Haemostatic Issues in Cancer Development and Progression:

"The Role of Coagulation and Haemostatic Factors in cancer Development and Metastasis"

Ph.D. Thesis

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2007

Contents

Dedication

To my mother for her gifts of love and life,

My father for his eternal support and encouragement,

My parents, brothers, sisters and family whose enthusiasm

and dedication continue to inspire me throughout my life

And, my friends & colleagues for being just that.

Foreword

When asking young physicians why they wish to embark on haematology-oncology as a speciality, a thoroughly rehearsed answer centres around their interest for a combination of clinical and laboratory work. This, of course, is precisely what has attracted many of us to haemato-oncology, and it has been a challenge to writers, editors and publishers. The major changes that have occurred in all fields of medicine over the last decade have been accompanied by an increased understanding of the biochemical, physiological, immunological, and pathogenic processes involved in malignant transformation, distant meastasis and disturbances that may occur in different diseases. At the same time, the range of treatment available for patients with malignant haemato-oncological diseases have widened and improved substantially as understanding of the disease process have increased and new drugs and means of support care have been introduced. I can only take pride in my association with Professor Shaker A. Mousa and Professor Hajna Losonczy over several years, and it is my great pleasure to wish them further success they richly deserves, and I sincerely thank both of them.

I. *Introduction:*

Thrombosis is a well-recognized and common complication in patients with malignant disease and can contribute significantly to the morbidity and mortality of this disease. The occurrence of thrombosis is heightened by therapeutic interventions such as operations or the use of radio-chemotherapy. It occurs both spontaneously, after surgery, after radiation therapy and in medical cancer patients receiving anti-cancer treatment. It may also be the first manifestation of underlying malignant disease. The magnitude of the risk for venous thromboembolism is well established for cancer surgery where rates twice that for abdominal surgery in non-cancer patients are described. Venous thromboembolism is the most common complication of cancer and the second most common cause of death in cancer patients (1, 2). Up to 60% of patients with cancer develop venous thromboembolism, depending on the type of cancer and the treatment given (3-6). Although the close relationship between tumour growth and the activation of blood coagulation has been known since 1865, when Professor Armand Trousseau first described the clinical association between primary or idiopathic venous thromboembolism and occult malignancy, only in the last two decade have significant advances in this field been achieved.

The association between malignancy and thromboembolic disease is well established and has been recognized in the medical literature for at least 135 years, since Professor Armand Trousseau and T. Billroth first presented their observations in publications by the New Syndenham Society (25). As Trousseau himself described, venous thromboembolism (VTE) may be a manifestation of occult malignancy and/or may be a complication of known malignancy. Billroth attributed the pathologic finding of tumour cells imbedded in thrombus as evidence for the role of venous thromboembolism in the metastatic process (25).

Several authors have demonstrated that patients with clinically apparent malignancy commonly develop thromboembolic disease (7-13). Patients with thromboembolic disease without a known diagnosis of malignancy have a higher rate of occult malignancy than the general population (13-18). Although supporting data are limited, patients who develop thromboembolic disease in association with malignant disease and are treated

with anticoagulation therapy are often thought to have a higher rate of recurrent thromboembolic disease in comparison with patients with thromboembolic disease that is not associated with malignancy (19-23).
It is now well known that the clinical manifestation of thrombosis in this condition can be very different and vary from localized venous thromboembolism to disseminated intravascular coagulation. In addition, a subclinical activation of blood coagulation or "hypercoagulable state" is present in almost all cancer patients, even without symptoms of thrombosis. A number of pathogenetic factors have been identified, showing that activation of coagulation in cancer is a complex phenomenon, involving many different pathways of the haemostatic system and numerous interactions of the tumour cells with other blood cells, including platelets, monocytes and endothelial cells. The activation of blood coagulation in those with malignant disease appears to be dependent upon the elaboration of tumour-derived tissue factors resulting in an activation of extrinsic pathway of coagulation cascade.
More recently prospective clinical trials have definitely demonstrated that patients with idiopathic venous thromboembolism are at significantly higher risk for a subsequent diagnosis of malignancy as compared to patients with secondary venous thromboembolism (i.e. VTE due to known causes, such as congenital thrombophilia, pregnancy, immobilization, and the use of oral contraceptives, among others).
Equally well demonstrated is the concept that patients with known cancer represent a particularly high-risk group for the development of secondary venous thromboembolism.

These patients have been stratified into the highest risk category for the development of secondary venous thromboembolism. This risk is significantly increased by the anti-tumour interventions, including surgery and chemotherapy. In addition to the thrombotic risk associated with cancer itself, therapeutic interventions such as cancer surgery, chemotherapy and central venous catheters have all been associated withy an increased risk of VTE (1-4). Evidence is accumulating to suggest the potential risk of cancer therapy in increasing the cancer-associated tendency to develop thrombosis. First of all, surgery, which is used to treat patients with localized tumours, is a well-known precipitating factor for thromboembolic disease, since the haemostatic system becomes

more activated before and after surgery (99). Secondly, some chemotherapeutic agents, such as L–asparaginase, mitomycin C, cisplatinum or high-dose chemotherapy-conditioning regimens for haematopoietic stem cell transplantation (veno-occlusive disease) have been associated with thrombo-embolic complications (100). Also the use of haematopoietic growth factors (i.e. G-CSF or GM-CSF) may be implicated in the hypercoagulation and increased thrombotic tendency of neoplastic patients (101, 102). Therefore, clinical trials have been conducted or are currently ongoing to define the best modalities to give thromboprophylaxis routinely to these patients at least during chemo-radio therapy or surgery. Another open question in the clinics of thrombosis and cancer concerns the treatment of thrombosis in those patients who have already experienced one episode of venous thromboembolism. Cancer patients are indeed at increased risk of both recurrences (even with adequate levels of anticoagulation) and bleeding complications. Therefore, much effort is devoted to developing studies in these patients to define the efficacy of anticoagulant drugs and the duration and intensity of anticoagulation.

Post-mortem studies have been demonstrated a markedly increased incidence of thromboembolic disease in patients who died of cancer, particularly those with mucinous carcinomas of the pancreas, lung, and gastrointestinal tract. Although VTE may be found in upwards of 50% of cancer patients at autopsy (50, 51, 52), the optimal study design for determining the true incidence of clinical VTE in cancer patients is a prospective cohort study. Cohort studies of surgical patients showed that the incidence of deep vein thrombosis was markedly higher in patients with malignant disorders than in patients with other, non-malignant diseases.

Although its frequency in different tumour types has not been described in detail, there appear to be tumour differences, with certain cancers associated with poor outcome (e.g. pancreas, lung) attended by a higher frequency of thromboembolic complications. The suggestion is that thrombosis may be associated with a poor outcome for patients with cancer is further supported by large epidemiological databases indicating that cancer patients who either develop a thromboembolic episode during the natural history of their cancer or are diagnosed with a thrombotic episode when their cancer is diagnosed have a poorer survival over time than cancer patients without thrombosis (80).

The first attempt at such a study was a retrospective analysis (53) of data derived from randomized clinical trials of therapy in patients with breast cancer (54-66); data that was collected prospectively.

Other patients with advanced cancers who are likely to be at higher risk for thromboembolism include patients with brain tumours receiving chemotherapy, those with locally recurrent rectal cancer receiving radiation, pancreatic cancer or advanced gastrointestinal cancers (particularly adenocarcinomas) [53]. However, precise estimates of thrombotic rates in these groups of patients are not available. Von Tempelhoff et al. (67) reported a 10.6% rate of VTE in women with advanced ovarian cancer receiving chemotherapy. The majority of data regarding the risk of VTE in patients with other cancers come from small case series and retrospective reviews. Rates of 8.4% have been reported in patients with germ cell tumours receiving chemotherapy (68), 24-60% in high grade gliomas (69, 70, 71), and 5-10% in patients with Hodgkin's or non-Hodgkin's lymphoma (72-75). In addition to chemotherapy, the use of haematopoietic growth factors may increase the risk of VTE, although no prospective data s available and meta-analysis by Barbui et al. (75) was inconclusive. Finally, cancer patients with indwelling central venous catheters are at increased risk for thrombosis of the axillary/subclavian vein (76, 77), with the catheters themselves susceptible to thrombotic occlusion despite the use of routine heparin flushes.

Surgical intervention in patients with cancer places them at increased risk of postoperative VTE (approximately 2-fold) in comparison to non-cancer patients undergoing the same procedures (78, 79). The case for routine thrombo-prophylaxis in patients receiving chemotherapy is less clear, and prospective studies investigating rates of thrombosis by tumour types, stage of malignant disease, and chemotherapeutic regimens are required.

In this thesis, the pathogenetic mechanisms of thrombosis in malignancy, thrombophilic state in cancer patients, changes in haemostatic parameters and their relation to cancer prognosis and also the thromboprophylaxis in the cancer patients are discussed and analyzed.

Pathogenesis-Pathophysiology:

Despite the fact that thromboembolism is a major problem in cancer patients, the study of the prethrombotic conditions in cancer have been relatively neglected or just given a phenomenological approach. Possible contributory mechanisms for blood clotting activation in tumour patients include general factors related to the host's response to the tumour (acute phase reaction, abnormal protein metabolism, neovascularization, necrosis, haemodynamic rearrangements) and more specific factors such as the activities expressed by tumour cells and tumour-associated macrophages. The history of medicine again reminds us that Virchow, over 150 years ago, postulated that three features may predispose to thrombus formation, that are abnormalities in -1) blood flow, -2) blood constituents and -3) vessel wall. Patients with cancer may show abnormalities of each of the three components of Virchow's triad, leading to a prethrombotic or hypercoagulable state. Pathogenetic mechanisms of thrombogenesis in the cancer patients are complex and involve multiple interdependent processes between the tumour and the patient's physiological response to the tumour. These act to promote a hypercoagulable state. These mechanisms accounting for the development of thrombotic disorders in patients affected by cancer were described by Virchow more than a century ago. They include procoagulant activity (hypercoagulability due to tumour cell activation of clotting and the pro-thrombotic properties of the tumour cells), host inflammatory responses, vessel wall injury, stasis, and extrinsic factors.

Abnormalities of blood flow may be associated with changes in blood resistance or viscosity. This may be due to risk factors occurring in malignancy, such as venous stasis due to a lack of mobilization, sepsis or external compression from a bulky tumor. Blood viscosity (high and low shear rates) measured preoperatively in cancer patients have been correlated with the incidence of postoperative DVT (88). The possibility that abnormal blood vessel formation (related to cancer angiogenesis and factors promoting it) may cause flow disturbances also has to be considered (89). Venous stasis predisposes to venous thrombosis by preventing activated coagulation factors from being diluted and cleared by normal blood flow. Moreover, hypoxic damage to endothelial cells due to stasis may produce pro-thrombotic alterations. Venous stasis develops as a consequence of immobility in severely debilitated cancer patients, in conjunction with cancer surgery,

or as a result of venous obstruction due to extrinsic vascular compression in patients with bulky tumour masses (49).

Cancer cells can activate coagulation directly through an interaction with platelets and/or clotting and fibrinolytic systems to generate thrombin. Clotting activation may be considered as a special type of inflammatory reaction to stimuli such as vessel wall damage, or intravascular cell aggregation or entry in blood of abnormal cells such as tumour cells (39, 90). The balance between the coagulation and the fibrinolytic system can easily shift to a prethrombotic state in cancer, through an excess of tissue factor (TF), other procoagulant proteins or of plasminogen activator inhibitor (PAI-1) or through deficiencies in inhibitory molecules (antithrombin, protein C, protein S) or in fibrinolytic principles (tissue plasminogen activator, t-PA). Consistent with the shift of the haemostasis balance toward hypercoagulation in cancer, several studies have shown reduced levels of natural inhibitors of coagulation such as antithrombin (AT), protein C (PC) and protein S (PS) in plasma of cancer patients. These decreased levels of inhibitors might result from an increased consumption as a consequence of the activation of coagulation or from a defective hepatic synthesis or both mechanisms combined. Chemotherapy and hormonal treatment can also induce an acquired deficiency in naturally occurring anticoagulants-inhibitors. In breast cancer, patients receiving cyclophosphamide, methotrexate and fluorouracil have decreased levels of PC and PS antigen and activity soon after the beginning of the therapy while plasminogen activator inhibitors increase (127). However, no relationship between these low levels of inhibitors and markers of hypercoagulability or thrombotic events could be found in these studies. Low levels of PC, PS and AT have also been reported after treatment with tamoxifen and *L*-asparaginase (128, 129, 130, 131, 132). Activated protein C (APC) resistance is the most common abnormality of the coagulation system in patients with hereditary venous thrombosis. The APC resistance phenotype is associated in a majority of patients with heterozygosity or homozygosity for a single-point mutation in the factor V gene which substitutes G with A at nucleotide 1691 (factor V Leiden, FVL). This mutation modifies one of the three APC cleavage sites. The slower degradation of the mutated activated factor V by APC leads to a higher rate of thrombin generation and a hypercoagulable state (133, 134, 135). Acquired resistance to APC is defined as a phenotypic resistance

that occurs in the absence of FV-Leiden mutation. Acquired resistance to APC was found to be much more common in patients with cancer and thromboembolism than in patients with thromboembolism without cancer (54% versus 19%). In contrast, resistance to APC due to FVL mutation was more common in thrombophilic patients without cancer than with cancer. These results provide evidence that FVL does not play a major role in the hypercoagulable state of cancer while acquired APC resistance contributes to the thrombotic resistance in these patients (136).

Venous thromboembolism is the most common complication of cancer and the second most common cause of death in cancer patients. Several types of cancer, including tumours of the pancreas, prostate, lung, breast, stomach, and colon, have been associated with a prethrombotic state characterized by clotting factor abnormalities, endothelial dysfunction, and abnormal blood flow due to increased viscosity or stasis. Among the most frequently studied characteristic properties of malignant disorders are the; cancer cell interactions with the haemostatic system and their procoagulant activities. Neoplastic cells can activate the clotting system directly, thereby generating thrombin and fibrin formation, or indirectly by stimulating mononuclear cells, platelets, and endothelial cells to produce and express pro-coagulants (27, 91, 92).

Thrombin generation and fibrin formation are constantly determined in patients with malignancy, who are at increased risk of thromboembolic complications. Most importantly, fibrin formation is also involved in the processes of tumour spread and metastasis. In addition to the thrombotic risk associated with cancer itself, therapeutic interventions such as cancer surgery, chemotherapy and central venous catheters have all been associated with hypercoagulable state and an increased risk of venous thromboembolism.

Malignant cells can interact with the haemostatic system in multiple ways. The principal pro-thrombotic properties of tumour cells are: -1) the capacity to produce and release pro-coagulant activities, fibrinolytic proteins, and inflammatory cytokines, i.e. interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF); and, -2) the capacity to interact with host cells, including endothelial cells lining the blood vessels, monocytes, platelets and neutrophils.

The best characterized tumour cell pro-coagulants are **tissue factor (TF) and cancer procoagulant (CP)**. These promote blood coagulation either directly, via activation of factors VII and X of the blood coagulation cascade, or indirectly by initiating an inflammatory response. The inflammatory response enhances the prethrombotic process via the action of potent inflammatory mediators, which induce further expression of procoagulants from tumour cells, activate platelets and induce endothelial expression of prethrombotic factors.

Tissue factor (TF) is a 47-kDa trans-membrane (integral membrane) glycoprotein which forms a complex with factor VII (FVII)/FVIIa: the TF/FVII complex triggers blood coagulation by proteolytically activating factor IX and X (26, 27, 81). Tissue factor is the cellular pro-coagulant found in normal cells, including endothelial cells and monocytes-macrophages. However, these cells do not express TF in normal resting conditions, but expose this pro-coagulant in response to pro-inflammatory stimuli, i.e. the cytokines IL-1β and TNF-α and bacterial endotoxin (82). Differently from normal cells, malignant cells constitutively express TF. In addition to activating blood coagulation, TF has the capacity of regulating VEGF expression in tumour cells and in vascular endothelium (83), which represents an important pro-angiogenetic mechanism. The expression of TF has been identified in some acute leukaemias (28) and in solid tumours of the stomach, ovary, and kidney (29). Direct factor X activation with the procoagulant cysteine proteinase has been found in some patients with prostate, lung, breast, lung, and kidney cancer and with leukaemia (30, 31). Mucin-secreting adenocarcinomas are frequently associated with thrombosis because the sialic acid moiety can cause non-enzymatic activation of factor X to its active form, factor Xa (32). Consequently, adenocarcinomas of the pancreas, lung, gastrointestinal tract, and ovary are often associated with venous thrombosis (33).

Cancer pro-coagulant (CP) is a 68 kDa cysteine proteinase with 674 amino acid residues and no detectable carbohydrates (84). It activates factor X independently of FVII and cleaves the factor X heavy chain at a different site compared to other known factor X activators. Cancer pro-coagulant (CP) has been found in extracts of malignant cells or in amnion-chorion tissues but not n extracts of normally differentiated cells (85). CP antigen has been identified in the sera of cancer patients and found to be elevated in 85% of the

study subjects. TF and CP have been identified in several human and animal tumour tissues and they are different in nature.

Fibrinolytic activities: Tumour cells can express all the proteins regulating the fibrinolytic system, including the urokinase-type plasminogen activator (u-PA), the tissue-type plasminogen activator (t-PA), and the fibrinolysis inhibitors plasminogen activator inhibitor (PAI)-1 and PAI-2 (86). Among the activators, u-PA is the most widely expressed within malignant diseases. Furthermore, cancer cells can carry the specific plasminogen activator receptor (uPAR) on their membranes. The presence of these receptors favours the assembly of all the fibrinolytic components on tumour cell membranes, facilitating the activation of the fibrinolytic cascade. The fibrinolysis proteins can play a role in tumour cell proliferation, invasion and metastasis.

Cytokines: Tumour cells produce inflammatory cytokines, including IL-1β, TNF-α and VEGF. Tumour cell-derived IL-1β and TNF-α can induce the expression of TF pro-coagulant activity by endothelial cells. The also down-regulate the expression of endothelial cell thrombomodulin (TM), the surface high-affinity receptor for thrombin. The TM-thrombin complex activates the protein C system, which in turn functions as a potent anticoagulant. Tissue factor up-regulation and TM down-regulation lead to a pro-thrombotic state of the vascular wall (86, 87). The same cytokines stimulate endothelial cells to produce the fibrinolysis inhibitor PAI-1. Inhibition of fibrinolysis further contributes to the pro-thrombotic potential of endothelial cells. In addition, the production of VEGF by malignant cells may significantly affect the functions of microvascular vessels in proximity of the tumour and may play an important role in tumour neo-angiogenesis (83). Further, VEGF is chemotactic for macrophages and can induce TF pro-coagulant activity of monocytes and endothelial cell. The expression of TF by tumour cells up-regulates the transcription of VEGF in these cells. TF modulates the expression of VEGF by endothelial cells, a function that can have important implications in tumour neo-vascularization (83). Regulation of VEGF synthesis by TF in malignant cells and vascular cells provides an important link in cancer patients between activation of coagulation, inflammation, thrombosis and tumour progression and metastasis.

Tumour cell-Host cell interactions: Based on studies of laboratory parameters, it has been demonstrated that there are activation/perturbation of several cellular systems 'in vivo' in cancer patients, i.e. elevated levels of plasma endothelial markers (e.g. von Willebrand factor, TM, soluble E-selectin, t-PA, PAI-1) and this leads to the activation of haemostasis at the endothelium site, especially during chemotherapy (83, 86). Further, the increase in tissue factor pro-coagulant activity (PCA) expressed by circulating mononuclear cells shows that this cellular compartment has become activated. Finally, the detection of high levels of platelet membrane specific glycoproteins (e.g. CD62 and CD63), which re exposed upon activation, provides evidence for platelet activation 'in vivo' in malignant conditions (87). Patients with metastatic cancers may exhibit increased activation as also indicated by enhancement of platelet turnover and a decrease in platelet survival time. These platelet abnormalities may be due to a greater degree of destruction of the lysosomal membranes and to involvement of platelets in the low-grade intravascular activation of clotting detectable in the majority f cancer patients (93). Tumour cells can interact with the endothelium essentially by their capacity to synthesize and release inflammatory cytokines. These cells can also directly adhere to endothelial cells and to the extracellular matrix through membrane adhesion molecules. Endothelial cells activated by IL-1β or TNF-α increase the exposure on their membranes of counter-receptors for the tumour adhesion molecules. The malignant cells attached to the vessel wall may play a key role in promoting localized clotting activation and thrombus formation by releasing their cytokine content and favouring the adhesion and arrest of other cells, including leukocytes and platelets. The adhesion of tumour cells to leukocytes or to vascular cells may also facilitate cell migration and extravasations. In addition, the TF-induced expression of VEGF by endothelial cells may have implications in tumour neovascularization.

Tumour cells can activate systemic coagulation by stimulating mononuclear cells to synthesize and express various procoagulant substances, including tissue factor and factor X activators. Normal monocytes and macrophages can be activated by tumour cells in the presence of lymphocytes (34). In patients with cancer, endothelial cells may be activated by cytokines such as tumour necrosis factor and interleukin-1 or interleukin-like substances that may induce tissue factor production (35). A peptide produced by a human

bladder cancer cell line stimulates tissue factor expression in endothelial cells (36). Clinical manifestation of increased thrombin generation may be accentuated by down-regulation of endothelial cell counter-regulatory mechanisms, such as decreased hepatic synthesis of antithrombin and protein C (37, 38, 41, 42). In addition, normal endothelial cells function may be disrupted by various defects in platelet function (37, 38, 41, 42). The enhanced clotting activation and the pro-thrombotic properties of the tumour cells in patients with cancer are confirmed by the demonstration of increased levels of systemic hypercoagulability markers, such as fibrinopeptide A, prothrombin fragment F1+2 and thrombin-antithrombin complexes in most patients (38, 39, 40). As expected, the risk of recurrent) venous thromboembolism is higher in those cancer patients who are also carriers of thrombophilia, such as the factor V Leiden mutation (41).

The role of endothelium in mediating a prethrombotic state is well known. Endothelial cells may be stimulated in cancer to produce procoagulant material (such as e.g. TF) either directly by tumour-specific antigens or indirectly by cytokines (82, 94, 95). The latter (IL-1, TNF-α) are able to suppress endothelial fibrinolytic activity, downregulate thrombomodulin expression while increasing the endothelial expression of leukocyte adhesion molecules, platelet-activating factor, as mentioned, TF (96, 97, 98).

There is increasing awareness that cancer cells can injure endothelium by direct vascular invasion, resulting in the onset of a pro-thrombotic state. Moreover, tumour cells may secrete vascular permeability factors which account for the extravascular accumulation of fibrinogen and other clotting proteins around tumour growth (36, 37, 38). The adhesion of tumour cells to endothelium was evaluated in vivo by Naschitz and associates, who observed a complex interaction between tumour cells, endothelium, and platelets (43). Direct vessel wall injury, in association with rheologic abnormalities and catheter-associated thrombin generation, is most likely the explanation for the occurrence of the upper extremity deep vein thrombosis arising as a complication of central venous lines (44). Among mechanisms responsible for thrombotic events arising during the use of chemotherapeutic drugs, vascular endothelium damage probably plays a major role besides the reduction in the plasma concentration of natural anticoagulants (45-48).

Thrombosis and disseminated intravascular coagulation (DIC) are common complications of cancer. Specific conditions associated with cancer such as stasis due to immobilization or blood flow obstruction, surgery, infections, endothelium damage due to chemotherapeutic agents, radiation therapy and abnormalities of blood coagulation contribute to the hypercoagulable and thrombophilic state of cancer patients. Several types of cancer, including tumours of the pancreas, prostate, lung, breast, stomach, and colon, have been associated with a prethrombotic state characterized by clotting factor abnormalities, endothelial dysfunction, and abnormal blood flow due to increased viscosity or stasis (103). This procoagulant state in cancer patients arises mostly from the capacity of tumour cells to express and release procoagulant activities, e.g.; TF and CP. Additionally, there are decreased levels of inhibitors of coagulation, impaired fibrinolysis, the presence of antiphospholipid antibodies and an acquired activated protein C resistance (APCR) contribute to the hypercoagulable state. The activation of coagulation is also implicated in tumour proliferation through interactions of coagulation with inflammation and increased tissue factor pathway inhibitor (TFPI).

Laboratory diagnosis of the procoagulant-thrombophilic state includes:

1. elevation of clotting factors, fibrinogen/fibrin degradation products, hyperfibrinogenaemia and thrombocytosis,
2. elevation of specific markers of activation of coagulation: fragment 1+2, fibrinopeptide A, D-dimers, thrombin-antithrombin (TAT) complexes

It is a well-known fact that none of these tests has any predictive value for the occurrence of thrombotic events in one individual patient. But, changes in these haemostatic parameters have been known to be associated with the prognosis of cancer patients. In patients with venous thromboembolism (VTE) a non-invasive screening for occult cancer is able to detect a relatively high incidence of hidden cancers and the search for thrombophilia seems important in patients without known cancer. Haemostatic abnormalities are found in more than 90% of cancer patients and are clinically expressed as DIC or VTE. The reported incidence of deep vein thrombosis (DVT) or pulmonary embolism (PE) ranges from 1 to 15% in autopsy (96, 97). However, the relationship between symptomatic VTE and the risk of subsequent occult malignant disease is still

controversial. Thrombosis is a multifactorial disease, where interactions between genetic and environmental factors result in the formation of an obstructive thrombus at a specific location. Among the acquired risk factors for VTE is the thrombophilic-hypercoagulable state in cancer patients, e.g.; procoagulant activities (TF and CP), changes in natural inhibitor levels (decreased antithrombin, Protein C and Protein S levels, increased tissue factor pathway inhibitor=TFPI), acquired APCR, increased expression of activator of fibrinolysis to a hyperfibrinolysis state, increased generation of thrombin-activatable fibrinolysis inhibitor (TAFI) which leads to impairment of fibrinolysis and will produce a hypercoagulable state in cancer patients, and development of antiphospholipid antibodies which produce an acquired hypercoagulable state. All these changes in the haemostatic parameters will lead to a hypercoagulable state in cancers patients which can be measured by different laboratory tests. Tissue factor pathway inhibitor (TFPI) is the physiological inhibitor of TF-induced coagulation by forming, in a two-step mechanism, a quaternary complex with the coagulation factors Xa and VIIa and TF. TFPI levels are high in cancer patients and increased levels have been found in advanced malignancy (130). The reason for these high values of TFPI is unknown. It could be a consequence of the hypercoagulability in cancer patients, since thrombin induces a re-distribution and acute phase release of TFPI in vitro as well as in vivo in animals (131, 132).
Thrombosis is a complication in patients with solid tumour malignancy (104), and the heightened risk is secondary to tumour elaboration of tissue factor, a physiologic procoagulant that is responsible for the genesis of a systemic hypercoagulable state (105). Once activated, coagulation proteases have a profound effect on tumour cell behaviour in experimental models (106), enhancing tumour cell motility, invasion, angiogenesis, and growth. Hence, interference with activated coagulation serine proteases may influence tumour biology.
Clinical evidence in support of anticoagulants having an anti-tumour effect was first reported in a multicenter, randomized, controlled trial in 1981 (107). In the Veterans Affairs Research Service Cooperative Study 75, warfarin was found to be associated with an improvement in median survival in patients with small-cell lung cancer who were receiving chemotherapy. Similarly, a randomized trial in the same patient population demonstrated a survival advantage for those patients treated with subcutaneous injections

of unfractionated heparin (108). However, despite compelling experimental evidence for a pathogenic role of blood coagulation in tumour growth and metastasis (109, 110, 111, 112), other studies in patients with solid tumours have failed to confirm a survival benefit for patients treated with anticoagulants (113, 114, 115, 116).

More recently, the question of whether anticoagulants can favourably influence the natural history f cancer has received renewed attention. Randomized controlled trials and meta-analyses of studies that compared low molecular weight heparins with unfractionated heparin for the initial treatment of venous thromboembolism have reported a reduction in the overall mortality of patients with cancer who were randomly assigned to receive a low molecular weight heparin (117, 118, 119, 120, 121). Although the reduction in mortality has been consistent across studies and could not be attributed to differences in fatal pulmonary embolism or bleeding, the observation that 5 to 7 days of low molecular weight heparin treatment reduced cancer mortality has been difficult to explain. A plausible biologic mechanism, however, is now emerging from experimental studies that show low molecular weight heparins can inhibit angiogenesis, a process that is critical for tumour growth and metastasis, in a dose-dependent fashion (109, 110,122, 123).

To date, two randomized, placebo-controlled trials designed to evaluate whether low molecular weight heparins can improve survival in patients with advanced or incurable cancers have been completed (124, 125).To examine the influence of a low molecular weight heparin relative to coumarin derivatives on the survival of cancer patients with venous thromboembolism (FAMOUS study) and to investigate the hypothesis that low molecular weight heparins have a greater impact on survival in cancer patients with limited disease than in those with disseminated cancer (MALT trial), the CLOT study was performed which was a posthoc analysis of the mortality data in patients with solid tumours who participated in the Comparison of Low Molecular Weight Heparin Versus Oral Anticoagulant Therapy for Long Term Anticoagulation in Cancer Patients With Venous Thromboembolism (126).

II. *Goal and perspective of Study:* Relationship between haemostatic parameters and cancer prognostic markers

Although the relationship between malignant diseases and thromboembolic complications has been convincingly demonstrated, the clinical implications of this association still have to be thoroughly elucidated. The most common abnormalities described in cancer patients are elevation of the clotting factors V, VIII, IX, and XI, increased fibrinogen/fibrin degradation products (FDP), hyperfibrinogenaemia, and thrombocytosis. The aim of this prospective study is to evaluate the changes in haemostatic-clotting parameters in patients with different types of non-haematological malignancies (solid tumours) and to asses the correlation between changes in coagulation parameters and the stage of tumour, imaging findings and also with changes in characteristic tumour markers. In our prospective study, we mainly focused on specific abnormalities of haemostasis in these groups of patients, the changes in haemostatic parameters and their relation to cancer prognosis. In addition, the thromboprophylaxis in the cancer patients are discussed and analyzed. The relationship between the hypercoagulable state and the tumour progression and the occurrence of metastasis has not been well established. Larger studies and further investigations on tests that could have a reliable predictive value in tumour progression are required to evaluate the correlation between changes in haemostatic parameters, tumour stage and progression-metastasis Much more information is needed and only large-scale clinical trials will unequivocally establish whether these hypercoagulable parameters have strong predictive values and whether the modulation of haemostatic system will modify the process of tumour progression and metastatic dissemination. This study also provides a rationale for the use anticoagulants for the prevention of thromboembolic complications and may change the course of tumour progression.

Inclusion in the study required tissue diagnosis for histopathology classification, detailed imaging techniques for exact staging, and absence of any medications that might interfere with the results of hypercoagulation markers. The study design and outcomes evaluations mirrored those used in prior studies, except that our study used more than one coagulation parameter for a more detailed assessment of the changes in haemostatic

system in cancer patients.

Except for a slight difference in the gender ratio, the analysis of patients who met the pre-specified criteria for evaluation, the hypercoagulable parameters were directly correlated with tumour progression and rise in characteristic tumour markers. It is well known fact that the tumour cells produce and express different procoagulant substances, such as tissue factor, cancer procoagulant, plasminogen activators and these factors interact with the vascular cells, blood cells, coagulation system and fibrinolytic system, and will lead to a disturbance in the normal haemostasis, which finally will result in an abnormal hypercoagulable status. We studied the prognostic values of F1 + 2, D-dimer, and natural inhibitors of abnormal coagulation in patients with solid tumours, but so far no convincing data have allowed identification of one of these hypercoagulability markers as reliable disease prognostic marker (Gouin-Thibault and Samama, 1999).

Parallel with the haemostatic parameters, the characteristic tumour markers were also measured. In all studied cases, there was a direct correlation between changes in the haemostatic parameters, tumour markers and radiological-imaging findings, e.g.: rise in D-dimer, F 1+2 was associated with a drop in AT, PS, PC, which was directly correlated with a rise in tumour markers and a progression of the malignant diseases in imaging findings.

We performed a prospective study to evaluate the relationship between changes in haemostatic parameters and cancer prognostic markers.

Method in selection of patients: Patients were eligible for enrolment in the study if they were over the age of 16 years and they had been diagnosed of having solid organ cancer. For patients to be eligible for enrolment in the study, the following requirements were necessary; tissue sample for establishment-confirmation of diagnosis and for histopathologic classification, detailed imaging investigations-techniques for exact staging (in some cases the imaging studies were reviewed by external radiologists and nuclear medicine specialists and therefore their report was considered satisfactory and no repeat examination was requested), and the patients could not take any medication or treatment that might interfere with the results of hypercoagulation markers. The study design and evaluations mirrored those used in prior studies, except that in our study we used more than one coagulation parameter for a more detailed assessment of the changes

in haemostatic system in this group of cancer patients. The patients were enrolled in this study prior to any type of treatment. Additional criteria for enrolment were absence of previous thromboembolic event in the past 12 months, absence of any heparin derivatives, oral anticoagulant agents and also anti-platelet drugs. Patients with suspected distant metastasis were excluded. Patients who received any type of anticoagulant or hormonal treatment in the past 6 months were also excluded. Patients having abnormal kidney and liver function tests were also not enrolled.

Patients diagnosed as having different types of non-haematological, solid organ tumour were enrolled in the study. Of the 54 patients enrolled in this study;

2 had oesophageal adenocarcinoma,

6 had gastric cancer,

16 had colorectal cancer,

4 had exocrine pancreatic carcinoma,

2 had adenocarcinoma of gallbladder,

6 had adenocarcinomatous type of non-small cell lung cancer, and

2 had small cell lung cancer,

12 had infiltrating breast carcinoma, and 2 had ovarian cancer.

Four patients were excluded because they developed VTE during the period of study. Detailed monitoring upon admission and prior to any cancer-related intervention and on a weekly basis post-intervention (chemotherapy, radiation or surgery) for up 18 weeks were carried out. The most important natural inhibitors of abnormal coagulation (PC, PS, and AT), and D-dimer and prothrombin activation peptide F 1 +2 as markers of the status of fibrinolytic and coagulation systems were studied in these group of patients prior to any form of therapy. These markers were repeatedly measured with each treatment course and their results were correlated with other markers of tumour prognosis.

Coagulation markers: The most common abnormalities described in cancer patients are the elevation of the clotting factors V, VIII, IX, and XI, increased fibrinogen/fibrin degradation products (FDP), hyperfibrinogenaemia, and thrombocytosis. Therefore, the following markers of coagulation system were examined; AT, PS, PC, D-dimer and Prothrombin activation peptide F 1 + 2

Design of study: Patients were stratified according to ECOG (Zubrod) performance status score and those with ECOG score of 0-1 were enrolled in the study. Baseline AT, PS, PC, D-dimer and prothrombin activation peptide F 1+2 as haemostatic parameters and characteristic tumour markers according to the type of tumour were measured (Green and Silverstein, 1996; Manucci, 1997; Tripodi and Manucci, 1996). Staging of cancer at various anatomic sites were done as developed by the American Joint Committee on Cancer (AJCC) in cooperation with the TNM Committee of the International Union Against Cancer (UICC). The International Histological Classification of Tumours provided by the World Health Organization (WHO) was used for pathologic classification and definition of tumour types. Physical examination, imaging, endoscopy, biopsy, and surgical exploration were used for clinical classification and staging. Histological grading was also used for qualitative assessment of the differentiation of the tumours. All the patients were treated according to the internationally recommended therapeutic regimens and they were closely followed up to evaluate the state of their malignant disease. The haemostatic parameters, the tumour marker and the imaging techniques were repeated after the completion of 2 full chemotherapeutic regimens. In those cases where surgical intervention were indicated prior to the start of chemotherapy, the coagulation parameters and tumour markers were measured prior to the surgery and the haemostatic values were repeated after the end of surgery. None of these patients were on any kind of medications which would interfere with the results of coagulation studies. During each visit physical examination, vital signs and medication history were taken and any changes in these findings were registered. If patient developed febrile neutropaenia as a complication of chemotherapeutic agent, the haemostatic parameters were measured at the onset of the diagnosis of febrile neutropaenia and thereafter.
At the end of study, 4 patients were excluded because they developed thromboembolic complications during the study period and they were started on antithrombotic therapy. The characteristics of the other patients are listed in ***Table 1***. The chemotherapeutic regimens that were used are all the standard internationally recommended protocols, as listed in ***Table 2***.

Table 1. Summary of tumour types & treatment

Tumour Type and Stage	Treatment
Non-small-cell lung cancer: two females with stage IIIA Bronchioalveolar adenocarcinoma, Stage IIIA (T3N1M0 & T2N2M0), 4 males; 2 with stage IIIA acinar adenocarcinoma (T2N2M0 & T3N1M0) and 2 with stage IIIB papillary adenocarcinoma (T3N3M0)	Neo-adjuvant chemo- (radio-) therapy followed by surgical resection or/and surgical resection followed by adjuvant chemo-(radio-) therapy
Small-cell lung cancer: two males with limited-stage disease (according to the Veterans Administration Lung Group staging system)	Surgical resection followed by adjuvant chemo (-radio) therapy and prophylactic cranial irradiation (PCI)
Breast cancer: 12 females (infiltrating ductal carcinoma); 7 had stage IIA (3 had T1N1M0 & 4 had T2N0M0), 4 with stage IIB (2 with T2N1M0 & 2 with T3N0M0) and one had stage IIIA (T1N2M0)	Surgical intervention, radiation treatment and adjuvant chemotherapy Neo-adjuvant chemotherapy followed by surgical resection, additional chemo (-radio) therapy

Ovarian cancer: two females with stage IIA (FIGO staging system), well-differentiated serous adenocarcinoma	Surgical resection followed by systemic chemotherapy
Colorectal adenocarcinoma, tubulovillous adenoma, Dukes B2 stage (16 males)	Surgical resection followed by adjuvant chemotherapy
Pancreatic adenocarcinoma, stage III (2 females, 2 males)	Surgical resection followed by systemic chemotherapy and radiotherapy
Oesophageal adenocarcinoma, stage IIB (2 males)	Primary systemic chemotherapy and radiation followed by surgical resection (oesophagectomy)
Gallbladder adenocarcinoma, stage II (1 male, 1 female)	Surgical resection followed by chemotherapy and local radiotherapy
Gastric adenocarcinoma, stage II (6 males)	Surgical resection (with lymphadenectomy) followed by chemotherapy and radiation therapy

Table 2. Most commonly used chemotherapeutic regimens

Tumour type	Regimen used
Non-small-cell lung cancer	Taxanes + platinum derivative, Vinorelbine and Gemcitabine every 3-4 weeks for maximum of 6 cycles
Small-cell lung cancer	Platinum derivative (mainly cisplatinum) + etoposide every 3-4 weeks for total of 4-6 cycles & PCI
Colorectal adenocarcinoma	5 (FU) + folinic acid for 8–12 cycles every 2–4 weeks
Gastric adenocarcinoma	Mainly 5 FU + Folinic acid + cisplatinum *or* ECF (Epirubicin + Cisplatinum + 5 FU), but ELF (Etoposide + Folinic acid + 5 FU) also used occasionally again every 3–4 weeks and for at least 6–8 courses (depending on the response)
Oesophageal adenocarcinoma	Cisplatinum + 5 FU every 3–4 weeks prior to surgery & irradiation for 2–3 cycles & after surgery to repeat the same regimen for 2–3 additional

	cycles; ECF protocol used in 1 case
Pancreatic adenocarcinoma	5 FU + Folinic acid every 4 weeks (plus Gemcitabine) for at least 6 cycles with radiation therapy
Breast cancer	Hormone (endocrine) therapy; SERMs, CMF and modifications, AC (Doxorubicin + Cyclophosphamide) followed by Taxanes, FAC (CAF) and modifications [5-FU, Doxorubicin, Cyclophosphamide], Trastuzumab, and many other protocols
Ovarian cancer	Taxanes (Paclitaxel) plus platinum

5 FU = 5 fluorouracil

The study was primarily designed to determine whether haemostatic parameters were correlated with changes in imaging findings, tumour stage, and characteristic tumour markers changes. The tumour markers which were used in this study are recommended in follow up of patients with different types of solid tumour. These tumour markers are not tumour-specific, but they might be used as a useful tool for both diagnosis and follow up these patients and also to assess the efficacy of the treatment. The pre-specified criteria for evaluation were usefulness of these hypercoagulability markers in the follow up of this group of patients and also their use as reliable cancer prognostic markers.

The study design and outcomes evaluation mirrored those used in prior studies, except that our study used more than 1 coagulation parameter for a more detailed assessment of the changes in haemostatic system in cancer patients.

Except for a slight difference in the gender ratio, in the analysis of patients who met the pre-specified criteria for evaluation, the hypercoagulable parameters were directly correlated with tumour progression and rise in characteristic tumour markers. It is well known that tumour cells produce and express different procoagulant substances (such as TF, CP, and plasminogen activators) and that these factors interact with the vascular cells, blood cells, coagulation system, and fibrinolytic system and will lead to a disturbance in the normal haemostasis, which finally will result in an abnormal hypercoagulable status.

We studied the prognostic values of F1 + 2, D-dimer, and natural inhibitors of abnormal coagulation in patients with solid tumours, but so far no convincing data have allowed identification of one of these hypercoagulability markers as reliable disease prognostic marker; however, a recent study has shown that D-dimer's property as a sensitive marker of fibrinolysis (which is an unfavourable clinical sign) can be used specifically in the prognosis and treatment of patients with lung cancer. To suppress the significant and apparently cumulative haemostatic activation that results from chemotherapy in patients with adenocarcinomatous malignancies, prophylactic dose of low-molecular-weight heparin has been recommended by some.

Parallel with the haemostatic parameters, the characteristic (but not specific) tumour markers were also measured. In all studied cases, there was a direct correlation between changes in the haemostatic parameters, tumour markers, and radiological imaging

findings; for example, rise in D-dimer and F 1+2 was associated with a drop in AT, PS, and PC, which was directly correlated with a rise in tumour markers and a progression of the malignant diseases in imaging findings. Likewise, a high level of D-dimer and low AT level was significantly ($P < 0.05$) correlated with short survival, which suggest that these may be a sign of poor prognosis in these group of patients.

The relationship between the hypercoagulable state and the tumour progression and the occurrence of metastasis has not been well established. Larger studies on tests that could have a reliable predictive value in tumour progression are needed. Much more information is needed, and only large-scale clinical trials will unequivocally establish whether these hypercoagulable parameters have strong predictive values and whether the modulation of haemostatic system will modify the process of tumour progression and metastatic dissemination.

The most important natural inhibitors of abnormal coagulation (protein C, protein S, and antithrombin), and D-dimer and prothrombin activation peptide F 1 + 2 as markers of the status of fibrinolytic and coagulation systems were studied in these group of patients prior to any form of therapy. These markers were repeatedly measured with each treatment course and their results were correlated with other marker of tumour prognosis. In all cases the levels of hypercoagulable markers were elevated directly correlated with tumour stage, tumour markers and radiological findings of cancer state. Additionally, the level of naturally occurring coagulation inhibitors were all decreased with progression of tumour. Also, these hypercoagulable parameters were raised after surgical resection of these tumours. Based on our findings, determination of D-dimer, F1 + 2, protein C, protein S and antithrombin levels can be used as markers to assess the tumour prognosis. Furthermore, hypercoagulation and haemostatic imbalances might accelerate DVT/PE, tumour growth, tumour (neo)-angiogenesis, and metastasis. This also suggests that early anticoagulation prophylaxis might prevent VTE and impair tumour progression.

Baseline Characteristics of the Patients: A total of 54 patients were enrolled in the study over a period of 18 months. Four patients were excluded from the final evaluation of study because of the development of thromboembolic events during the period of study. The baseline characteristics of the eligible patients are summarized in ***Table 3***.

Table 3: Baseline characteristics of eligible patients

Age (yr)	
-Median	54
-Range	26–72
ECOG performance score	0–1
Average number of chemotherapy cycles	8
Family history of cancer	
-Female	2
-Male	1
Smoking	
-Female	2
-Male	14
Alcohol intake	
-Female	0
-Male	4
Other malignancy	
-Female	1
-Male	0

Statistical Analysis:

The statistical analysis was performed by two-way analysis of variance (ANOVA) comparing the markers of haemostasis activation at admission to post-admission for each subject and with respect to average control values; differences were considered significant at ***p*** value of 0.05 or less.

The Cox proportional hazards regression model was used to adjust the treatment effect on survival for baseline factors in all patients with solid tumours, and for the subgroups with and without metastasis. The variables identified as potentially important predictors, and recorded at the time of enrolment, included age, gender, ECOG performance status, smoking status (ever vs. never), type of cancer treatment (radiation vs. none, chemotherapy vs. none), and major primary site (breast, lung, colorectal, pancreas, and gynaecologic).

D-dimer, prothrombin fragment 1 + 2 (F1 + 2), antithrombin, protein C, and protein S activities were also measured at the onset of diagnosis, pre- and post surgery, and after the completion of each chemotherapy course. Their levels were correlated with the levels of tumour markers. The normal ranges of various haemostatic parameters and abbreviations are summarized in ***Tables 4-5***, respectively.

Table 4: Normal ranges of various haemostatic parameters

Haemostatic parameters	Normal reference values
AT-activity	**Ref. range: 65–140%**
PC activity	**Ref. range: 70–140%**
Free PS antigen	**Ref. range: 65–140%**
PS activity	**Ref. range: 60–140%**
D-dimer	**Ref. range: 0.0–0.3 mg/l**
Prothrombin fragment 1 + 2	**Ref. range: 0.32–1.1 nmol/l**

Table 5: Abbreviations used

AJCC: American Joint Committee on Cancer, UICC: International Union Against Cancer
AT: Antithrombin
CP: Cancer Procoagulant, TF: Tissue Factor
DIC: Disseminated Intravascular Coagulation
FPA: Fibrinopeptide A
PAP : Plasmin-Antiplasmin
PC: Protein C
PS: Protein S
TAT: Thrombin-Antithrombin
TFPI: Tissue Factor Pathway Inhibitor
VTE: Venous Thromboembolism
WHO: World Health Organization

III. *Results* of study: The Relationship between haemostatic parameters & cancer prognostic markers

1. Gastric carcinoma (Stage II & IIIA)

Prothrombin F1 + 2 and D-dimer levels increased over time post-treatment in 4 of 5 gastric adenocarcinoma patients, with a peak increase at weeks 8–11 (*Figure 1A, 1B*). In 1 of the 5, the levels of F1 + 2 and D-dimer were normalized (*Figure 1A, 1B*). In contrast, the natural anticoagulants PC-activity, PS-activity, and AT-activity levels showed progressive decrease in 4 of 5 patients, with a peak decrease at 8–11 weeks (*Figures 1C–1E*). In 1 of the 5 patients, the levels of those natural anticoagulants were normalized (*Figure 1C–1E*).

Figure 1: Haemostatic activation markers at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks in gastric adenocarcinoma (stage II/IIIa) patients. *Figure 1A*: F1+2 (nmol/L), and *Figure 1B*: D-dimer (mg/L). Natural anticoagulant markers at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks are shown in *Figure 1C*: PC activity (%), *Figure 1D*: PS activity (%), and *Figure 1E*: AT activity (%).

Figure 1A.

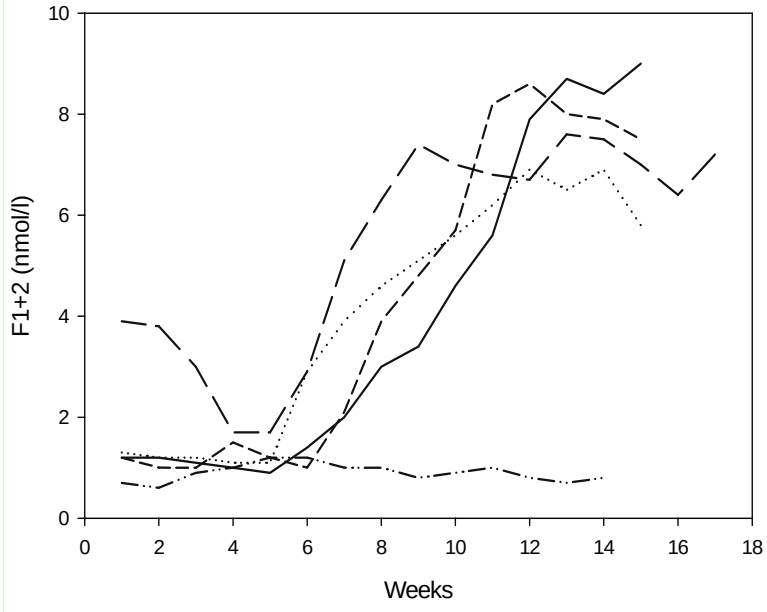


Figure 1B.

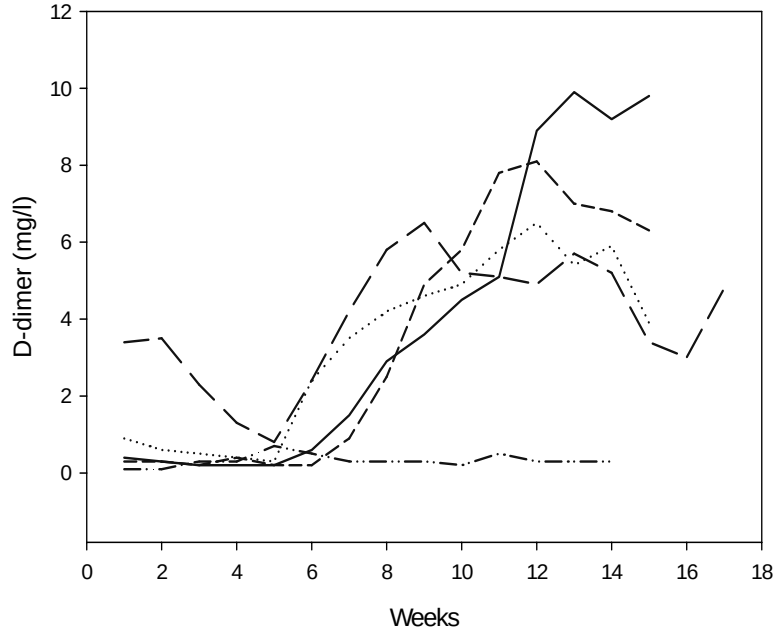


Figure 1C.

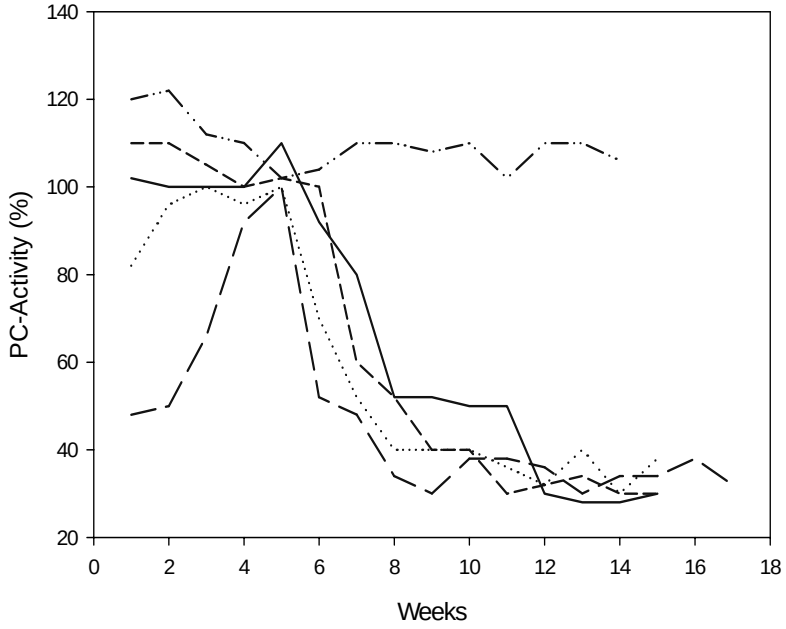


Figure 1D.

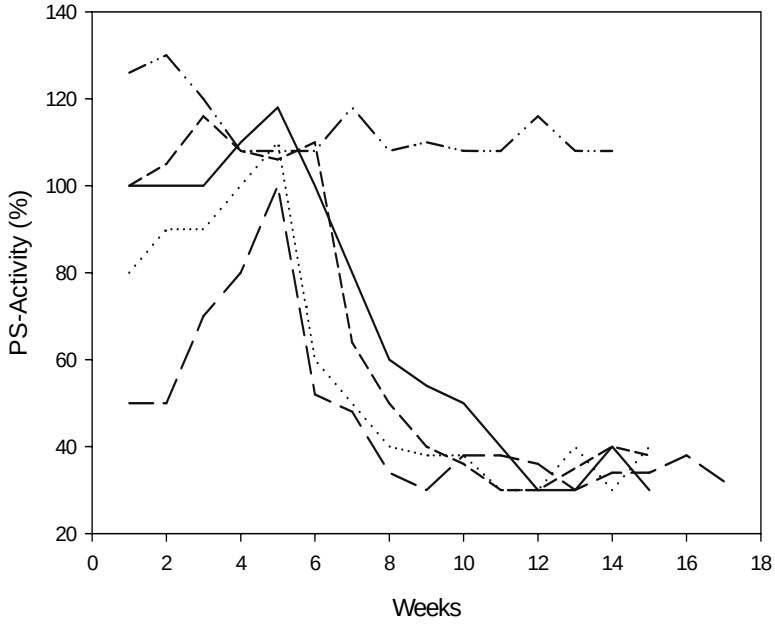
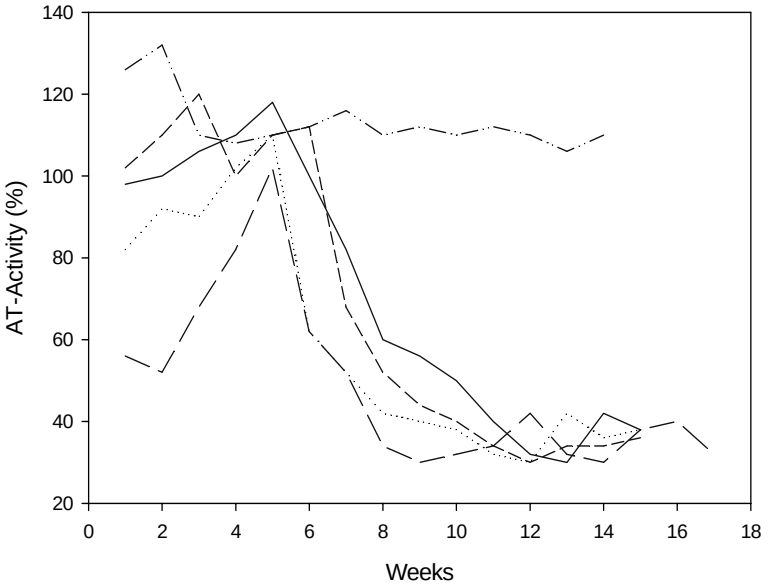


Figure 1E.



CT imaging in gastric adenocarcinoma patients:

An excellent trend between tumour mass and haemostasis activation was also shown. A representative case of preoperative CT in a male patient with stage II/IIIa showed the existence of a large obstructive tumour blocking the gastric lumen and infiltrating the stomach wall (*Figures 1H-I*). Early elevation in haemostasis activation markers was also noticed. In *Figures 1H-I*, CT imaging after surgery and chemotherapy illustrates the excellent response and the normalization of haemostasis markers.

Figures 1 F-G: At diagnosis—CT preoperative imaging in male patient with stage II/III gastric adenocarcinoma. The tumour as shown is obstructing the gastric lumen and infiltrating the wall of the stomach. Early changes in haemostatic activity markers were shown. *Figures 1 F-G* are postoperative CT imaging after surgery and adjuvant chemotherapy showing very good response.

Figure 1 F.

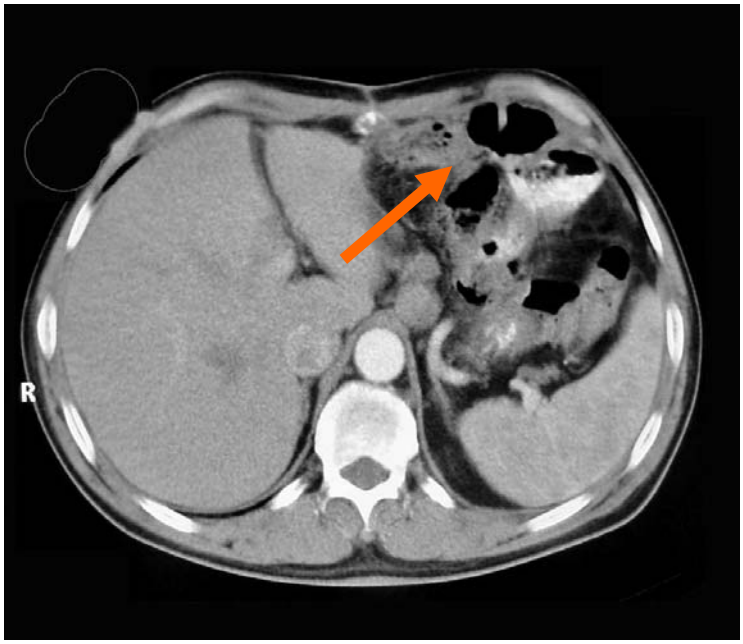


Figure 1 G.

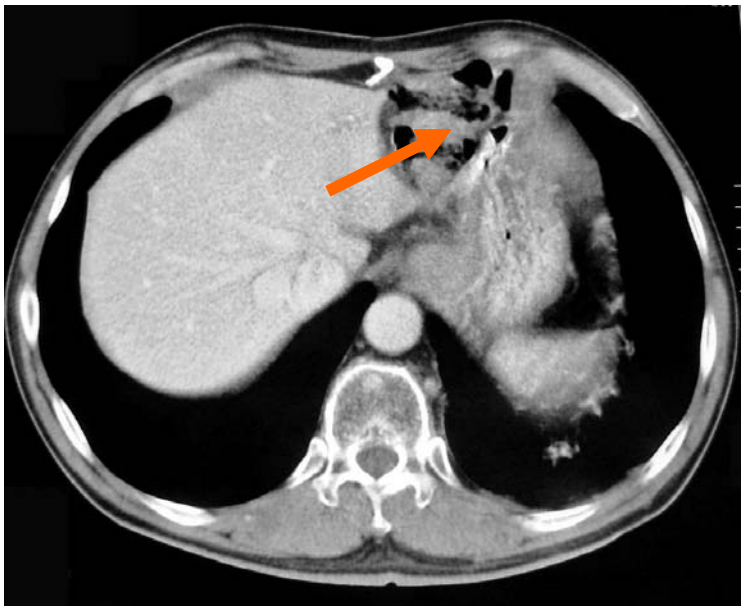


Figure 1 H.

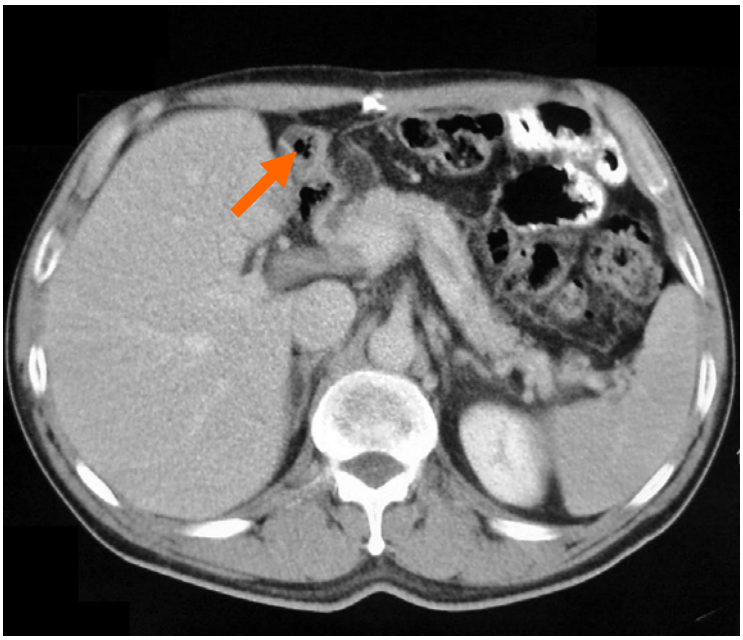
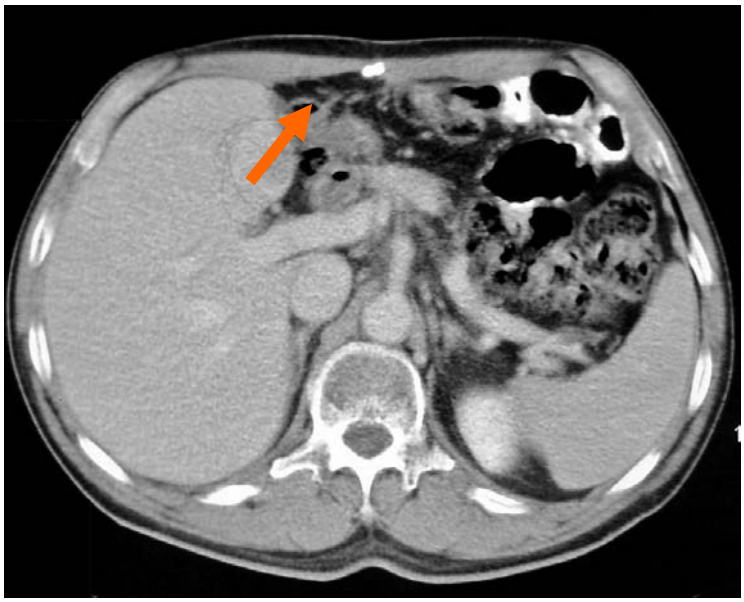


Figure 1 I.



2. Colorectal adenocarcinoma in male patients (Dukes B2 stage):

Plasma levels of F1+2 and D-dimer showed highest level at admission, with a significant decline post-treatment. About 50% of the patients (6 of 12) started with normal ranges of F1+2 and D-dimer, and the remainder showed 100% response post-treatment, with normalized levels of both markers by weeks 6–10, depending on the starting deficit (*Figure 2A, 2B*). In contrast, the natural anticoagulants demonstrated the reverse trend to that of F1+2 and D-dimer (*Figures 2C–2E*). About 50% of the patients showed significantly lower levels at admission, with 100% response to treatment and full normalization by week 6 (*Figures 2C–2E*).

Figure 2: Haemostatic activation markers at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks Colorectal Adenocarcinoma in male patients (Dukes B2 stage). *Figure 2A* shows F1+2

(nmol/L), and *Figure 2B* shows D-dimer (mg/L). Natural anticoagulant markers at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks are shown in figures 2C, 2D, and 2E. *Figure 2C*: PC activity (%), *Figure 2D*: PS activity (%), and *Figure 2E*: AT activity

Figure 2A.

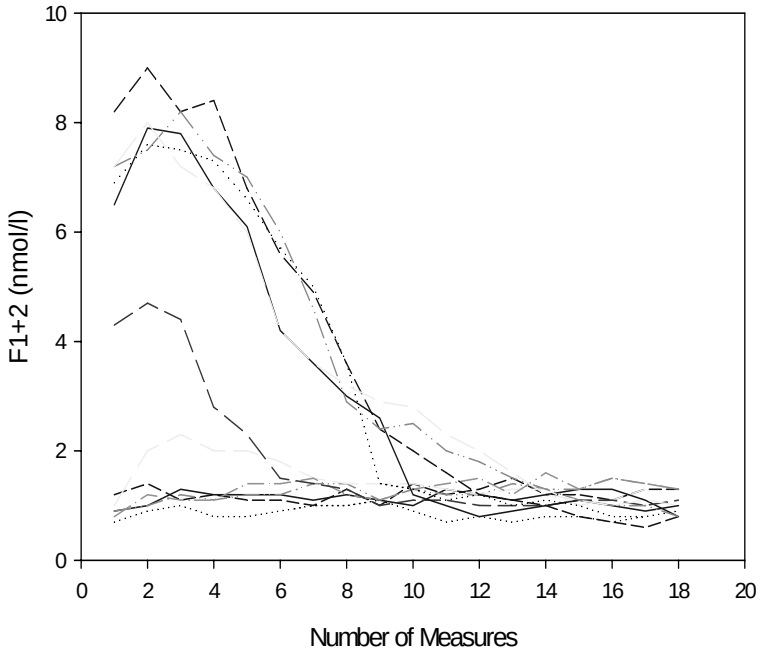


Figure 2B.

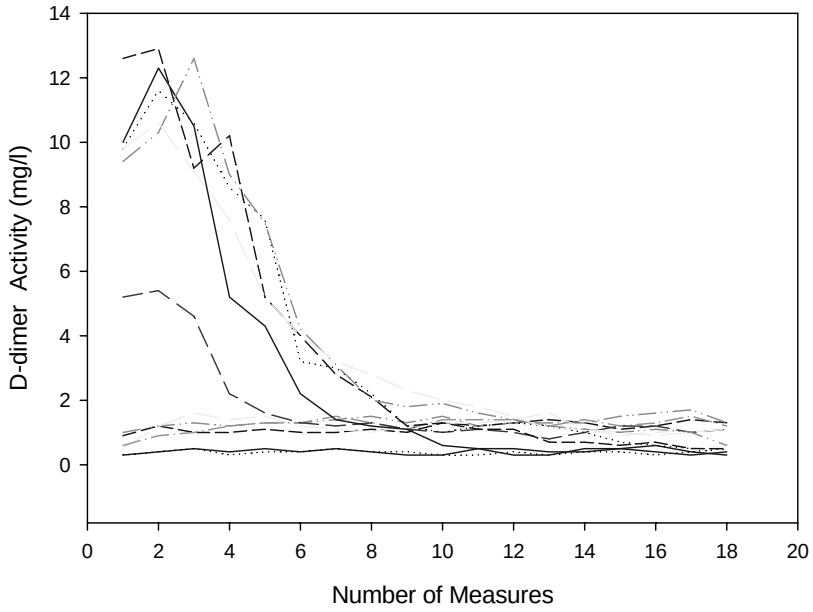


Figure 2C.

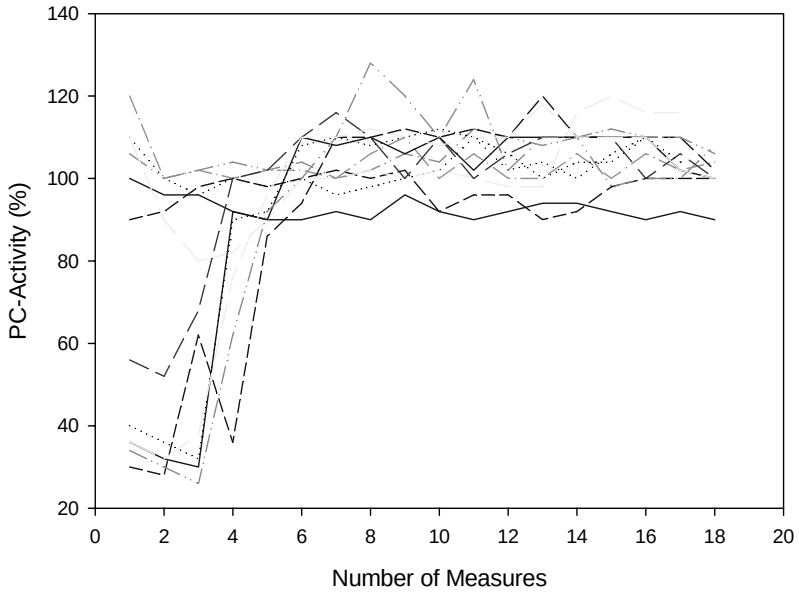


Figure 2D.

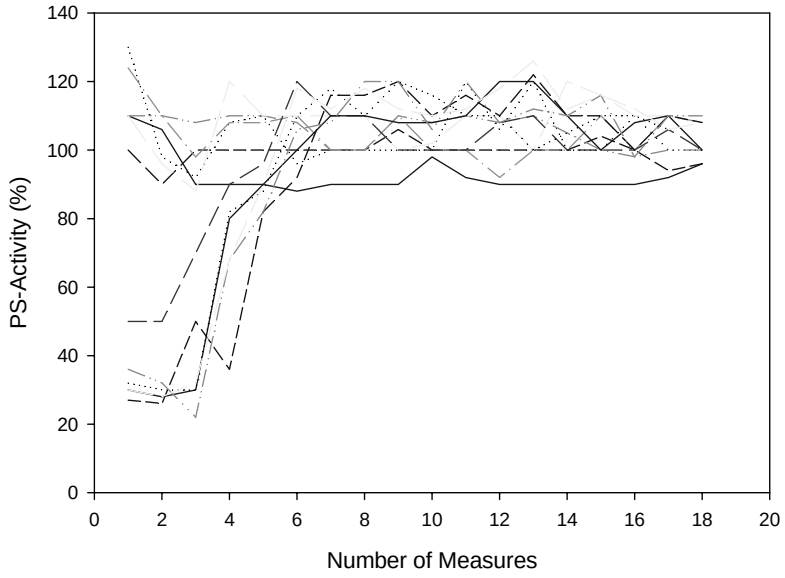
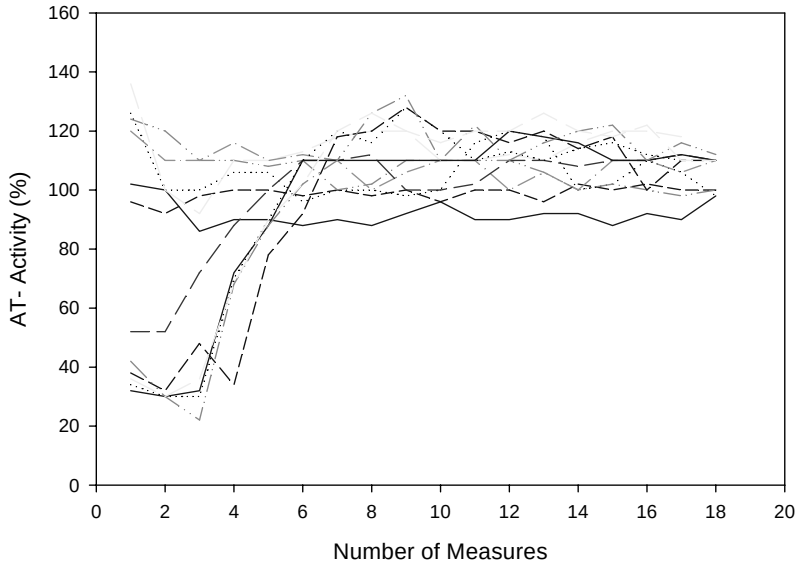


Figure 2E.

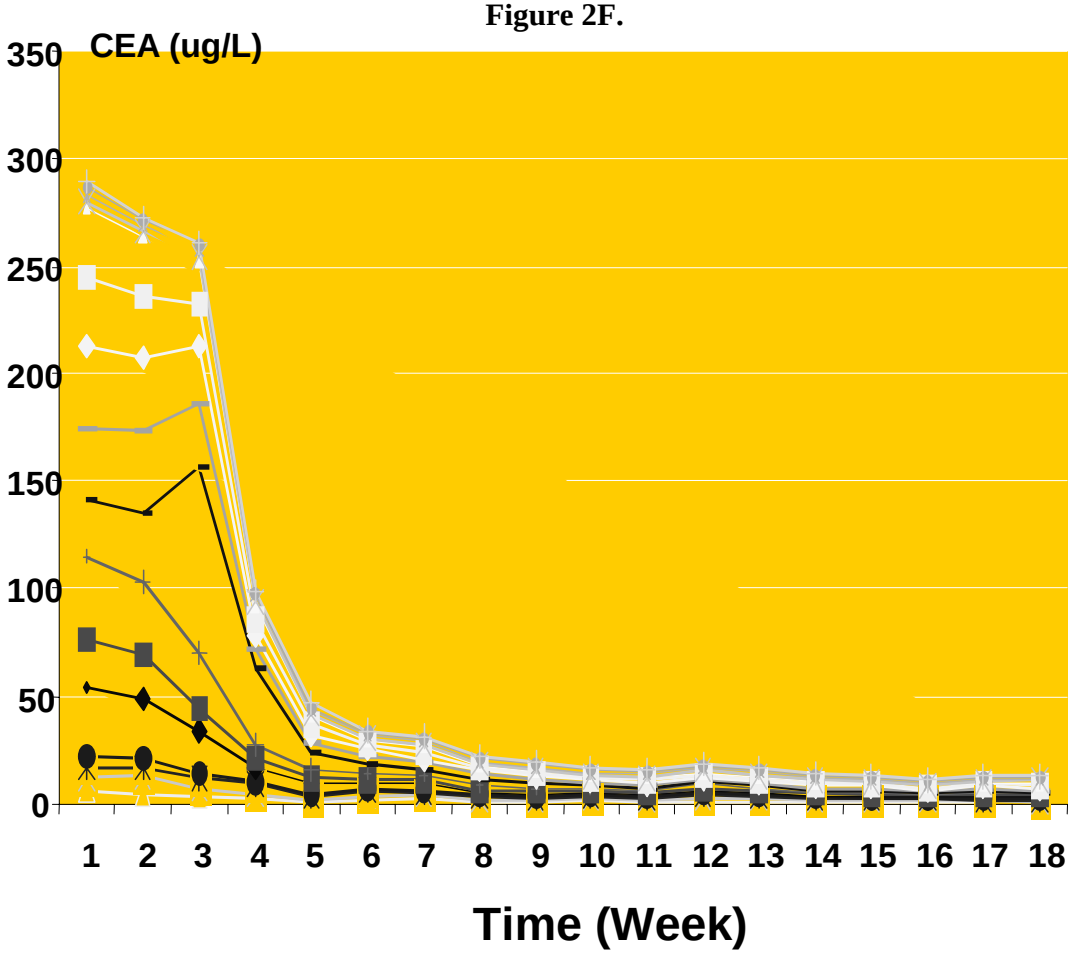


Tumour marker (CEA) in colorectal cancer patients:

Different levels of elevation (20–300 ug/L) above normal ranges are shown at admission. A 100% response to treatment in the reversal of elevated levels of CEA by weeks 5-10 depending on the initial levels are shown. Patients with highest levels of CEA at admission showed the highest F1+2/D-dimer and the largest deficit in natural anticoagulants.

Tumour marker (CEA, ug/L) at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks in colorectal carcinoma in female patients are shown in figure 2 F.

Figure 2F.



CT imaging in a female patient with Dukes Stage B2 colon adenocarcinoma:

This illustrates a large obstructive tumour mass (*Figures 2 G-H*) that was also seen with colonoscopy. At 4 weeks post-surgery followed by chemotherapy, no tumour was detected (*Figures 2 I-J*). Normalization of tumour marker and haemostasis marker levels was also demonstrated at 4–18 weeks.

Figures 2 G-H; show representative CT images in woman with Dukes stage B2 colon adenocarcinoma (tubular) preoperative, showing large obstructive tumour masses. Elevated levels of tumour markers and haemostasis activation markers were documented at admission. *Figures 2 I-J;* show representative CT images at 4 weeks post-surgery showing successful removal of tumour, which coincides with normalization of tumour markers and haemostasis markers.

Figure 2 G.

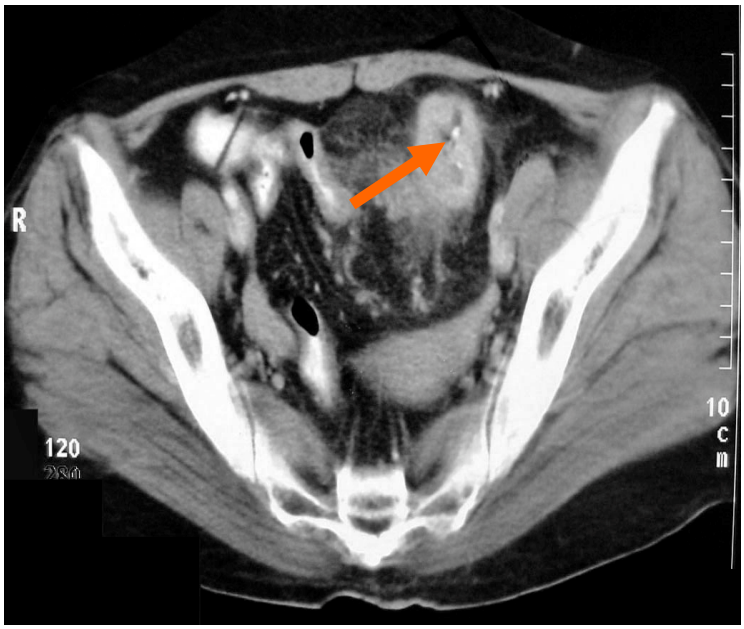


Figure 2 H.

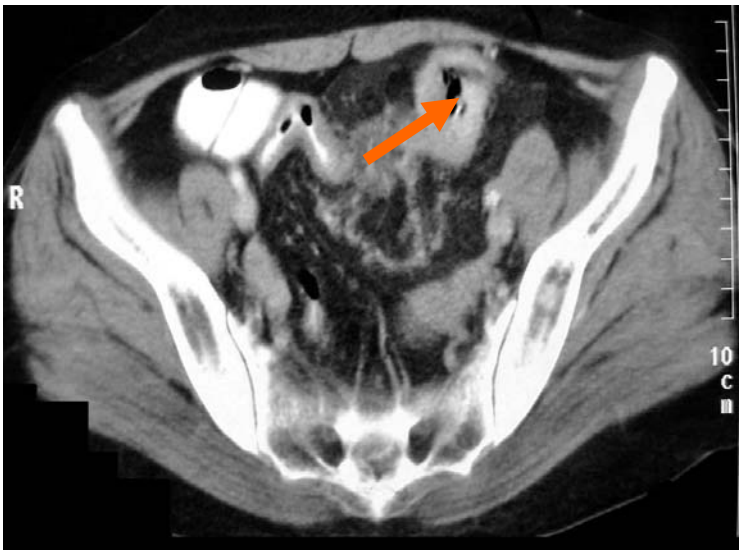


Figure 2 I.

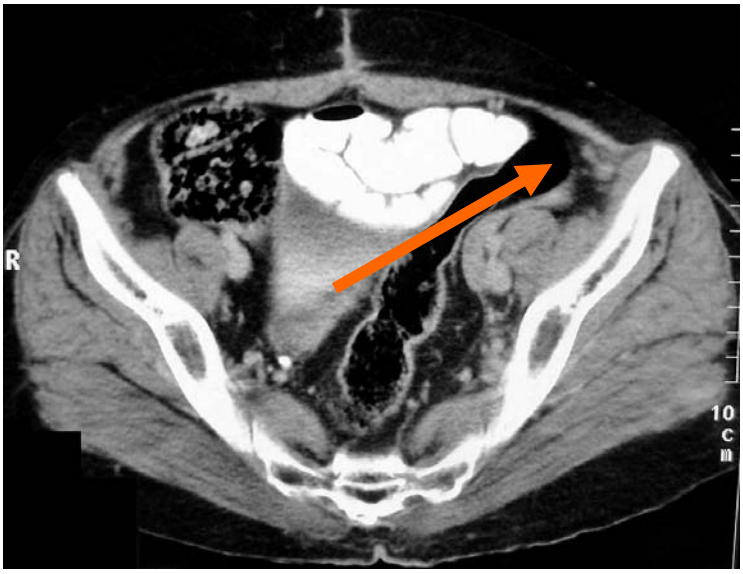
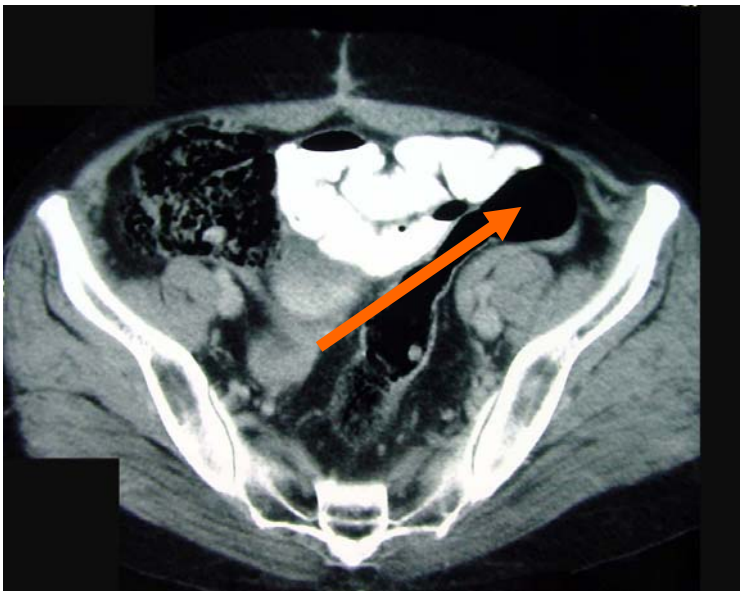


Figure 2 J.



3. Pancreatic adenocarcinoma (Stage III):

A progressive increase in F1+2 and D-dimer in 3 of 4 patients post-treatment was shown (*Figure 3A, 3B*). In one case, a decline in both F1+2 and D-dimer were noticed up to the 12th weeks post-treatment, followed by rapid increase that caught up with other cases. In contrast, the natural anticoagulants (PC, PS, and AT activities) demonstrated the reverse trend to that of F1+2 and D-dimer and these are seen in *Figures 3C–3E*. Most of these patients (3 out of 4) showed no response to treatment and full normalization in 1 of the 4 by week 7, but this was followed by a rapid decline at weeks 12–14 and these changes are nicely seen in *Figures 3C–3E*.

Figure 3A.

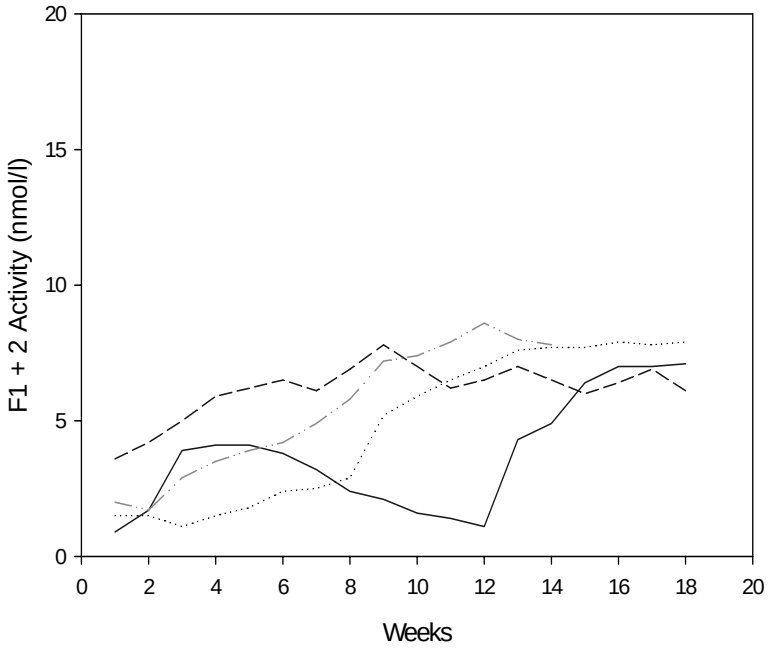


Figure 3 A-E; show haemostatic activation markers at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks in pancreatic adenocarcinoma patients (stage III). *Figure 3A*: F1+2 (nmol/L), and *Figure 3B*: D-dimer (mg/L). Natural anticoagulant markers at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks are shown in *Figure 3C*: PC activity (%), *Figure 3D*: PS activity (%), and *Figure 3E*: AT activity (%)

Figure 3B.

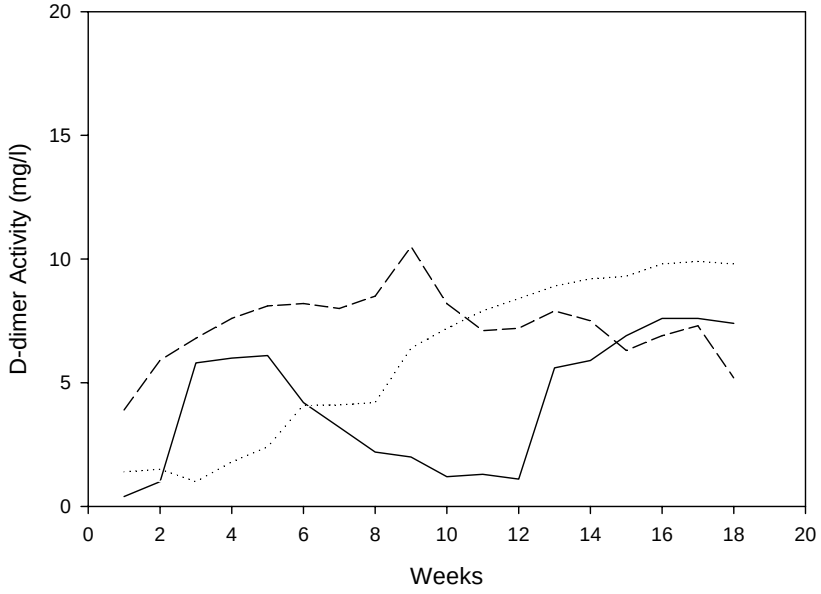


Figure 3C.

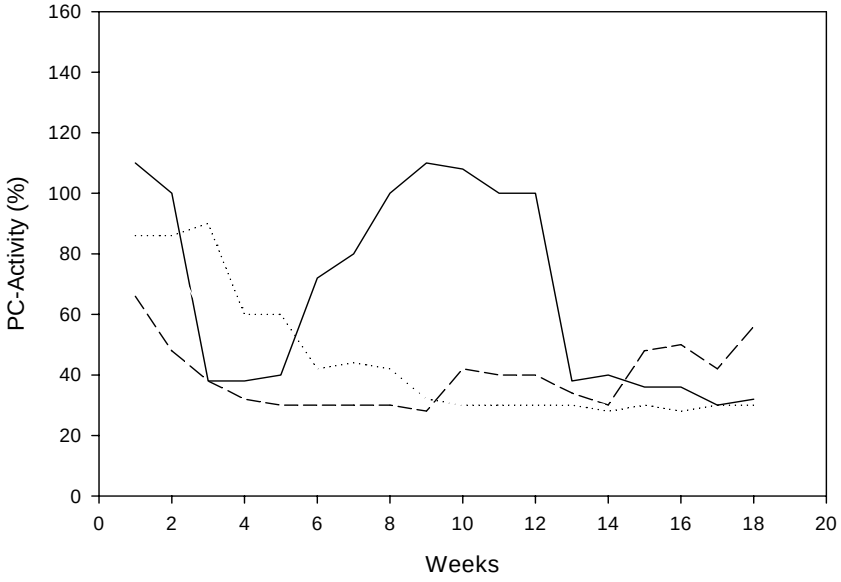


Figure 3D.

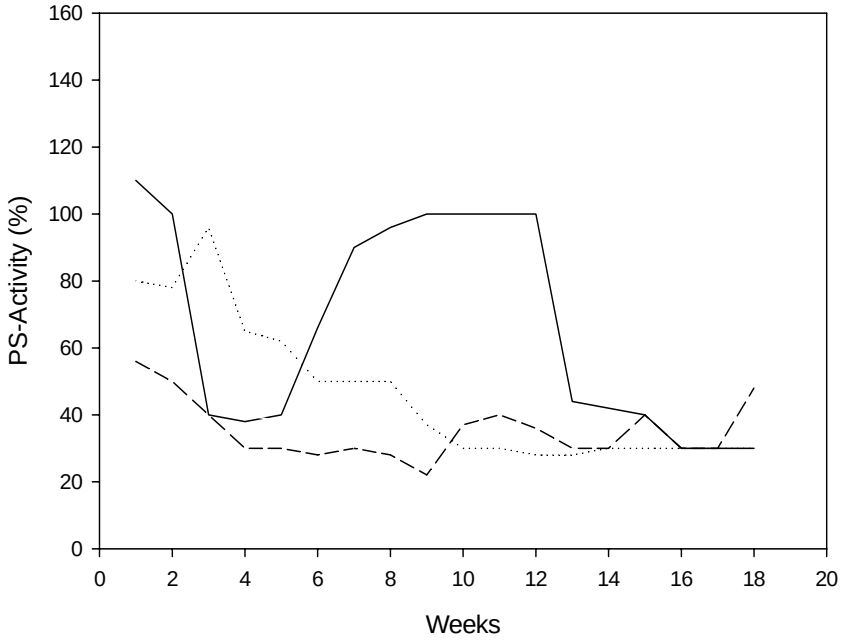
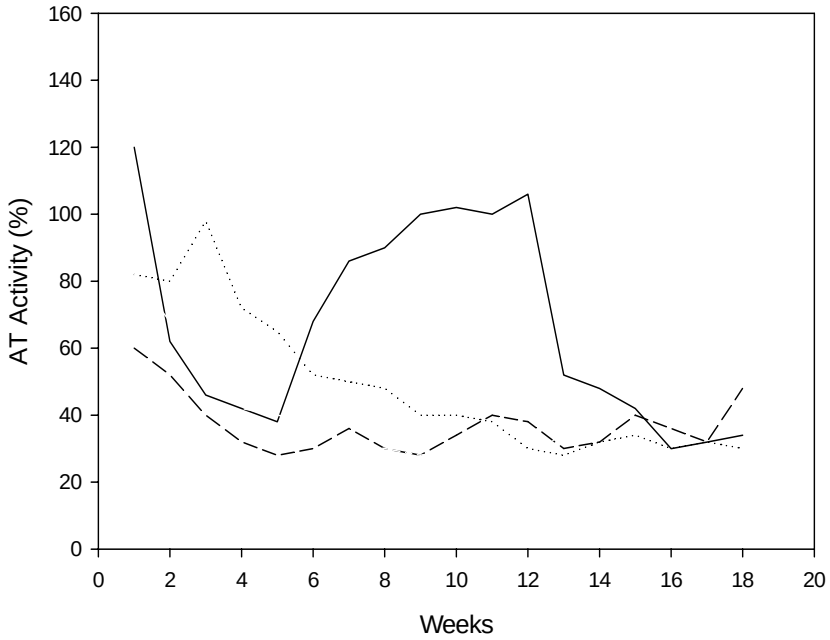


Figure 3E.

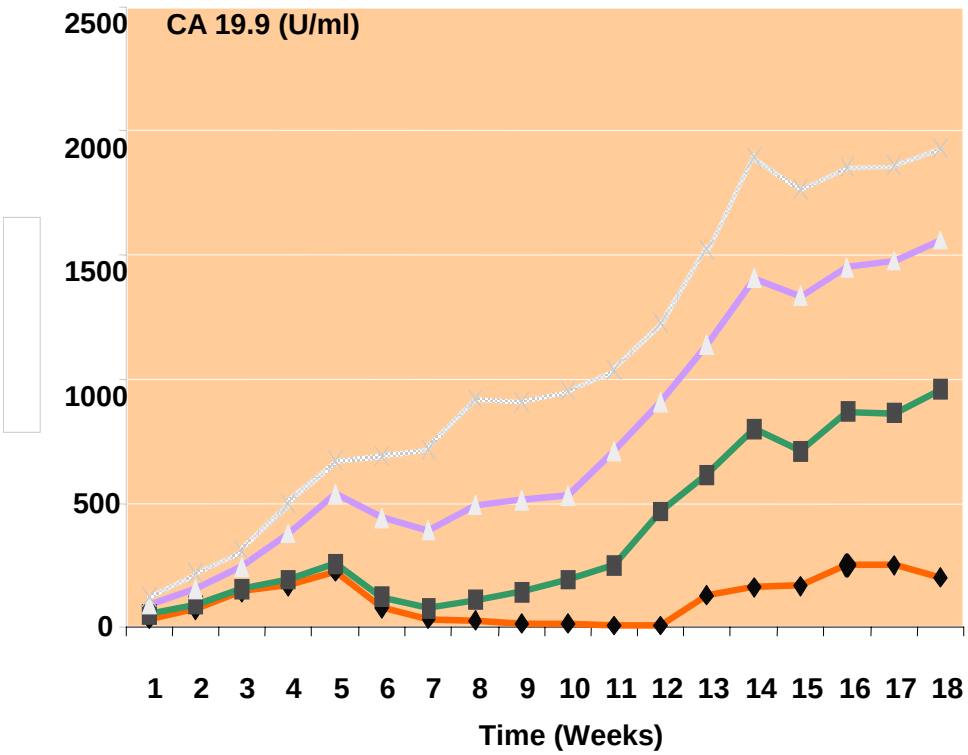


Tumour marker (CA 19.9) in pancreatic cancer patients:

Different levels of progressive elevation above normal ranges are shown post-treatment. An excellent response to treatment is shown in 1 of 4 patients. Patients with highest levels of CA 19.9 showed the highest F1+2/D-dimer and the largest deficit in natural anticoagulants.

The results of tumour marker (CA 19.9, U/ml) at admission or at initial presentation or at time of diagnosis (Week 1) and post-treatment on a weekly basis for up to 18 weeks in pancreatic cancer patients, are seen in figure 3 F.

Figure 3F.



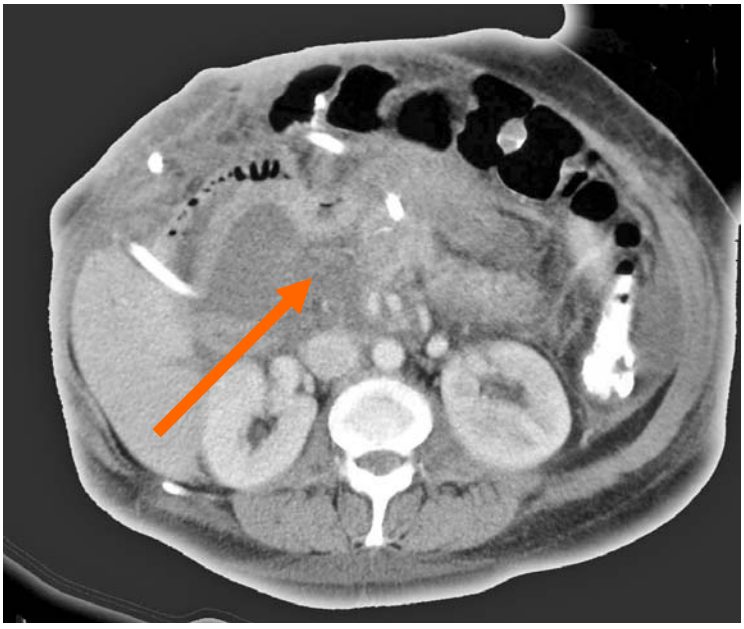
CT imaging of patient with (exocrine) pancreatic adenocarcinoma:

The imaging techniques used for diagnosis and staging were based on international recommendations for each disease category. These findings were reviewed by 2 different radiologists.

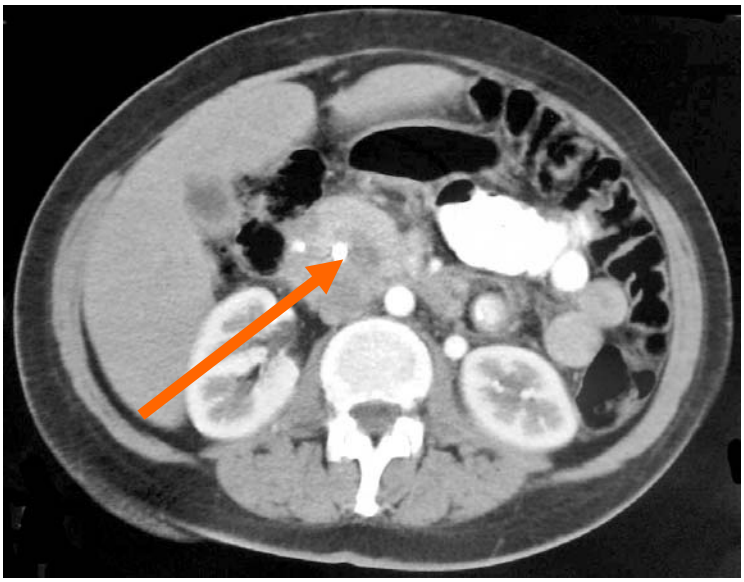
Pancreatic cancer patients with highest tumour marker (CA19.9), highest F1+2 or D-dimer, and lowest natural anticoagulants (PC, PS, AT activities) showed the largest tumour mass. A representative CT imaging of a patient with a relatively large tumour mass at admission is shown in *Figure* **3 G.** This patient showed a good response to treatment (week 8), as shown in *Figure* **3 H**. This patient showed the lowest CA19.9 levels, as well as a normalized level of F1+2/D-dimer and PC/PS/AT activities at week 8.

***Figure* 3 G**: At diagnosis—CT imaging in female patient with pancreatic adenocarcinoma, large tumour mass, with highly elevated tumour marker CA19.9 and haemostatic markers. Figure 3 H, shows the post-treatment CT imaging in a female patient with pancreatic adenocarcinoma who showed reduction in tumour mass and the tumour marker CA 19.9. Improvements in haemostatic markers are shown as well.

Figure 3 G.



Ffigure 3 H.



4. Lung carcinoma:

Patients diagnosed as having small and non-small cell lung cancer were also enrolled in the study. Detailed monitoring upon admission and prior to any cancer-related intervention and on a weekly basis post-intervention (chemotherapy, radiation or surgery) for up 18 weeks were carried out as mentioned earlier. The laboratory investigation including haemostatic parameters and tumour markers were repeatedly measured with each treatment course and their results were correlated with other marker of tumour prognosis in a manner similar to the previously mentioned gastrointestinal tract tumours. Ten patients were enrolled; 6 of them had adenocarcinomatous type of non-small cell lung cancer and 4 had small cell lung cancer. Two patients were excluded from the final evaluation of study because of the development of thromboembolic events during the period of study. In all remaining cases, the levels of hypercoagulable markers were elevated directly correlated with tumour stage and radiological findings of cancer state. Additionally, the level of naturally occurring coagulation inhibitors were all decreased with the progression of tumour. As mentioned

earlier, determination of D-dimer, F1 + 2, protein C, protein S, and antithrombin levels can be used as markers to assess the tumour prognosis. This also suggests that early anticoagulation prophylaxis might prevent VTE and impairs tumour progression.
The base-line characteristics of the types of cancers are shown in *Table 1*. And, the chemotherapeutic regimens are shown in *Table 2* .
Lung cancer is the leading cause of cancer death among men and women, AND approximately 60%-65% of lung cancer patients present with advanced disease in which the majority of newly diagnosed lung cancers are non-operable at time of diagnosis.
The markers of haemostatic system activation which included prothrombin F1 + 2 and D-dimer were elevated in all except one patient (the patient had non-small cell lung cancer=NSCLC) and this had a progressive course (the rise was by factor of 3–4 folds) at 6 to 18 weeks post-treatment. In contrast, the natural anticoagulants activity of PC-activity, PS-activity, and AT-activity levels showed a reverse profile in these 7 of the 8 patients. In 5 patients with NSCLC, the levels of those natural anticoagulants were progressively reduced by weeks 6–18 in parallel with disease progression.

The average values of haemostatic parameters in 7 patients with lung cancer

Time in weeks	AT	PS-Ag	PS-Act	PC-Act	D-dimer	F1+2
Baseline	39	37	35	38	9.2	7.1
4th week	69	75	73	78	7.2	6.3
8th week	118	117	114	113	2.1	3.2
12th week	114	113	110	106	1.4	1.96
18th week	110	107	105	102	1.1	0.9

CT imaging: The non-small cell lung cancer patient with the highest F1+2 or D-dimer and lowest natural anticoagulants (PC, PS, AT activity) showed the largest tumour mass. A representative CT imaging of a patient with a relatively large tumour mass at admission is shown in Figure 4 A. Unfortunately, no CT imaging was done at follow up, but this patient showed a sustained high level of F1+2/D-dimer and low levels of PC, PS, and AT activities at weeks 4–18.

Initial CT imaging at admission in a small cell lung cancer patient demonstrated a large tumour mass (Figure 4 B.). In contrast, CT images post-surgical procedure and treatment showed successful removal and remission (Figure 4C -1, 4C -2)

Figure 4 A.

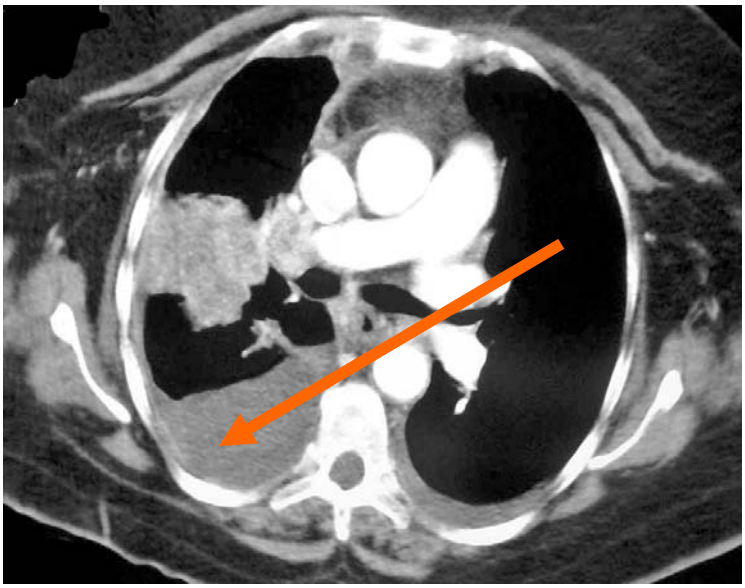


Figure 4 B.

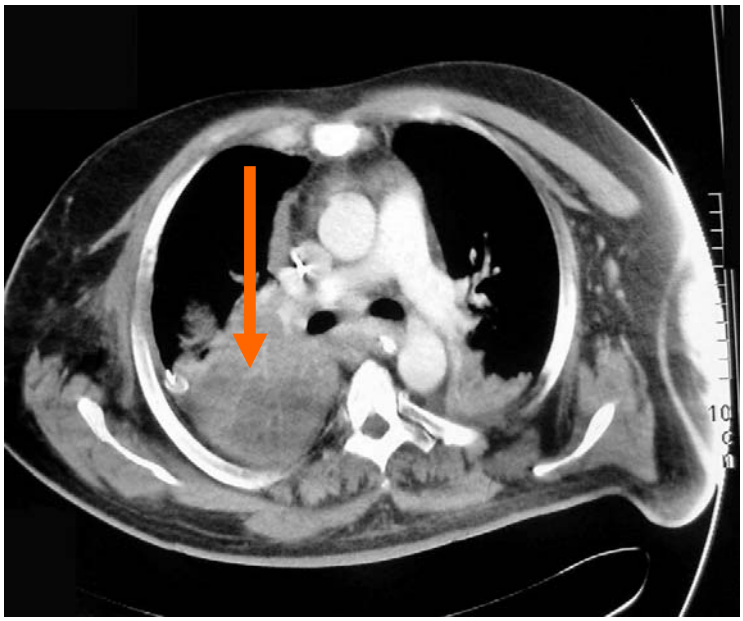
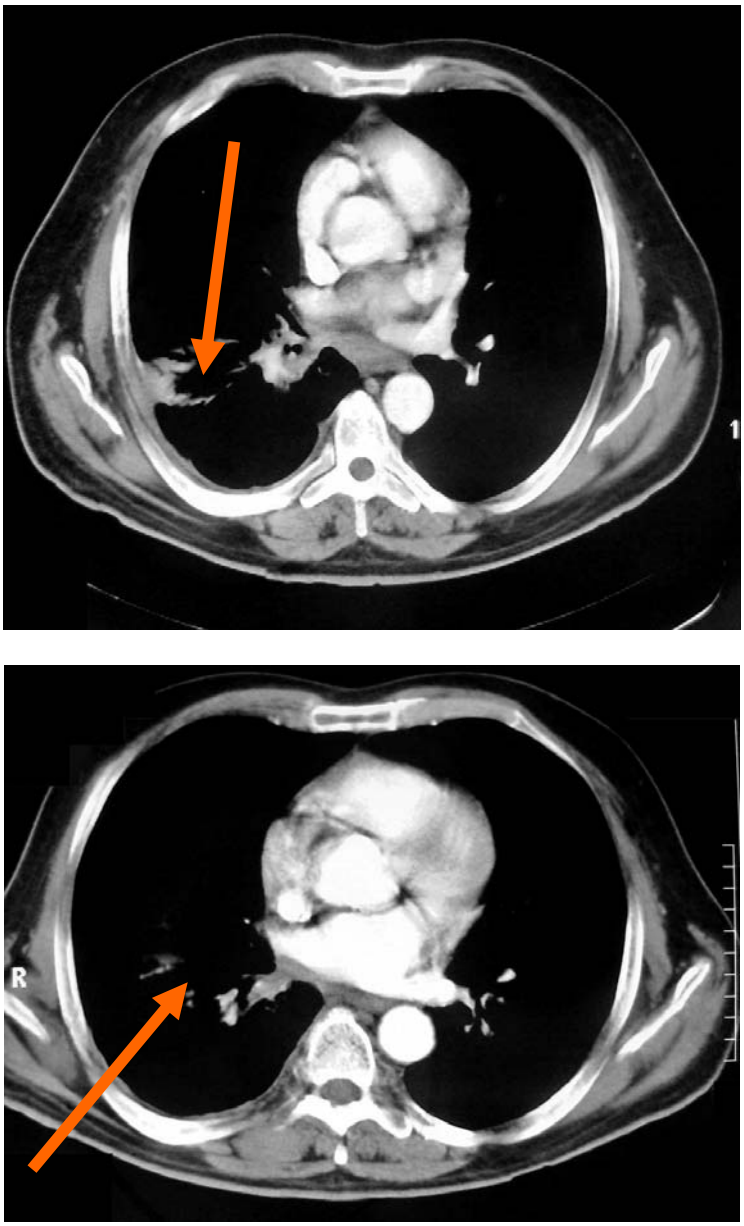


Figure 4 C-1, 4C-2



5. Breast and ovarian cancers:

Fourteen patients were enrolled in this study, 12 with infiltrating breast carcinoma and 2 with ovarian cancer. The laboratory work-up and follow-up were similar to other cases of previously mentioned non-haematological malignancies. In all studies cases, the levels of hypercoagulable markers were elevated in a direct correlation with tumour stage, tumour markers, and radiological findings of cancer state. Additionally, the level of naturally occurring coagulation inhibitors were all decreased with progression of tumour. These hypercoagulable parameters were also raised after surgical resection of these tumours.
Breast cancer is the second-leading cause of cancer-related deaths in women in the United States. About 20%–25% of patients with breast cancer have HER2/*neu* over-expressing disease, which not only increases the risk of disease recurrence, metastasis, and death, but also might lead to a poor response to conventional therapy with tamoxifen and alkylating agents. Similarly, a significant percentage over-express epidermal growth factor receptor (EGFR) also associated with poor prognostic features.
In case of beast cancer, the haemostatic parameters, the tumour marker, and the imaging techniques were repeated after the completion of 2 full chemotherapeutic regimens. In those cases in which surgical intervention was indicated prior to the start of chemotherapy, the coagulation parameters and tumour markers were measured prior to the surgery and the haemostatic values were repeated after the end of surgery. None of these patients were on any kind of medications that would interfere with the results of coagulation studies. During each visit physical examination, vital signs and medication history were taken, and any changes in these findings were registered. If the patient developed febrile neutropaenia as a complication of chemotherapeutic agent, the haemostatic parameters were measured at the onset of the diagnosis of febrile neutropaenia and thereafter. At the end of study, 2 patients were excluded because they developed thromboembolic complications during the study period; they were started on antithrombotic therapy. And, after a follow up of 18-20 months, 12 patients were eligible for final evaluation.
The haemostatic parameters were measured at the onset of diagnosis, pre- and post surgery, and after the completion of each chemotherapy course. Their levels were correlated with the levels of tumour markers as in other cases.

CT imaging: With regard to the patients with ovarian cancer (stage IIA, well-differentiated ovarian adenocarcinoma), no preoperative images were available (patients were referred to us after surgical intervention). The postoperative CT scans that were done as follow up after chemotherapy are normal, except for minor free fluid in the lesser pelvis. The tumour marker CA 125 decreased significantly after surgery and remained low thereafter; more significantly, the haemostatic parameters (mainly AT, PS, and PC) normalized after surgery and with chemotherapy.

Patients with breast cancer always come to the care of the medical oncologist after surgery and neo-adjuvant (before any surgery) or adjuvant chemotherapy; plain x-ray imaging and mammography are performed at our institutions, but because CT scans are only performed in cases in which there are metastases to lung, bone, or liver, there are no CT scans with which to compare these. There was a clear correlation between tumour marker CEA and CA15.3, and haemostatic parameter.

Fourteen patients were enrolled in the study over a period of 18-20 months. Two patients were excluded from the final evaluation of study because of the development of thromboembolic events during the period of study. *Table 1* summarizes the base-line characteristics of the types of cancers, whereas *Table 2* summarizes the baseline characteristics of the 12 eligible patients.

The most frequently used tumour markers were as follow: HER-2/*neu* expression, ER/PgR status and CA 15-3 (mainly in breast cancer) and CA 125 (in ovarian cancer). These markers were measured at the initial diagnosis and following each chemotherapy cycle in manner mentioned earlier.

The chemotherapeutic regimens (either neo-adjuvant prior to surgery or adjuvant after surgery or palliative after initial treatment failure) that were used included standard internationally recommended regimens and they are mentioned in table 2.

At the time of study, Taxanes and targeted (Monoclonal antibody) therapy were not frequently used and therefore regimens containing these new agents are not mentioned.

Haemostasis markers in breast cancer patients:

Prothrombin F1 + 2 and D-dimer levels showed 6–10-fold elevation at diagnosis in 5 of the 12 patients and normal levels in 7 of the 12 patients at admission *(Figure 5a, 5b)*. In the elevated coagulation markers in 5 of the 12 patients, the levels of F1 + 2 and D-dimer were normalized by 8–10 weeks post-treatment *(Figure 5a, 5b)*. In contrast, the natural anticoagulants activity of PC-activity, PS-activity, and AT-activity levels showed a reverse profile in these 5 of the 12 patients *(Figures 5c–5e)*. In those 5 of the 12 patients, the levels of those natural anticoagulants were normalized by weeks 6–8 *(Figure 5c–5e)*. Breast cancer is the second-leading cause of cancer-related deaths in women in the United States. About 20%–25% of patients with breast cancer have HER2/*neu* over-expressing disease, which not only increases the risk of disease recurrence, metastasis, and death, but also might lead to a poor response to conventional therapy with tamoxifen and alkylating agents. Similarly, a significant percentage over-express epidermal growth factor receptor (EGFR) also associated with poor prognostic features.

Figure 5a.

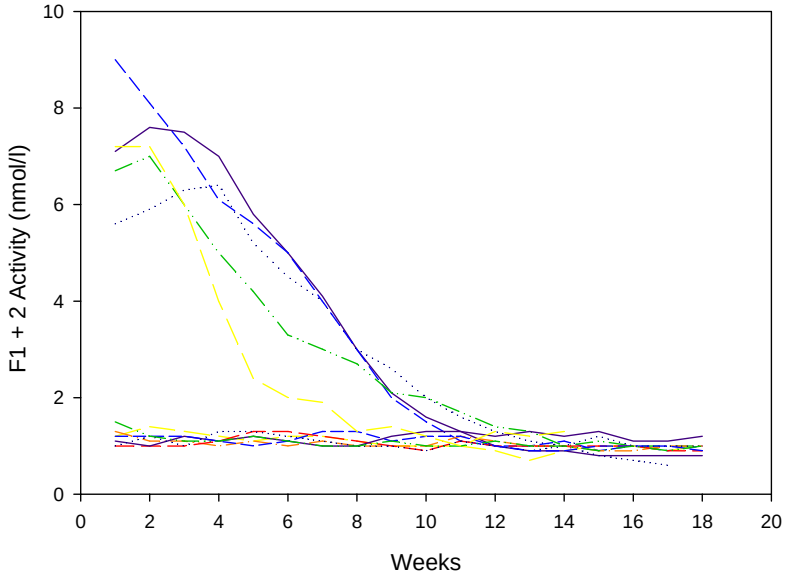


Figure 5b.

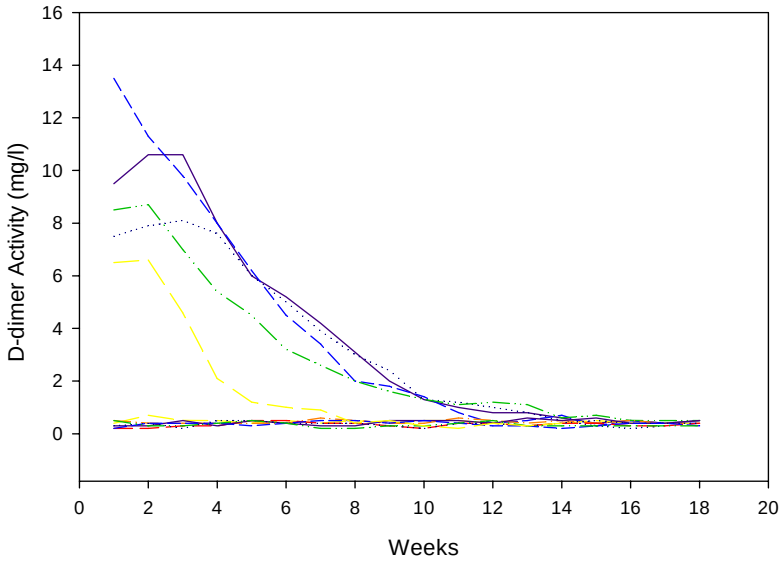


Figure 5c.

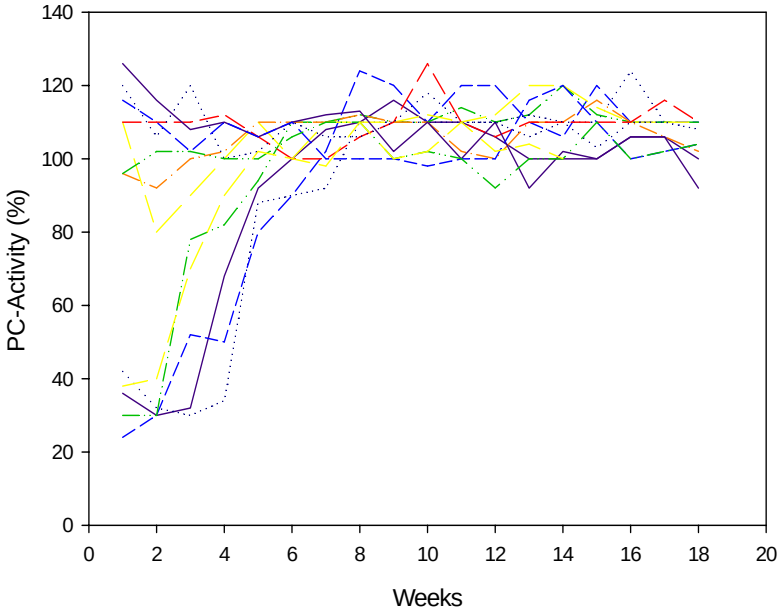


Figure 5d.

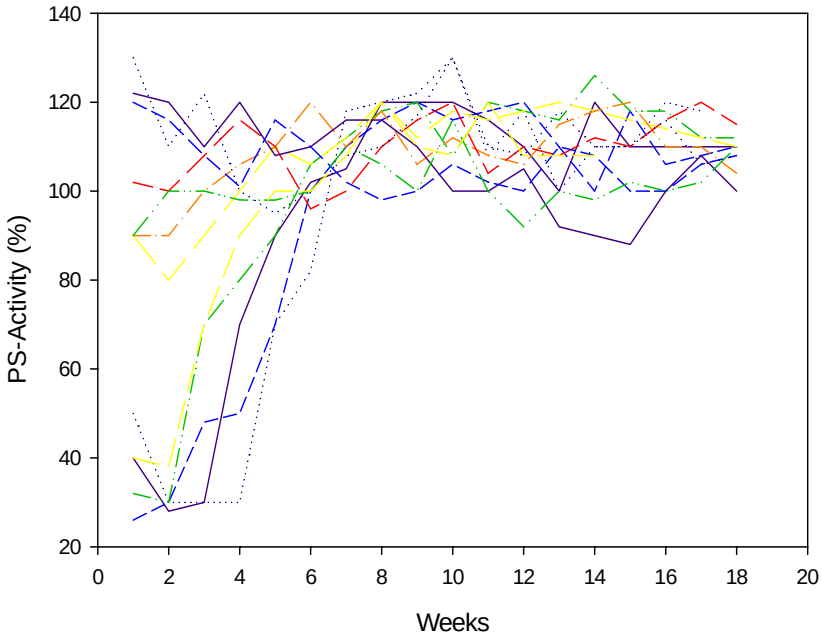
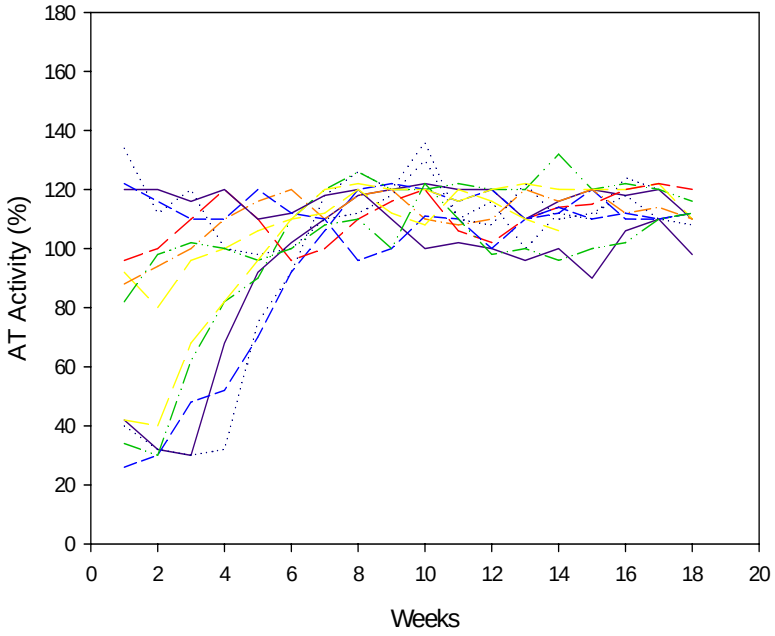


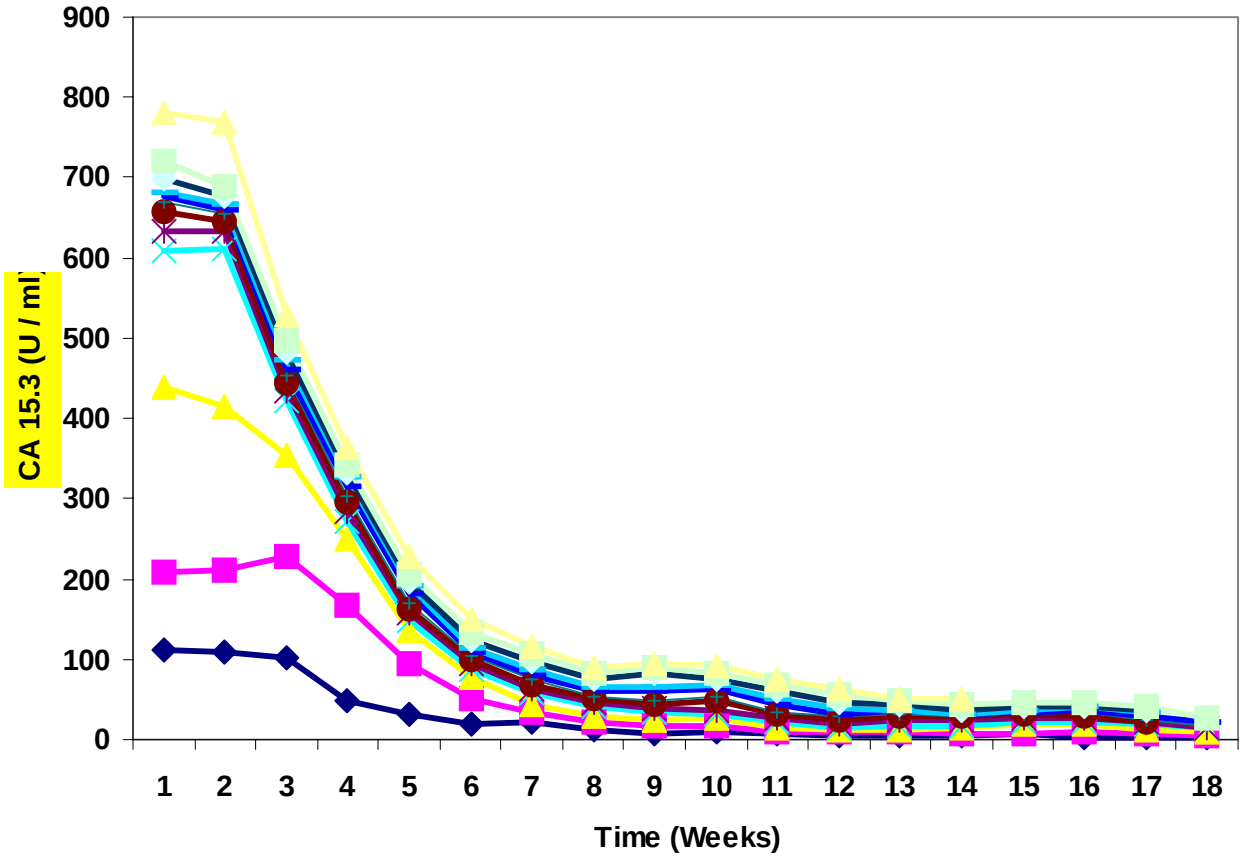
Figure 5e.



Tumour marker (CA 15.3) in breast cancer patients

Different levels of progressive elevation above normal ranges were shown at the initial diagnosis (*Figure 6*). An excellent response to treatment was shown in all patients by weeks 6–8 (*Figure 6*). Patients with highest levels of CA 15.3 showed the highest F1+2/D-dimer and the highest deficit in natural anticoagulants.

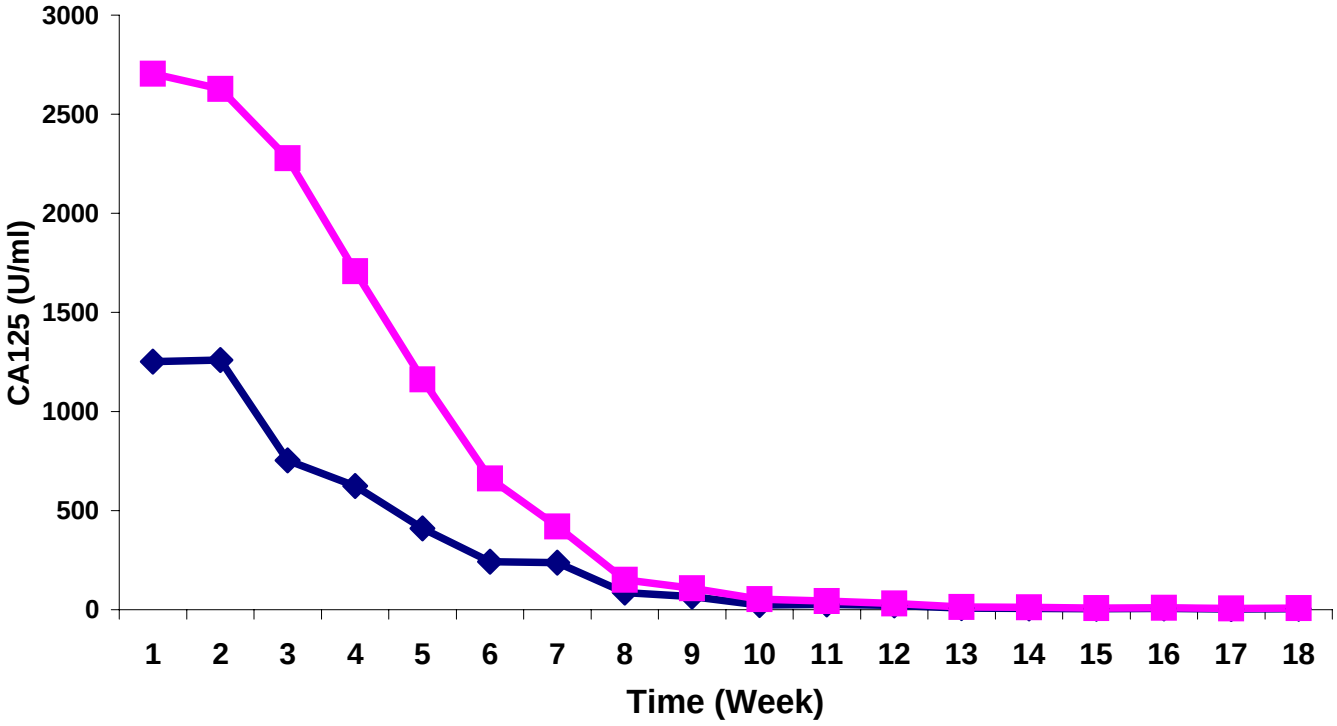
Figure 6. Tumor Marker (CA15.3) in Breast Cancer Patients at Admission and Post-treatment (Reference Range: 0-28 U/ml)



Tumour marker (CA 125) in ovarian cancer patients

A variable level of CA125 elevation was shown at diagnosis, but a 100% response rate was achieved by week 8 (*Figure 7).*

Figure 7. Tumor Marker (CA125) in ovarian cancer patients with well-differentiated Ovarian adenocarcinoma (stage IIA) at admission and Post-treatment - (Reference Range: 0 - 37 U/ml)



IV. Discussion:

In the nearly more than one century since Trousseau first described migratory thrombophlebitis-thrombosis in patients with cancer, thromboembolism has become a well-recognized initial presenting sign and complication of cancer. The clotting system is activated in cancer patients and this is further amplified by different therapeutic interventions, e.g.: surgery, systemic chemotherapy, hormonal treatment or radiation therapy. Hypercoagulation is a well-documented in virtually all cancer types, albeit at different ranges and severity, and is considered the second leading cause of mortality in cancer patients. The relationship between the activation of clotting system and carcinogenesis supports the idea of cancer as a hypercoagulable state and holds great implications for the development of thrombosis, enhancement of tumour growth, development of tumour progression-metastasis and risk of poor clinical outcomes and response to treatment. Although it is well documented that cancer status can activate the clotting cascade (the ability of tumour cells to produce and secrete procoagulant/fibrinolytic substances and inflammatory cytokines), it is less known that activation of the coagulation system may also support tumour progression and metastasis. In addition, the physical interaction between tumour cells and blood elements (neutrophils, monocytes, platelets) and/or vascular beds may play additional role in the development and progression of tumour. The platelet activation in cancer patients and its impact on tumour progression and metastasis further expand the role of the coagulation system in malignancy. Other possible mechanism of thrombosis initiation and promotion in malignancy include non-specific factors such as; abnormal protein metabolism (e.g.: paraproteinaemia), generation of acute phase reactants, and haemodynamic compromise (e.g.: stasis), and production and secretion of different substances which promote generation of new and abnormal vasculature (neo-angiogenesis).

Different types of anti-tumour treatments may significantly increase the risk of thromboembolic events by different mechanisms, e.g.: endothelial damage, release of procoagulants, or stimulation of tissue factor production by host cells. Thrombo-embolic complication in patients with cancer and in those with metastatic diseases is considered a major problem for oncologists. A hypercoagulable or prothrombotic state of malignancy

occurs due to the ability of cancer cells to activate the clotting system and therefore the hypercoagulation or the thromboembolism accounts for one of the most common complications in cancer patients and it has serious consequences. The exact molecular basis for cancer associated thromboembolic events remains unknown (although considerable advances have been achieved in our understanding of pathomechanism-pathobiology of thrombogenesis in cancers, particularly in the field of interaction between the clotting cascade activation and the process of tumour growth and metastasis), and the relative contribution of chemotherapeutic-cytostatic agents, tumour cells, endothelial cells, and circulating procoagulants in promoting thrombus formation, their attribution to tumour progression and dissemination continues to be investigated and studied. Additionally, the thromboembolic events contribute for a significant percentage of mortality and morbidity in cancer patients. Laboratory markers of coagulation activation such as prothrombin fragments 1+2 or thrombin-antithrombin complex or D-dimer support the premise that cancer is a hypercoagulable state and different coagulation proteins, procoagulant microparticles and inflammatory cytokines may be raised in patients with cancer. In parallel, the naturally occurring anti-thrombotic (anticoagulants) proteins are decreased in malignancy. Despite the common occurrence of coagulation abnormalities in patients with cancer, the utility of coagulation markers in determining either the extent of malignant disease or their value in predicting those who will develop thrombosis during therapy remains questionable. At present, it is difficult to identify the relative impact of these interactions on the basis of the well-recognized clinical evidence of hypercoagulable status in tumour patients. In term of disease progression, only fibrinopeptide A levels have been shown to correlate with advancing disease and increasing tumour burden. Although the relationship between malignant diseases and venous thromboembolism has been convincingly demonstrated, the clinical implications of this association still have to be thoroughly elucidated.

Based on these well-established data, we planned a study in which patients with different types of solid tumours were enrolled and they were closely followed up from the time of initial diagnosis till either the completion of planned treatment or until disease progression or dissemination. Patients above 16 years of age, a life expectancy of more than 3 months, and histopathologically proved tumour were enrolled in the study. The

study was approved by the regional Ethic Committee, and informed written consent was obtained from patients. The changes in clotting parameters in patients with different types of solid tumours were evaluated, and the correlation between these parameters and tumour stage, changes in known characteristic tumour markers, imaging findings, and the changes in haemostatic parameters which occur with different types of therapeutic intervention were assessed. The markers of hypercoagulability were monitored from the time of initial presentation and during each planned visit when the patients received the due cycle of treatment. Our main focus was on specific abnormalities or changes of the haemostatic parameters in this group of patients with different types of solid malignancies. And, because it is not possible to accurately predict those with cancer who will develop thrombosis, the results of this prospective study can be used as additional clinical evidence to recommend routine thrombo-prophylaxis is cancer patients. It is very important to mention that the magnitude of risk for development of venous thromboembolic complication for a given anti-tumour therapy is sufficiently great, and the thromboprophylaxis method is safe and effective. Patients were stratified according to ECOG (Zubrod) performance status score, and those with ECOG score of 0-1 were enrolled in the study. Baseline AT, PS, PC, D-dimer and prothrombin activation peptide F1+2 as haemostatic parameters and characteristic tumour markers according to the type of tumour were measured. Staging of cancer at various anatomic sites was done as developed by the American Joint Committee on Cancer (AJCC) in cooperation with the TNM Committee of the International Union Against Cancer (UICC). The International Histological Classification or Tumours provided by the World Health Organization (WHO) was used for pathologic classification and definition or tumour types. Physical examination, imaging, endoscopy, bronchoscopy, biopsy, and surgical exploration were used for clinical classification and staging. Histological grading was also used for qualitative assessment of the differentiation of the tumours. All the patients were treated according to the internationally recommended therapeutic regimens (the regimens were those which were recommended during the period of study), and they were closely followed up to evaluate the state of their malignant disease. The haemostatic parameters, the tumour markers, and the imaging studies were repeated after the completion of each full cycle of chemotherapeutic regimen. In those cases where surgical intervention was

indicated prior to the start of chemotherapy, the coagulation parameters and tumour markers were measured prior to the surgery and the haemostatic values were repeated after the end of surgery. None of these patients eligible for final analysis were on any kind of medication which could interfere with the results of coagulation studies. During each visit, physical examination, vital signs and medication history were taken and any changes in these findings were registered. If the patient developed febrile neutropaenia as a complication of a chemotherapeutic agent, the haemostatic parameters were measured at the onset of the diagnosis of febrile neutropaenia and thereafter.

The statistical analysis was performed by two-way analysis of variance (ANOVA) comparing the markers of haemostasis activation at admission to post-admission for each subject and with respect to average control values; differences were considered significant at $\boldsymbol{p}$ value of 0.05 or less.

Except for a slight difference in the gender ratio (in gynaecological cancer group), the analysis of patients who met the pre-specified criteria for evaluation showed that the hypercoagulable parameters were directly correlated with tumour progression, rise in characteristic tumour markers and with progression of tumour on imaging investigations. We studied the prognostic values of F1+2, D-dimer, and natural inhibitors of abnormal coagulation in this group of patients with solid tumours, however, no convincing data have thus far identified one of these hypercoagulability markers as a reliable prognostic marker [144], except F1+2 which has been shown to correlate with advancing disease and tumour burden. Parallel with the haemostatic parameters, we also measured the characteristic tumour markers; in all cases, there was a direct correlation between changes in the haemostatic parameters, radiological imaging findings, and tumour markers (e.g., rise in D-dimer or F1+2 was associated with a drop in AT, PC, and PS, which was directly correlated with a rise in characteristic tumour markers and a progression of the malignant diseases in radiological imaging findings). In cases of breast and ovarian cancers, data on the plasma levels of D-dimer illustrated excellent correlation with the tumour markers CA15.3, CA125, respectively, and tumour volume. The correlation between haemostatic parameters, radiological findings, tumour stage, and the characteristic tumour markers were analyzed. The tumour markers used in the studies cases were those which are recommended by different oncology societies. These tumour

markers are not tumour-specific, but they might be used as a useful tool for both diagnosis and follow up of these patients and also to assess the efficacy of the treatment. Additionally, the hypercoagulability parameters which were used in the study reflect a broad spectrum combination of known markers of coagulation cascade activation and each of these markers was used in previous studies separately. The following parameters were measured in all enrolled patients; plasma levels of D-dimer and F1+2, activity of Antithrombin (previously called ATIII), Protein C and protein S in addition to the measurement of Free PS antigen level. All these parameters were determined and measured at the; onset of diagnosis (at the time of enrolment into study), pre- and post-surgical intervention, after the completion of each cycle of chemotherapy, and pre- and pos-radiation therapy (depending on the type of therapeutic intervention which was planned for each specific case). The levels of aforementioned haemostatic parameters were correlated with the levels of characteristic tumour markers. Each blood sample taken from the enrolled patients was tested for AT activity using thrombin (AT-IIa) as the substrate; PC activity, using both clotting (PC-CI) and chromogenic (PC-Chr) methods; PS activity; D-dimer; and F1+2 using immunoassays, ELISA, clotting, and chromogenic methods [137-142]. The reagents and kits used for these different assays were obtained from Dade Behring, Inc. (Deerfield, IL), Stago International (Parsippany, NJ), and R&D Systems, Inc. (Minneapolis, MN).

We documented a direct correlation between the markers of hypercoagulable status, tumour stage, changes in tumour markers, and the degree of radiological response in all examined cases. This correlation was most obvious in all examined cases except that of lung and gastric cancers. This can be explained by the fact that patients with gastric and lung cancers usually present to oncologists at advanced stage and despite initial response of tumour to treatment, most of these cases relapse or progress during or shortly after the completion of treatment. These findings are mostly observed in patients with adenocarcinoma, e.g.: breast, gastro-intestinal tract including pancreatic cancer, non-small-cell lung cancer, prostate, and brain and these have been previously published in multiple studies and trials. The imaging techniques that were used for diagnosis and staging were based on international recommendations for each disease category. These

findings were reviewed by two different radiologists and in some cases additional help of external radiologist when the patient was referred from a different hospital.

The relationship between the hypercoagulable state and both the tumour progression and occurrence of metastasis has not been well established. Larger studies are needed to determine those tests that could have a reliable predictive value in tumour progression. Much more information is needed, and only large-scale clinical trials will unequivocally establish whether these hypercoagulable parameters can be used reliably to predict the progression of disease and whether modulating the haemostatic system will have an impact on the process of tumour progression and metastatic dissemination.

Patients with cancer represent 15-20% of all patients with thrombosis. Furthermore, about 10% of patients presenting with unprovoked or idiopathic thrombosis are diagnosed with early or advanced malignancy within the next 1-2 years of the thrombosis event (Prandoni *et al*, 1992; Lee & Levine, 2003). Hence, approximately one quarter of all thrombosis cases are related to underlying malignancy. Given the ageing population and the rising incidence of cancer in industrialized nations, venous thrombo-embolism in patients with cancer will become an increasingly common health care issue.

According to a population-based study by Sorensen *et el* (2000), the 1-year survival rate in patients diagnosed with cancer at the time of their thrombo-embolic event is 12%, as compared with 36% in cancer patients who are free of thrombosis but otherwise are matched for sex, age at the time of cancer diagnosis, tumour type and the duration of cancer. The higher mortality rate in cancer patients with venous thrombo-embolism may indicate that venous thrombo-embolism is a marker of aggressive malignancies or that these patients are dying prematurely from thrombotic complications, or both.

Unfortunately, the true incidence of venous thrombo-embolism associated with various tumour types remains unknown for the majority of cancers because the appropriate cohort studies have not been conducted. On the contrary, the most common tumour types found in patients with venous thrombo-embolism are cancers of the lung, colon, breast and prostate. This reflects the high prevalence of these cancers in the general population.

Recent reports of very high rates of thrombotic complications in patients receiving experimental therapy with inhibitors of vascular endothelial growth factor (Kuenen *et al*, 2002; Marx *et al*, 2002; Kbabbinavar *et al*, 2003) and other anti-angiogenic agents, such

as thalidomide (Zangari *et al*, 2001; Desai *et al*, 2002; Rajkumar *et al*, 2002), have re-emphasized the importance of cancer therapy in the pathogenesis of the thrombosis in cancer patients.

Effective and safe antithrombotic therapy- the mainstream in prophylaxis and treatment of thromboembolism- remains very challenging clinical task in cancer patients- a population with high rate of treatment failure, haemorrhagic and thromboembolic complications recurrences and relapses. Prospective randomized clinical trials have documented that with advent of LMWH, new possibilities for thrombosis treatment and prevention with more convenient and safe antithrombotic have been emerged. But, the problem of thromboembolic events in patients with disseminated and advanced stage of malignancy remains a serious concern for haemato-oncologists. Based on the available evidence-based clinical data regarding the role of coagulation system in cancer development and progression, a new challenge for modern therapy in oncology has been built and this is appreciated in the hypothesis of anti-neoplastic effects of antithrombotic-anticoagulants which could influence the outcome of treatment in human cancers. Anti-tumour effects of anticoagulants agents have been observed and documented in various experimental models (cancer cell lines). Heparin and its derivatives mainly low-molecular-weight heparin in addition to Warfarin, have been the most extensively studied and the results are very promising in terms of; reduction in the primary tumour size, its growth and metastatic progression. Joint evidences from basic research and from multiple prospective, randomized clinical studies, showing beneficial impact of antithrombotic therapy (mainly LMWHs) on cancer patients' survival, confirm the need for further and more extensive scientific steps to confirm the biologic benefits of antithrombotic therapy in human cancers. Warfarin and unfractionated heparins (UFH) have been in clinical use for many years, and both are effective anticoagulants, but they are associated with many impediments, including the need for intensive monitoring of their effects, multiple drug-drug interactions (mainly in case of Warfarin), wide variations in dose-response relationships, and serious immune-mediated thrombocytopaenia (in the case of UFH). The development of LMWH advanced anticoagulation therapy by enhancing efficacy and eliminating the need for intensive monitoring. The evidences are started to accumulate, that clinically approved LMWHs have different abilities to influence some steps of

metastatic spread. The experimental works toward the development of such heparin derivatives with low anticoagulant activity, but with potential inhibitory effects on tumour cells spread are in progress. At present, most clinical haemato-oncologists report thromboprophylactic use of different types of antithrombotics in less than 5% of cancer patients. One of the reasons for this very limited use of thromboprophylaxis is probably the lack of haemato-oncology specific guidelines. The available excellent guidelines for treatment of thromboembolism must be adapted to the specific context of cancer patients. Anticoagulant prophylaxis is routinely recommended for patients undergoing major surgery because the risk of post-operative thrombosis is substantial. For patients having surgery for cancer, the risk of venous thrombo-embolism may be as high as 50% without prophylaxis (Geerts *et al*, 2001). Compared with the thromboprophylaxis in the surgical oncology setting, prophylaxis in cancer patients on chemotherapy has received even less research attention. Clinical trials have not been performed to evaluate primary prophylaxis specifically in hospitalized medical oncology patients. To date, multiple randomized trials and meta-analyses of these trials have confirmed that, for initial therapy, LMWHs are at least as efficacious as UFH in reducing recurrent thrombosis and are likely to be associated with a lower risk of major bleeding (Koopman *et al*, 1996; Levine *et al*, 1996; The Columbus Investigators, 1997; Gould *et al*, 199; Dolovich *et al*, 2000; Merli *et al*, 2001; van Dongen *et al*, 2004). In many developed countries, outpatient LMWH has become the standard of care for the initial treatment in patients with deep venous thrombosis or haemodynamically stable pulmonary embolism. Several studies were conducted to evaluate the efficacy and safety of LMWH as initial treatment, secondary prophylaxis and long-term therapy. Two meta-analyses of these studies showed a statistically non-significant reduction of approximately 30% in the risk of recurrent VTE favouring LMWH (van der Heijden *et al*, 2001; Iorio *et al*, 2003), while one of these studies found a significant reduction of 62% in the risk of bleeding with LMWH (van der Heijden *et* al, 2001). To date, three published clinical trials have examined the use of long-term LMWH as an alternative to coumarin derivative therapy in cancer patients with acute VTE (CANTHANOX, CLOT, and FRONTLINE). Two unpublished studies have also evaluated LMWH for long-term use in cancer patients and the results have been presented in abstract form. A subgroup analysis of the long-term

Innohep® treatment evaluation study reported improved efficacy with tinzaparin over Warfarin in the 167 patients with cancer (Hull *et al*, 2003). Tinzaparin reduced the rate of recurrent VTE by 50%, but this was not statically significant because of the small number of patients.

Evidence from the CLOT trial is promising with respect to the efficacy of long-term LMWH in the management of VTE in cancer patients, and its implications are addressed in a new paradigm in the management of thrombosis in cancer patients. There is strong evidence that long-term LMWH is efficacious and safe for preventing recurrent VTE in cancer patients. Bleeding does not appear to be increased compared with Warfarin therapy but this remains a major concern in cancer patients receiving long-term, anticoagulant therapy because of their co-morbid conditions. Daily injections appeared to be well tolerated but the main practical limitation of using LMWH for long-term use is the drug cost. A cost-effective analysis has suggested that LMWH might be a cost-effective drug for secondary prophylaxis of VTE, especially in patients at high risk of recurrence and where the drug cost is lower (Marchetti *et al*, 2001). Although recurrent VTE is relatively frequent in patients with cancer while on oral anticoagulant therapy, treatment in this setting has not been investigated in clinical trials. Traditionally, four options are available: continue with oral anticoagulant therapy aiming for higher INR target; switch to UFH; use of LMWH; or insertion of an inferior vena caval filter. None of these alternatives have been compared or rigorously evaluated. Duration of anticoagulant therapy has not been addressed in cancer patients. Based on accepted concept that the risk of recurrent VTE is increased in the presence of any ongoing risk factor, it is generally recommended that patients with metastases continue with "indefinite" therapy because metastatic malignancies are considered a persistent risk factor. In those without metastases, anticoagulant treatment is recommended for a long as the cancer is 'active' and while the patient is receiving anti-tumour therapy. In general, it is advisable to re-evaluate frequently the risk-benefit ratio of ongoing anticoagulant therapy in individual patients, taking into consideration the overall clinical status of the patient, including the quality of life and life expectancy.

To date, the studies evaluating new anticoagulants have included few or no patients with cancer. Given the differences in the natural history and response to therapy between

patients with and without cancer, research is needed to study the efficacy and safety of these agents especially in the various oncology settings.

Fondaparinux, the first selective factor Xa inhibitor, represents yet another advance in anticoagulation therapy. Efficient inhibition of factor Xa activity impairs the activation of tissue factor/factor VIIa complex which finally leads to downregulation of procoagulant state, pro-angiogenesis, and pro-inflammatory factors induced by TF/FVIIa complex However, despite compelling experimental and clinical evidences for a pathogenic role of blood coagulation in tumour growth and metastasis (Mousa SA, ref. 110), other studies in patients with solid tumours have failed to confirm a survival benefit for patients treated with anticoagulants (Smorenburg et al, ref. 116). More recently, this topic has received renewed attention and several randomized controlled trials and meta-analyses of studies have reported a reduction in the overall mortality of patients with cancer who were randomly assigned to receive a low molecular weight heparin (Prandoni P. et al, ref. 117, and Lensing AW et al, ref. 121). A plausible biologic mechanism is now emerging from experimental and *in vitro* studies that show LMWHs can inhibit angiogenesis, a process that is critical for tumour growth and metastasis, in a dose-dependent fashion (Nash GF. et al, Lancet Oncol 2:608-613, 2001, Norrby K, Haemostasis 23:141-149, 1993, and Hejna M. et al, J Natl Cancer Inst 91:22-36, 1999).

The findings in our study which show strong association between haemostatic parameters from one side and the tumour progression-metastasis (tumour biology), and response to treatment from other side, are consistent with previous observations from multiple clinical studies, including two randomized, placebo-controlled trials designed to investigate the influence of low molecular weight heparin on cancer survival (FAMOUS trial by Kakkar et al, MALT trial by Klerk et al, and CLOT trial by Agnes YYL et al). In all three studies, the use of low molecular weight heparin was associated with improved survival in patients with relatively good prognosis. Finally, we attempted to describe the current theory about the pathophysiology of the hypercoagulable status in cancer patients, and we also tried to discuss whether or not to screen elder patients (patients above age of 45 years) with idiopathic deep venous thrombosis for an underlying malignancy, and whether this would be potentially beneficial to patients and to the ongoing arguments regarding economic background for prophylactic and therapeutic strategies in cancer

patients. We hope that a better and more scientific understanding of these mechanisms (to be explored by further randomized clinical trials) will ultimately lead to the development of more targeted treatments to prevent and to treat thromboembolic complications in cancer patients. We also hope that guidelines for antithrombotic treatment may also have a positive effect on the process of tumour growth and metastasis.

Based on the findings from this small cohort of patients, we may conclude that antithrombotic therapy interfere with various processes involved in cancer development, growth and dissemination. Based on available data from multiple clinical studies, clinically relevant and improved efficacy of LMWHs as compared to Warfarin and UFH, on the survival of cancer patients with thromboembolic events, have been well documented and established. Our study has demonstrated a significant role for LMWH on tumour growth, tumour progression and dissemination and currently we are working on the role of LMWH on release of TFPI as a possible regulatory mechanism of angiogenesis and on potent inhibition of matrix-degrading enzymes by LMWHs. The anti-angiogenesis effect of LMWHs has been clearly documented and therefore LMWHs have been used in those clinical conditions in which angiogenesis has been associated with the clinical disorder, including human tumour growth and dissemination. Our findings can be used as documented basis for beneficiary effect of LMWH in inhibition of neo-angiogenesis in different cancer types in addition to their primary role as antithrombotic agents. More studies are required in this population to look at antithrombotic therapy, duration of therapy, bleeding, quality of life, cost-effectiveness and the influence of anticoagulants on cancer survival. These results might be useful as basis for future larger scale trials in which additional markers of hypercoagulable status will be evaluated in order to identify the most sensitive marker with highest prognostic impact on patients' survival. The significant finding of the study in which a direct correlation between hypercoagulability markers and tumour stage was confirmed, can be further assessed in future trials to compare the efficacy of different doses of low molecular weight heparins and to determine the effect of LMWHs on tumour progression. In cancer patients with good prognosis and longer life expectancy, the treatment with LMWHs could result in survival benefit. Based on experimental data, there is a significant difference between treatment regimens (dose of LMWHs) and their

effect on tumour biology (tumour development and metastasis). The duration of anticoagulation with LMWH in cancer patients is a very important issue which has received great attention in the recent years. Further studies at large scale are needed to evaluate the optimal duration and dosing of different anticoagulation therapy during the active phase of anti-cancer therapy and after the end of all therapeutic interventions in patients with different types of solid tumours especially in those with ECOG score of 0-1 and with no clinical evidence of secondary VTE. The relationship between the hypercoagulable state and the tumour progression and the occurrence of metastasis has not been well established. Much more information is needed and only large-scale clinical trials will unequivocally establish whether these hypercoagulable parameters have strong predictive values and whether the modulation of haemostatic system will modify the process of tumour progression and metastatic dissemination. The mechanisms for a potential anti-neoplastic effect of LMWHs remain unknown and will require further investigations in well-designed experimental studies. An anti-angiogenic effect is an appealing possibility and it is evident in patients with limited disease and could be persistent beyond the administration of the agent. It can be hypothesized that in patients with disseminated cancer, tumour-related vasculature is sufficiently developed so that an anti-angiogenic agent would have minimal impact, whereas impairing the establishment of such vasculature by an anti-angiogenic agent could exert an inhibitory effect on tumour growth even beyond the time of drug exposure. Although it has been suggested that the improvement in cancer survival associated with LMWHs observed in previous studies may be due to a reduction in fatal pulmonary embolism as compared with unfractionated heparin, the survival benefit beyond the period of administration of low molecular weight heparin observed in CLOT trial would argue against this hypothesis. A very strong association between cancer and disturbances in haemostatic parameters has been demonstrated consistently in experimental and clinical trials. Our findings offer additional evidence that the coagulation system and its cascade of activation, is intrinsically involved in tumour development and/or tumour progression-metastasis. But, additional, well-balanced studies designed to confirm the anti-tumour effects of low molecular weight heparins and explore the pathophysiologic mechanisms are warranted to further investigate these findings.

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