristine. Mitomycin was associated with thrombotic microangiopathic syndrome, which in most patients was a lethal event.[59] Several authors documented that patients with operable, primary breast cancer, when receiving the cyclophosphamide, methotrexate, fluorouracil (CMF) regimen as an adjuvant chemotherapy,[60,61] developed thrombosis in 2.2 to 6.8% of the cases, increasing to a risk of 17.6% in patients with metastatic breast cancer.[62] Evaluation of coagulation factors through a 6-month period before and after the initiation of chemotherapy in the plasma of patients with stage II breast cancer revealed a significant decrease in the PC and protein S antigen and activity, whereas the PAI-1 levels increased.[63]

Chemotherapeutic drugs can alter endothelial activity to platelets by inducing the release of IL-1, which in turn facilitates adhesion molecule expression on the endothelial cell surface.[64] This may contribute to the

elevated risk of thrombus formation. In anticancer drug-induced thrombosis, patients are observed to have decreased PC/AT ratio as well as increased endothelial reactivity. Other effects noted were release of TF from monocytes and endothelial cells and downregulation of thrombomodulin and a decreased fibrinolytic response.[57]

Tamoxifen, a selective estrogen receptor modifier drug, has been used as the therapeutic agent of choice in patients with breast cancer in adjuvant setting and in metastatic disease, depending on the expression of hormonal receptors by the tumor. Tamoxifen has been associated with substantially increased risk of thrombosis. In the long-term use of tamoxifen for a period of 5 years, the incidence of thrombosis was evaluated.[65] Of the patients treated with tamoxifen, 5.62% suffered venous thrombosis, whereas none was reported in the control group. Patients receiving tamoxifen have significantly lower activities of AT and PC compared with control subjects. Jankowski et al[66] reported that the α_2-antiplasmin level was decreased and the levels of plasminogen were increased, whereas the AT and fibrinogen levels remained unchanged. Activated PC (APC) resistance related to F V Leiden mutation has been reported as a major cofactor for thrombosis in women receiving estrogen analogues.[60] The relative ratio of venous thrombotic events in patients with current tamoxifen use is 7.1 as compared with control. The combination of chemotherapeutic agents and tamoxifen significantly increase venous and arterial thromboembolic events as compared with chemotherapy alone in premenopausal women.

Aromatase inhibitors are associated with lesser thromboembolic events. The recent finding of increased thrombogenicity of apoptotic cells is relevant in such increased thromboembolic events. Apoptosis is induced by chemotherapeutic drugs, radiation, and hormones and is likely to be contributing to the thrombogenesis.

Tumor Lysis Syndrome

Acute tumor lysis syndrome (ATLS) was formally recognized in 1980,[67] giving a name to a hyperuricemic syndrome in the setting of lymphoid malignancies but now is recognized to be a specific oncological disorder that occurs when tumor cells are destroyed rapidly by natural means, chemotherapy, or radiation therapy. This syndrome is another example of increased thrombogenicity of apoptotic and necrotizing tumor cells. The classic laboratory findings include hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. The potassium and phosphate result from the intracellular contents of dying cells; the uric acid results from the metabolism of purines in the degradation of nucleic acids; and the calcium is downregulated in the presence of high phosphate levels. Renal failure results from tubular precipitation of uric acid in acid pH and calcium phosphate in alkaline pH and classically shows evidence of calcification on renal ultrasound.[68] Clinically, the patient may have nausea, lethargy, clouding of the urine, renal colic, or, rarely, joint discomfort or cardiac arrhythmias. Exacerbating factors include heavy tumor burden, high tumor growth fraction, dehydration, concurrent renal insufficiency, and initiation of rapidly effective therapies, including radiation therapy, corticosteroids, and cytotoxic chemotherapy.[69] The time of greatest risk of ATLS is during initiation of therapy,[70] especially rapidly effective therapies such as induction therapy for acute lymphoblastic leukemia in children. It has been postulated that ATLS develops as a consequence of the high rate of tumor cell death associated with aggressive therapy. The resultant release of cellular contents leads to profound metabolic abnormalities. It is therefore not surprising that this syndrome is most commonly associated with hematologic malignancies, including lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myelogenous leukemia, and classically with Burkitt's lymphoma. The severe degree of thrombogenicity often leads to microvascular coagulation and a DIC picture.

Modification in Management

Because the association of increased risk of thrombosis in malignant diseases is well-recognized, stress has been laid on the prophylaxis of deep vein thrombosis, especially in hospitalized patients. In light of the findings of increased thrombogenicity of tumor cells undergoing apoptosis, prophylactic measures should be more aggressive, particularly during periods of increased apoptosis in the clinical course of cancer. These periods include those when the patient is receiving chemotherapy, radiation therapy, hormonal therapy, and hematopoietic growth factors singly or in combination.

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