Endothelium and Cancer: Basic and Clinical Aspects

P 01
Expression of Tissue Factor Pathway Inhibitor-2 (TFPI-2) in Human Tumor Tissue
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Thromboembolic complications as well as laboratory abnormalities in hemostatic parameters are frequently observed in cancer patients. Tissue factor (TF) has been documented to play a crucial role in the pathophysiology of thrombosis and, among other coagulation proteins, in tumor growth and metastatic spread. The principal inhibitor of TF-dependent pathway of blood coagulation is tissue factor pathway inhibitor (TFPI). Its presence in cancer tissue was not detected in previous studies. There exists a second inhibitor of tissue factor dependent pathway, TFPI-2, also known as placental protein (PP5) or matrix-associated serine protease inhibitor (MSPI). TFPI-2/PP5 inhibits the amidolytic and proteolytic activity of TF/VIIa complex. TFPI-2/PP5 is a strong inhibitor of human factor Xa amidolytic activity, human plasma kallikrein, plasmin, trypsin and chymotrypsin. TFPI-2/PP5, synthesized predominantly by the endothelial cells of small blood vessels, is secreted primarily (60–90%) into the extravascular matrix. However, information on the presence TFPI-2 at the tumor burden still remains obscure. The purpose of this study was to evaluate the expression of the TFPI-2 in situ in several human neoplasms. The study employed immunohistochemical procedures. TFPI-2 expression was observed in neoplastic cells of laryngeal, breast, gastric, colon, pancreatic, renal, endometrial cancer and non-small cell lung cancer (NSCLC), as well as glial neoplasms. The intensity of staining was inconsistent. Higher intensity of staining for TFPI-2 was detected in more differentiated tumors. Breast, gastric, endometrial and colon cancer cells revealed populations of cells that were either TFPI-2 positive or negative. Antigenic TFPI-2 was also demonstrated in tumor infiltrating macrophages in the case of NSCLC, gastric and renal cancers. In gastric cancer a staining for TFPI-2 in tumor stroma was revealed. TFPI-2 was also observed in normal tissue of the larynx, breast, stomach, colon, pancreas, kidney, lung and endometrium. The data demonstrate that the expression of TFPI-2 diminishes with an increasing degree of malignancy, which may suggest a role for TFPI-2 in the maintenance of tumor stability and inhibition of the growth of neoplasms.

P 02
Up-Regulation of Cyclin D1 in Cultured Human Endothelial Cells in Response to Exogenous Tissue Factor
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Tissue factor (TF) a cell surface receptor, known as the principal initiator of blood coagulation, is also of great interest for its ability to induce signalling mechanisms involved in angiogenesis, tumour growth and metastasis. The ability of TF to influence cellular behaviour may arise either directly from this protein, or via the activation of protease activated receptors mediated through TF-factor VIIa (FVIIa) or factor Xa (FXa) activities [1]. In this study, we examined the influence of TF on endothelial cell proliferation and its ability to regulate the expression of one of the key regulator protein of the cell cycle: cyclin D. Cyclin D upregulation facilitates the entry into S phase and hence progression through the cell cycle. Human umbilical vein endothelial cells (HUVEC) were cultured in serum-free media and treated with combinations of TF and FVIIa. Cell proliferation was examined using a chromogenic assay and the expression of the cyclin D1 mRNA, was assessed by semi-quantitative RT-PCR measurement and in comparison to β-actin. Following a 24h incubation, the level of cyclin D1 mRNA increased with the concentration of TF (5–500nM) reaching a maximum at TF concentration of 500nM (2.5-fold enhancement). These data are in agreement with the observed rate of cell proliferation which increased proportionally on stimulation with TF. The addition of recombinant FVIIa (5nM), together with TF (50nM) had little effect on cell proliferation and expression of cyclin D mRNA, over and above that of TF alone. In conclusion, the presence of high concentrations of TF, indicative of severe injury, trauma or disease state, initiate cell proliferation by targeting cyclin D, one of the most important regulatory proteins of the cell cycle.

The support of The Yorkshire Cancer Research is acknowledged.

Reference
Tumors activate the hemostatic system of the host, which in turn influences tumor growth and dissemination. In this setting, the role of tumor cell/endothelial cell (EC) interactions play a key role, including the induction of EC tissue factor (TF) by tumor cell-derived products. TF can play a dual role, both as a prothrombotic and a proangiogenic factor. LMWHs are effective anti-thrombotic agents, which may show beneficial effects on survival in cancer patients, therefore, in this study, we evaluated whether a LMWH (enoxaparin, ENX) may interfere with EC TF expression elicited by various tumor cell types, and identify the cytokines possibly involved in this process. Methods: Human EC from both micro- (HMEC-1 line) and macro-vascular (HUVEC) beds were incubated for 4h with tumor conditioned media (CM) obtained from two human breast carcinoma cell lines [i.e. MDA.MB.231 (highly metastatic), and MCF-7 (low metastatic)] and the human promyelocytic leukemia cell line NB4, with or without ENX (0.01–10IU/ml). Then, EC samples were tested for TF expression (both as activity and antigen). Results: All tumor CM significantly increased TF expression by both EC: in HMEC-1, TF induced by all three CM types was dose-dependently counteracted by ENX, in HUVEC, TF induced by NB4 and MCF-7 CM, but not from MDA.MB.231, was significantly reduced. To identify the soluble mediators possibly involved, CM samples were screened for their cytokine content, including IL-1β, VEGF, b-FGF and TNF-α. These cytokines were differentially distributed according to CM types, as follows: VEGF: MDA.MB.231 > NB4 > MCF-7; IL-1β: NB4 > MCF-7 > MDA.MB.231; TNF-α: NB4 > MDA.MB.231 > MCF-7; and b-FGF: NB4 > MDA.MB.231 > MCF-7. The relative role of each cytokine was explored by means of specific MoAbs. A study with purified standard cytokines showed that IL-1β, TNF-α, and b-FGF significantly (p < 0.05) increased TF in both EC types, whereas VEGF significantly induced TF only in HUVEC (p < 0.05). ENX counteracted TF induced by standard IL-1β, TNF-α, and b-FGF in both EC types, while did not significantly affect VEGF-induced TF in HUVEC. Conclusions: The LMWH enoxaparin reduces the EC TF induction triggered by a number of tumor cytokines. This effect may vary depending on both the malignant cell and the EC types involved. TF regulation may represent one mechanism for non-anticoagulant effects of LMWH.

Tumor growth involves reciprocal signaling events between endothelial cells (EC), cancer cells and the surrounding environment. An example of this cross-talk is angiogenesis, the induction of tumor vasculature required for tumor progression. To understand the influence of the tumor/angiogenic environment on endothelial cells, HAMEC (human adrenal gland microvascular endothelial cells) were plated onto ED-B-FN (oncofetal fibronectin, Blood 1999;94:192–198) and exposed to VEGF (Vascular Endothelial Growth Factor), FGF-2 (basic Fibroblast Growth Factor) and EGF (Epidermal Growth Factor). These are cancer associated factors known to be involved in angiogenesis in vivo, and shown to be important for the in vitro survival and growth of tumor derived endothelium (Clin Exp Met 1999;17:655–662). We were able to identify a differentially expressed transcript and to clone its complete coding sequence. It was expressed by human EC isolated from umbilical veins, adrenal glands, as well as kidney and ovarian carcinomas. The transcript was not ubiquitously expressed in a panel of human adult tissues. In addition to ED-B-FN, its expression was modulated in EC by collagen I, collagen IV and thrombospondin-1, all extracellular matrix proteins involved in angiogenesis. In contrast, the level of expression of the transcript was not modulated in a panel of tumor cell types. The encoded protein showed an estimated molecular weight of 22kD including four transmembrane domains. The analysis of the protein sequence predicted that it was the human homolog of an integral peroxisomal membrane protein previously characterized in rat and mouse. A GFP chimeric construct was originated and used for transfection of EC. The fluorescence of the fusion protein localized in intracellular organelles, compatible with the peroxisomal localization. Superimposition of the green fluorescence with the catalase staining, specific for peroxisomes, revealed co-localization.

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ZD6126 is a novel tubulin-binding compound in development as a vascular-targeting agent. In vivo, ZD6126 is rapidly converted into N-acetylcolchinel. In vitro N-acetylcolchinel induces rapid changes in the morphology of human umbilical vein and lung microvessel endothelial cells. Within 40 min, the compound induced endothelial...
cell retraction, destabilization of the tubulin cytoskeleton, induction of actin stress fibers, and membrane blebbing. These effects occurred at non-cytotoxic concentrations, and were rapidly reversed upon removal of the drug. Non-confluent endothelial cells were more sensitive than confluent, quiescent cells. Among different cell types, endothelial cells were the most sensitive to the induction of morphological changes, whereas smooth muscle cells were not affected. In an in vitro cord formation assay onto Matrigel, N-acetylcolchinol disrupted the network of newly formed cords in a rapid and reversible manner. In vivo, ZD6126 caused the shutdown of neo-formed vessels elicited by FGF-2 in a pellet of Matrigel. One hour after a single treatment, vessels in the Matrigel plug were no longer functional, as shown by the lack of perfusion with the FITC-isolectin B4. Twenty-four hours later, functional vessels were again observed, mainly at the periphery of the pellet, indicating the reversibility of the effect. In the human tumor xenograft model MDA-MB-435, a singe injection of ZD6126 caused extensive necrosis at the center of the tumor mass, with viable cells remaining at the tumor periphery. Maximal necrosis (up to 35–50% of the total tumor area) was observed 24h after treatment, while at later times (96h) the tumor resumed its growth, and the percentage of necrotic area was reduced. Consistently with these findings, a single treatment with ZD6126 only marginally affected tumor growth, though multiple treatments had a significant antineoplastic effect. This study indicates that rapid alteration of endothelial cells morphology caused by ZD6126 might trigger a cascade of events leading to vascular shutdown, rapid loss of tumor blood vessel integrity, and extensive tumor necrosis.

**P 06**

**Tumour Related Gene Expression in Head and Neck Squamous Cell Carcinoma**


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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the developed world and even more frequent in Asia. Overall survival rates for these patients have not significantly improved over the last three decades. Since tumorigenesis and their response to treatment involve various genes and pathways, the analysis of large number of genes need to be established. Our investigation focuses on characterisation of tumour target genes in HNSCC by cDNA array. Sixteen HNSCC tumour biopsies and their autologous unaffected normal oral epithelial biopsies were used for this purpose. In spite of their important role in various cancer types, HNSCC expressed only marginal levels of Bcl-2, IFG1 and uPAR genes. Majority (>50%) of these tumours up-regulated angiogenesis related genes, VEGF, EGFR, Integrin a2/a3. Some of these tumours (≥25%) down-regulated CD95, DR3, TNFR and Endostatin genes. Thus, HNSCC tumour related gene expression profile might be use as the indicator for individual treatment program.

**P 07**

**Impact of Surgery and Chemotherapy on von Willebrand Factor (vWF) and Vascular Endothelial Growth Factor (VEGF) Plasma Levels in Colorectal Cancer (CRC) Patients**

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**Introduction:** Several molecules play a role correlating haemostasis, angiogenesis and cancer, vWF is one of those proteins mediating tumour cells to platelets binding process. Due to this union, tumour cells gain mobility and infiltration capacity needed for cancer metastasis. In addition, platelets are involved in tumoural angiogenesis being able to release VEGF under determined stimuli. Moreover serum vWF and VEGF seem to correlate with stage in CRC. **Purpose:** To determine the impact of surgery and chemotherapy on vWF and VEGF plasma levels in CRC patients at different stages and its clinical implication. **Patients and Methods:** 20 healthy volunteers (group 0), 14 locally advanced (group 1) and 12 metastatic (group 2) CRC patients were enrolled in our study. All patients have signed an informed consent. Blood samples were taken as follows: at the time of joining the study in group 0; before and after surgery in group 1, before and after three chemotherapy cycles in group 2. All samples were stocked at −80°C until further processing. vWF, VEGF, platelet counts, C-reactive protein (CRP), ceruloplasmine and CEA plasma levels were measured in blood samples using standardised techniques. **Results:** At baseline, there were differences between groups in vWF and VEGF levels, showing higher concentrations of vWF in group 2 compared to groups 0 and 1 (p < 0.001 and p = 0.002 respectively). In the same way, lower levels of VEGF were observed in group 0 compared to group 2 (p = 0.040). In group 1, there were no changes in mean levels of platelets and VEGF after surgery. However, an increase in vWF was apparent (p = 0.016), not correlated with changes in CRP nor ceruloplasmine levels after treatment. Conversely, CEA levels decreased after surgery (p = 0.011). In group 2, mean levels of CEA, platelets and vWF decreased after chemotherapy, but not significantly. Interestingly, a decrease in VEGF was observed after chemotherapy in these patients (p = 0.018). **Conclusions:** In metastatic CRC patients serum vWF and VEGF levels were higher compared to healthy subjects and locally advanced patients before any treatment. In addition, surgery increases vWF plasma levels independently of inflammatory status. VEGF showed a dramatic reduction after chemotherapy in metastatic patients.

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Effect of Cytostatic Drugs on the Endothelium and Platelets

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Background: Chemotherapy induces often the release of procoagulants or activators of fibrinolysis from disintegrated cells in the tumor. Cytostatic drugs by themselves may, however, induce the activation of various mechanisms and they affect not only the number of platelets, but also their function as well as the functions of endothelial cells. Aim of the Study: We evaluated the effect of cytostatic therapy on the endothelium and platelets in hemato-oncological patients. Patients and Methods: A total of 37 patients with recently diagnosed hemato-oncological diseases (acute leukemia: n = 8; non-Hodgkin's lymphoma: n = 15; Hodgkin's disease: n = 12; myeloma multiplex: n = 2) were enrolled. Before entering the study patients were neither treated with cytostatic drugs nor exposed to the radiotherapy. The control group consisted of 38 healthy individuals. The first cycle of chemotherapy including 6 cytostatic treatments was determined as the follow-up period. Venous blood was taken 12 times: the first sample was taken before the beginning of polychemotherapy and the followed blood specimens were collected before and after each cytostatic treatment. The plasma levels of the endothelial and platelet markers (BTG, PF4, TSP, vWF, TM, FN, tPA and PAI-1) were measured by EIA. Results: There were no significant differences found in the patient group between the values of individual parameters in the 1st and 12th sampling. Correspondingly, no trends indicating neither increase nor decrease in the values of measured parameters were observed over the examined period in the patient group. The medians of BTG, PF4, vWF, FN and TM plasma levels were significantly higher (p < 0.001) and the median those of PAI-1 significantly lower (p < 0.05) in the patient group compared to the control one. Conclusion: Our results suggest that hematological malignancies affect the function of both platelets and endothelium already before the initiation of polychemotherapy.

P 09

Expression Levels of Soluble VEGF in Plasma of Patients Affected by Ovarian Cancer

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Ovarian cancer is the most common gynecological malignancy in women older than 50 years. The most common dissemination of epithelial ovarian cancer is by exfoliation of malignant cells from ovary capsule with wide spread intraperitoneal tumors and ascitic fluid formation. The production of growth factors and cytokines are responsible for ovarian cancer-associated angiogenesis and dissemination. Vascular endothelial growth factor (VEGF) is highly expressed in ovarian cancer and this presence suggests that targeting VEGF might be useful approach to control angiogenesis and tumor spread. In this study we measured the levels of VEGF in plasma of patients with ovarian cancer (n = 40) and other gynecological pathologies (n = 30) and in healthy volunteers (n = 26). We also studied the levels of VEGF in the ascitic fluids of the patients with ovarian carcinoma. An initial statistical analysis of the correlation between VEGF, clinical parameters and outcome is described. ELISA was used to assay soluble VEGF. Preoperative plasma VEGF levels were significantly higher in patients with ovarian cancer (median 109.1 pg/mL) than in healthy volunteers (median 0 pg/mL, p < 0.0001) or patients with non-malignant gynecological pathology (median 18.8 pg/mL, p < 0.0001). Elevated levels of VEGF was found in ascitic fluid, but no correlation was found between ascites VEGF levels and clinical parameters. Subgroup analysis showed that VEGF was higher in plasma of patients with serous ovarian carcinoma than other histological types. Furthermore, plasma VEGF levels, was high in plasma of cancer patients with thrombocytosis although the rise was not significant. No correlation was found between plasma VEGF levels with tumor stage, grading, residual disease and poor survival. The limited number of patients might account for this. A larger numbers of patients are needed to establish whether high levels of VEGF is predictive of more aggressive tumor.

Coagulation Activation and Tumor Cell Biology

P 10

Pattern of Coagulation/Fibrinolytic System Components in loco in Gastric Cancer

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Thromboembolic complications frequently accompany gastric cancer. The purpose of this study was to evaluate the solid phase interactions among gastric cancer and coagulation/fibrinolytic proteins in situ that may contribute to tumor progression in this tumor type. Immunohistochemical techniques were applied to tissues from 37 cases of adenocarcinoma of the stomach obtained at surgical resection. Fibrinogen was present throughout the tumor stroma. Fibrin and its subunit ‘a’ of factor XIII and factors VII, IX, X and XII were observed on tumor-associated macrophages. Protein C was observed on cancer cells and small blood vessels, while protein S was present only in the vascular bed. There was no staining for TFPI. High molecular weight...
urokinase plasminogen activator (HMW-uPA) antigen was not detected, but weak and inconsistent staining for low molecular weight urokinase plasminogen activator (LMW-uPA) was demonstrated on cancer cells. Weak staining for tissue plasminogen activator (tPA) occurred on cancer cells and in the tumor stroma. In contrast, plasminogen activator inhibitor-1 (PAI-1) expression was strong in the tumor stroma along with PAI-2 and PAI-3. The endothelium of small stromal blood vessels, particularly near the host-tumor interface, demonstrated vonWillebrand factor antigen (vWF Ag). These results demonstrate extravascular coagulation activation in situ in gastric cancer, which is TF-dependent and does not appear to be counterbalanced by TFPI or sufficient fibrinolytic activity.

P 11
Increased Blood Clotting in Murine Breast Cancer Treated with Sodium Cromolyn

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Human breast cancer is extensively infiltrated by mast cells that contain powerful anticoagulants such as heparin, tryptase, and chymase. In order to determine if human breast cancer is associated with mast cell activation, we measured the levels of mast cell tryptase (an indicator of mast cell activation) in the blood of 20 women with varying stages of breast cancer. The mean level of tryptase in women with breast cancer (10.3 ± 4.2 μg/L) was significantly higher than in 50 normal healthy women without breast cancer (3.0 ± 2.5 μg/L, p < 0.05 by two-tailed t-test). To explore the role of mast cells in breast cancer in more detail, we then performed experiments that were aimed at determining if an inhibitor of mast cell function, sodium cromolyn, could increase blood clotting and hypoxia within subcutaneous implants of the 4T1 mammary adenocarcinoma cell line in mice. We treated tumor-bearing mice with five consecutive daily doses of sodium cromolyn (10 mg/kg, i.p.). An average of 30% of the periphery of the tumors from the five drug-treated mice contained large lakes of clotted blood that were not evident in any of the tumors from the control (untreated) mice. By computerized image analysis of tumors immunostained for a hypoxia marker (pimonidazole), the tumors from the treated mice had significantly more hypoxia (35 ± 12% hypoxic regions, n = 5) than the tumors from untreated (control) mice (16 ± 7%, n = 5). We conclude that sodium cromolyn enhanced peri-tumoral blood clotting and intratumoral hypoxia. These results suggest that mast cells may play an important role in regulating blood clotting and hypoxia within breast cancer.

P 12
Assessment of Cancer Procoagulant Activity in Patients with Multiple Myeloma

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Haemostatic disorders are the important clinical problem in patients with multiple myeloma (MM). The most common are bleeding symptoms due to platelet dysfunction, inhibition of fibrin polymerisation and increased clearance of coagulation factors. Much less frequent are thrombotic complications. The aim of the study was to assess the cancer procoagulant (CP) activity and disturbances of coagulation system in different types and clinical stages of MM patients. Twenty seven patients, in this number 19 with IgG MM, 5 with IgA MM and 3 with light chain disease were studied. Twenty one patients were in third clinical stage of the disease according to Durie and Salmon classification while 6 patients were ranked to the second stage. The control group was 20 healthy volunteers. The activity of cancer procoagulant, D-dimer concentrations and standard coagulation tests were analysed. The CP activity was increased above baseline level in 8 patients, including 6 cases with IgG MM and 2 cases with IgA MM. However the mean cancer procoagulant activity did not differ significantly in reference to the control (10.49 mU/ml vs 9.82 mU/ml, p = 0.67). There was no correlation between levels of monoclonal immunoglobulins and CP activity. The mean CP value was higher in IgA than in IgG patients (13.2 mU/ml vs 9.96 mU/ml) but it was not statistically significant. The serum level of CP activity was not dependent on the stage of the disease. In 4/8 patients with CP activity above baseline and in 6/22 patients with CP activity below baseline the elevated concentrations of DD were observed. In none of the studied patients the thrombotic symptoms were found. Conclusions: Increased cancer procoagulant activity can be observed in different types and clinical stages of multiple myeloma.

P 13
Procoagulant Activities Expressed on Tumors in a Rat Model of Spontaneous Prostate Cancer

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The Lobund-Wistar (LW) rat combines histologic and clinical features resembling clinical human prostate cancer; androgen-modulated growth, age-dependent spontaneous onset, and metastatic potential. Metastasis is common in both spontaneous and induced prostate cancer in the LW rat and typically involves the lungs. In contrast to rat strains commonly used in carcinogenicity studies that only develop tumors in the ventral lobe, the LW rat has been shown to develop tumors of the anterior, dorsal, and lateral lobes of the prostate and therefore represents a particularly useful model of human prostatic carcinogenesis. Most significantly, the disease culminates in autochthonous metastatic prostate adenocarcinoma. Tumors often express...
Tissue factor (TF) is constitutively expressed by many tumour cells and is implicated in peri-tumour fibrin deposition and hypercoagulability in cancer. TF and other coagulation factors enhance tumour growth and play a role in tumour metastasis. We examined the influence of exogenous tissue factor, factor VIIa, factor Xa and TFPI, on the ability of cultured Tumour cells to proliferate and adhere. Human adenocarcinoma cell lines LoVo (expressing a high level of TF) and HT29 (expressing a low level of TF) were cultured in serum-deprived media and supplemented with combinations of TF, factor VIIa, factor Xa and TFPI. After 24h incubation, cell proliferation and the percentage of adherent cells in the samples were assessed. Our data suggest that despite the clear increases in cell proliferation, samples containing either TF alone or TF-FVIIa combinations, exhibited a greater percentage of adherent cells (Å = 13% and 11% in LoVo and HT29 cells respectively). However, while the supplementation with factor Xa and/or TFPI had little additional effect on LoVo cells, the percentage of adherent HT29 cells was reduced to below that of the control sample (Å = −15%). The data indicate that tissue factor may be able to induce cell adherence, probably through the up-regulation of adhesion receptors and may contribute to the pro-metastatic properties of TF expressing tumour cells.
Inflammation, Clotting and Cancer Growth: New Insights from Pentraxin PTX3?


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Both inflammatory responses and blood coagulation activation have long been proposed to enhance tumor development. PTX3, the novel inflammatory acute phase reactant which belongs, together with C-reactive protein (CRP) and serum amyloid P component (SAP), to the family of the pentraxins, is synthesized by monocytes and endothelial cells following exposure to a variety of inflammatory agents. Monocytes and endothelial cells, when properly stimulated, synthesize tissue factor (TF), the transmembrane glycoprotein that triggers blood coagulation. We have previously shown that PTX3 could enhance TF activity by activated human monocytes and endothelial cells. When mononuclear cells (MN), obtained from peripheral blood of healthy donors, and human umbilical vein endothelial cells (HUVEC) were incubated with endotoxin (LPS) and highly purified PTX3, the resulting TF activity, assessed by a one-stage clotting time and characterized by anti-TF monoclonal antibodies, was higher than in the presence of LPS alone. The amplification in activity was paralleled by an increase in TF antigen, and required mRNA synthesis, as assessed by reverse transcriptase PCR for monocytes and Northern blot analysis for HUVEC. Interestingly, the increase in activity in MN was specific for LPS, since in the presence of other TF-inducing agents, such as IL-1β, and TNF-α, PTX3 was not effective. In contrast, PTX3 was effective on HUVEC also when IL-1β and TNF-α were used instead of LPS, suggesting a different regulatory mechanism for these cells. To assess the specificity of PTX3, the effect of other acute phase reactants such as CRP and SAP was studied. These proteins, when incubated with the cells in the presence of LPS, did not modulate TF activity, assigning PTX3 a unique role among the components of the pentraxin family. We studied the translocation of the transacting factor c-Rel/p65 into the nucleus by EMSA (electro mobility shift assay). Translocation of c-Rel/p65 induced by LPS in MN, and by LPS, IL-1β, and TNF-α in HUVEC was increased in the presence of PTX3. Western blot analysis revealed that the increased c-Rel/p65 activity was dependent upon enhanced degradation of the inhibitory protein I-kBα. Preliminary results suggest that the inflammatory responses here described in monocytes and endothelial cells may represent a model of tumor cell responses: some tumor cells (from a breast cancer line), indeed, have been found to respond to PTX3 treatment with an enhancement of their constitutive TF expression. Taken all together, these data suggest a novel biological role for PTX3: this molecule could modulate cell-mediated fibrin deposition which is a common feature not only of the inflammatory response but also of tumor growth.

Assembly and Regulation of Prothrombinase Complex on B16F10 Melanoma Cells

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For more than a century, a correlation between cancer and alterations of the coagulation system, especially those related to prothrombotic states have been described. Melanoma is a highly metastatic cancer, and there is evidence that thrombin contributes to this aggressive pattern. However, few studies correlate this type of cancer with formation of the prothrombinase complex, which is responsible for conversion of prothrombin into thrombin in the coagulation system.

Objective: The aim of this study was to investigate the assembly and regulation of prothrombinase complex on the murine melanoma cell line, B16F10. Methods and Results: B16F10 cells were grown in 96-well plates for 24h and further assayed for prothrombin activation using the synthetic thrombin substrate, S-2238. B16F10 cells were unable to activate prothrombin except when previously incubated for 10 min with 10nM factor Xa. This effect was dependent on factor Xa binding to cell membrane, since no activation was observed with Glu-domainless factor Xa. The thrombin formation by B16F10-bound factor Xa was enhanced ~10-fold in the presence of factor Va, indicating the assembly of prothrombinase complex. Differently from platelets, B16F10-assembled prothrombinase complex was inhibited by prothrombin fragment 1 but not by fragment 2. In addition, bothrojaracin, a specific proexosite ligand of prothrombin decreased the zymogen activation. Conclusion: Our data demonstrate that B16F10 melanoma cells generate thrombin through the assembly of the prothrombinase complex. This ability might be correlated with the increased metastatic potential of this cell line. Moreover, B16F10-assembled prothrombinase complex seems to be modulated in a distinct way from that found for the physiological complex assembled on platelets.

Pentolame, Estradiol and Ethinylestradiol Induce Differential Changes on Hemostatic Parameters in Male and Ovariectomized Wistar Rats

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Estradiol (E2) and synthetic estrogens administered at high doses increase the incidence of thromboembolic events. We have observed contrasting effects in blood clotting times of rodents treated with E2 or 17β-aminosterogens. The effect of E2, ethinylestradiol (EE) and the 17β-aminosterogen pentolame on some hemostatic tests of the male (M) and ovariectomized (Ovx) Wistar rats were studied. Different groups of animals received subcutaneously (s.c.) for
21 consecutive days; E2 or EE (1, 10 and 100 μg/animal/day) or pentolame (1, 10, 100, 500 μg/animal/day) or vehicle (propylene-glycol 0.1 ml/animal/day). After 24 h from the last injection the hemostatic parameters: prothrombin time (PT), activated partial thromboplastin time (APTT)/thrombin time (TT) were determined. The same patterns of the effects on PT and APTT induced by the estrogens were observed in M and Ovx animals. Significant differences were observed in TT among M and Ovx animals. Pentolame parameters were similar to those of the vehicle group in PT, APTT and TT in M rats. In the Ovx group, pentolame showed a 10% increment in TT. Whereas E2 and EE induced significant decrease on TT. The maximum response (~30%) was obtained at the 100 μg dose with E2. We observed contrasting effects of pentolame and the estrogens E2 or EE in the TT parameter. Additional work is in progress about the influences of these estrogens on fibrinogen concentration.

This supports the hypothesis of an increased risk of thrombosis in advanced cancer.

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**Platelets, Fibrinolysis and Cancer**

**P 19**

**Increased Platelet Adhesion in Metastatic Breast Cancer**


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The increased risk of thromboembolism in cancer may relate to a prothrombotic or hypercoagulable state, with abnormalities of platelet activation and adhesion, such as raised plasma P-selectin and fibrinogen, and thrombocytosis. To investigate the role of platelets in cancer, we developed and then applied a new microplate assay to quantify platelet adhesion to fibrinogen. Recruiting 8 patients with metastatic breast cancer, 12 free of metastases, and 9 age-matched controls, we applied $2 \times 10^9$ platelets to microwells ( precoated overnight with $5 \text{mg/ml fibrinogen}$) for 1 h at room temperature. Unbound platelets were aspirated off and pelleted. The pellet, and the platelets bound to the plate, were solubilised (lysed) in 200 μl 5% Triton. Soluble P-selectin was measured in lysates by ELISA.

<table>
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<td>53 [6]*</td>
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</table>

Data (mean [SD]) are P-selectin (ng/ml) in the cell lysates; p values from ANOVA; *higher than the other two groups.

Patients with metastatic disease also had more soluble P-selectin in their plasma (44 [7] ng/ml than patients without metastases (34 [11] ng/ml, p = 0.02, t test). Compared to patients without metastases, those with metastatic breast cancer are characterised by increased platelet adhesion in an in vitro assay, as well as raised P-selectin.

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**Role of Megakaryopoiesis during Haematogenous Dissemination of Cancer: An Experimental Model on Murine Lewis Carcinoma**

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Role of platelets in the process of metastasising has been a subject of disputes and it is not yet clearly understood. It has been known recently that interactions between tumour cells, platelets, and endothelial wall contribute to haematogenous dissemination of cancer by activation of local coagulation and thrombus formation with generation of platelet-tumour cell emboli. Not only the role of platelets but participation of megakaryopoiesis in early stages of tumour dissemination is not yet well investigated and estimated. The aim of our study is to experimentally research megakaryopoiesis during the period of metastasising using a murine model-25 male mice, line C-57-BL, with Lewis lung carcinoma. Newly formed platelets are tagged by Se 75-Methionine and thus thrombopoiesis and platelet survival in the circulation are measured in the 24th hour and during the period of haematogenous dissemination of the cancer (between the 4th and the 27th day). Cytological bone marrow investigation is concomitantly conducted in order to appraise megakaryopoiesis. On the 4th day the percentage of enclosed Se 75-Methionine is not differing from the control group of healthy animals. On the 9th, 10th, 11th day a statistically reliable elevation of the included Se 75-Methionine has been detected. Next 12th, 16th, 18th, and 25th day the percentage of incorporated Se 75-Methionine diminishes and becomes similar to that of the control group. On the 27th day a rapid increase in the included Se 75-Methionine in the newly formed platelets has been observed. On the one hand, morphological investigation of bone marrow aspirates reveals excessive megakaryocytosis between the 9th and the 16th day. On the other hand, bone marrow cytology shows suppressed megakaryopoiesis and respectively myelopoiesis with hypoplastic bone marrow and neoangiogenesis on the 25th and 27th day. Role of megakaryopoiesis in the so-called early and late period of metastasising is discussed. Excessive megakaryocytosis in the early stage is probably due to the enormous needs of circulating platelets as a defense mechanism against tumour dissemination. This compensatory mechanism is exhausted in the later stage. Consumptive coagulopathy and suppressed megakaryopoiesis by malignant tumour line are opened up for discussion.
Erythromelalgia is presenting symptom in patients with essential thrombocythemia. For erythromelalgia to develop arachidonic acid metabolites, microvascular inflammation and thrombosis are necessary. In erythromelalgic areas are present a local platelet consumption evidenced by higher levels of platelet activation markers (β-thromboglobulin, thrombomodulin, increased urinary thromboxane TXB2 excretion), a shortened platelet survival times, arteriolar fibromuscular intimal proliferation and occlusions by platelet-rich thrombi in skin biopsies. We had in study a lot of 52 patients with essential thrombocythemia, 36 females and 16 males with ages between 37 and 72 years. In this patients, the diagnosis of essential thrombocythemia was established concordant with TESG criterions (1998). All patients from this study have shown at least one of the following complications: erythromelalgia (61%), digital ischemic phenomenon (pelvic member) (56%), suggestive transitory ischemic stroke phenomenon (21%), cerebral infarct (4%), myocardial infarct (4%). The patients with an initial number of platelet between 670,000/mmc and 1,000,000/mmc who initially have hemorrhagic complications higher digestive hemorrhage 32.2%, cerebral hemorrhage 11.4%, the start of the specific treatment (Interferon-a-2b in doses by 3 × 3,100 u.i. or Hydroxyurea 1.5–2.5 g/day) and the symptomatic treatment with aspirin (40–50 mg/day) have significantly decreased the appearance of hemorrhagic complications (in average with 86.6%) in these patients. In conclusion, the treatment with low doses of cyclooxygenase inhibitors (aspirin) may control successfully the release of arachidonic acid metabolites involved in microvascular inflamations and thrombosis in essential thrombocythemia. The association with specific therapy in case of very high number of platelets, decreased the risk of hemorrhagic complications possible promoted by aspirin administration.

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The Effect of Cyclooxygenase Inhibitors on Microvascular Inflammatory and Thrombotic Phenomenon in Essential Thrombocythemia

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It has already been recognized that leukocyte/platelet cross-talk may represent a model of tumor cell/platelet interaction in the metastatic process. Adhesion molecules such as von Willebrand factor (VWF) may act as a bridge between tumor cells and blood cells with an impact on the tumor arrest at the vascular level. In this context, as a part of a larger study, we report here data on VWF present on PMN surface, which acts as a bridge between platelets and PMN. It has been recently shown that Mac-1 is the receptor for the immobilized form of VWF, such as that present in vascular subendothelium. The purpose of the present work was to characterize VWF present on PMN. An analysis of the multimeric pattern of VWF, showed that VWF of leukocytes only has the small multimers (n = 8). Lysates of PMN or platelets were analyzed by reducing and non-reducing SDS-9% PAGE, blotted and probed with an antibody against VWF, followed by immunoenzymatic stain and evaluated by densitometric tracing (n = 6). At variance with the pattern of solubilized platelets, VWF bound to PMN showed only two bands of 80 and about 30 kD, while the pattern of VWF on PMN samples in reducing conditions yielded a single band of about 30 kD. A similar proteolytic pattern was found after electrophoresis under non-reducing conditions of a recombinant VWF that only contains A1-A2-A3 domains. The possibility that structural characteristics of leukocyte VWF would be due to the proteases derived from PMN was also considered and analyzed. Purified VWF was coincubated with PMN stimulated with 1 uM of fMLP in the presence of cytochalasin B (2.5 μg/mL) at 37°C for 10 min and western blot was performed as described above. Results showed that the exposure of VWF to proteases derived from PMN did not generate the VWF pattern of 80 and 30 kD observed in leukocyte VWF. In order to elucidate when VWF is incorporated to PMN surface, we analyzed its presence in PMN precursors (isolated from human bone marrow). Flow cytometry studies showed that VWF was completely absent in PMN precursors (22 ± 12 mean ± SEM of anti-VWF fluorescence, n = 4) as compared with circulating (mature) PMN (221 ± 28, mean ± SEM of anti-VWF fluorescence, n = 8, p < 0.01). Simultaneous determination of Mac-1 demonstrated that the receptor of VWF on PMN is expressed in precursors (285 ± 32 mean ± SEM of anti-CD11b fluorescence, n = 4) and circulating (567 ± 26 mean ± SEM of anti-CD11b fluorescence, n = 8) PMN. In conclusion, our observations indicate that the VWF present on PMN (1) has very low molecular weight, (2) could have a dimeric structure, (3) this structure is not due to the effect of proteases derived from PMN and (4) is incorporated to PMN after maturation, probably during the trans-endothelial migration from bone marrow to the circulation.
Induction of TNF-α, uPA, IL-8 and MCP-1 by Doxorubicin in Human Lung Carcinoma Cells

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Purpose: We previously demonstrated doxorubicin-induced urokinase (uPA), tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8) expression in human H69 small cell lung carcinoma (SCLC) cells by the microarray technique. Furthermore, the doxorubicin-induced macrophage chemoattractant protein-1 (MCP-1) expression was demonstrated by the RNase protection assay. We, therefore, extended the study by testing the effects of doxorubicin on the induction of uPA, TNF-α, IL-8 and MCP-1, in other types of lung carcinoma cells. Methods: We examined the effects of doxorubicin on the expression of uPA, TNF-α, IL-8 and MCP-1 in 12 human lung carcinoma cell lines, including 5 SCLC, 3 adenocarcinoma and 4 squamous cell carcinoma cells. Furthermore, the surface expression of their receptors was also examined. Results: uPA was significantly induced in 5 cell lines, H69, SBC-7, EBC-1 (squamous cell), EBC-2 (squamous cell), and Sq-1 (squamous cell), TNF-α in 3 cell lines, H69, SBC-7 (SCLC) and PC-9 (adenocarcinoma), IL-8 in 3 cell lines, H69, PC-9 and EBC-1, and MCP-1 in 5 cell lines, H69, SBC-3 (SCLC), SBC-7, PC-9 and Sq-1. In H69, uPA antigen levels were increased approximately 3-fold by doxorubicin treatment, in parallel with the increase of mRNA levels. Similarly to the case of TNF-α and IL-8, the maximum induction was observed at a 'sublethal' concentration such as 2 or 4 μM where cell-growth was slightly inhibited 24 h after treatment. Furthermore, the cells did not express receptors such as uPA receptor (uPAR), types I and II TNF-α receptors, C-X-C-chemokine receptor-1 (CXCR-1), or C-C-chemokine receptor-2, corresponding to uPA, TNF-α, IL-8 and MCP-1, respectively, that were induced by doxorubicin in the cells, although SBC-7 expressed uPAR, and EBC-1 expressed CXCR-1. Conclusions: uPA, TNF-α, IL-8 and MCP-1 induced and secreted from tumor cells upon doxorubicin stimulation may activate surrounding cells expressing those receptors such as neutrophils and monocytes/macrophages as in a paracrine fashion. TNF-α is a major proinflammatory cytokine, and IL-8 and MCP-1 are major chemoattractants for neutrophils and monocytes/macrophages, respectively. Furthermore, uPA activates matrix metalloproteinase-9 which can truncate and activate IL-8. Thus, the simultaneous induction of uPA, TNF-α, IL-8 and MCP-1 may enhance the interaction between tumor and inflammatory/immune cells, and augment cytotoxicity.

Plasmin-Induced Chemotaxis Requires Signaling Through Protease-Activated Receptor 1 and Integrin αβ1

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Plasmin is a major extracellular protease that transduces intracellular signals to mediate platelet aggregation, chemotaxis of peripheral blood monocytes, and release of arachidonate and leukotriene from several cell types in a G protein-dependent manner. We have previously reported that angiostatin, a fragment of plasminogen, is a ligand and an antagonist for integrin αβ1 (Tarui et al., 2001). Here we report that plasmin specifically interacts with integrin αβ1 using CHO cells expressing recombinant αβ1 (α9-CHO cells), and that plasmin induced migration of α9-CHO cells. We have found that anti-α9 antibody, anti-plasminogen kringle antibodies, angiostatin, and a serine protease inhibitor effectively blocks plasmin-induced chemotaxis of α9-CHO cells. These results suggest that this process requires the catalytic activity of plasmin, the kringle domains, and αβ1. We studied whether the cleavage of G-protein coupled protease-activated receptors (PARs) are involved in plasmin-induced αβ1-dependent signaling. Although angiostatin itself did not induce migration of α9-CHO cells, PAR-1 activating peptide induced migration on angiostatin, while PAR-2 or PAR-4 activating peptides were without effect. Notably, a small chemical inhibitor of PAR-1 (RWJ 58259) blocked the plasmin-induced migration of α9-CHO cells. These results suggest that plasmin-induced signaling requires both binding to αβ1 through its kringle domain and activation of PAR-1 by its catalytic activity.

Different Expression of Laminin-5 in Metastatic and Non-Metastatic Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third tumor death-related cause in the world. In European and North American countries HCC develops on cirrhotic liver, and its frequency is constantly increasing, mainly because of the growing spread of hepatitis C. The occurrence of metastasis is the main problem in patients with HCC, since it severely affects prognosis and survival. Laminin-5 (Lm-5), is an extra-cellular matrix component that promotes adhesion and migration, mainly present at the basement membrane (BM) levels in human tissues. Although Lm-5 has been shown to promote motility and scatter of epithelial rat liver cells, it has never been found in the liver. Recently, over-expression of Lm-5 has been implicated in the growth and diffusion of different epithelial malignancies. This study provides the first report that Lm-5 is present in the HCC primary nodule, but not in normal or peritumoral cirrhotic
tissues. In particular, the γ2 chain is strongly associated with the occurrence of metastasis (96%) and with a worse prognosis. In peritumoral tissues, Ln-5 has been detected along the advancing edge of the metastatic nodule, with the same pattern of expression as that observed in the primary lesion in the same patient. In conclusion, Ln-5 could promote HCC cell invasion due to different mechanisms including γ2 chain remodeling by matrix metalloproteases (MMPs), or migratory activity of this chain, as described by others. In addition, the role of the γ2 chain as an unfavorable prognostic factor, and as a diagnostic marker for detecting micrometastasis in surrounding peritumoral tissues could be an important finding. In this regard more studies are required to better define the biological and clinical role of Ln-5 in patients with HCC.

Risk Assessment of Thrombosis in Cancer (1)

P 27
Are D-Dimer and Residual Vein Thrombosis Predictive of Recurrence after a First Episode of Venous Thromboembolism in Cancer Patients?
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Background: Recently D-dimer and residual vein thrombosis (RVT) have been shown to be predictive of recurrence after VTE. Aim of the Study: To evaluate whether D-dimer and RVT are predictive of recurrent VTE in cancer patients after the withdrawal of OAT. Experimental Design: Prospective cohort study. Patients and Methods: Consecutive cancer patients who experienced a first episode of deep vein thrombosis (DVT) and/or pulmonary embolism (PE) were evaluated. DVT was objectively documented with compression ultrasound (CUS) and/or phlebography and PE was documented with pulmonary V/Q. All patients were treated with an initial course of heparin or low molecular heparin followed by OAT. Clinical and instrumental follow-up was performed at 3, 9, 15 and 21 months after OAT withdrawal. D-dimer and RVT on compression ultrasound were measured at 3 months after OAT withdrawal. Recurrent events were diagnosed by objective methods. Results: Eighty-seven patients (M/F: 36/51, mean age: 71; range: 34–88) were evaluated. The site of cancer was the prostate (10), breast (25), gastrointestinal (15), hematological (15), other (27). The mean duration of OAT was 18 months, (range 3–28). RVT was present in 66% of patients (58/87), while D-dimer was positive in 53% (46/87). Seventeen patients (19.5%) developed recurrent VTE during 106 patients/year follow-up after OAT withdrawal. D-dimer and RVT on compression ultrasound were measured at 3 months after OAT withdrawal. Recurrent events were diagnosed by objective methods. Results: Eighty-seven patients (M/F: 36/51, mean age: 71; range: 34–88) were evaluated. The site of cancer was the prostate (10), breast (25), gastrointestinal (15), hematological (15), other (27). The mean duration of OAT was 18 months, (range 3–28). RVT was present in 66% of patients (58/87), while D-dimer was positive in 53% (46/87). Seventeen patients (19.5%) developed recurrent VTE during 106 patients/year follow-up after OAT withdrawal. In patients with RVT, the rate of recurrence was 29.1% (14/48) when compared with 7.6% (3/39) in patients without RVT (p < 0.0145). In patients with a positive D-dimer the rate of recurrence was 30% (14/46) when compared with 7.3% (3/41) in patients with negative D-dimer (p < 0.001).

Conclusions: Residual vein thrombosis and D-dimer at 3 months after the withdrawal of OAT are predictive of recurrent VTE in patients with cancer.

P 26
Fibrinolytic Activity in Nigerian Breast Cancer Patients
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Introduction: Reduced blood fibrinolytic activity (FA) has been postulated in cancer but fibrinolytic role in Africans with breast cancer who are known to possess enhanced fibrinolysis is not clear. This study is designed to assess the effect of breast cancer on the fibrinolytic activity and the consequence of mastectomy in African women. Patients and Methods: Fifty breast cancer patients aged between 25 and 35 years were compared with 50 age-matched controls and 10 histologically proven breast cancer patients from surgical out-patients. Plasma fibrinogen levels (PFC) and euglobulin lysis time (ELT) were estimated with standard techniques. Results: Patients with breast cancer had significantly increased fibrinogen levels and ELT than the control group (p < 0.001). There was significantly mean difference between pre- and post-mastectomy fibrinogen and ELT (p < 0.05). Progressive significant decrease in fibrinogen levels and ELT were also observed over 5 weeks study respectively in post-mastectomised patients (p < 0.05). Conclusion: African women with breast cancer may have defective fibrin clearing system which may predispose them to thrombotic diathesis with increasing plasma fibrinogen concentration and early mastectomy may be beneficial.
Evidence of haemostatic activation has been demonstrated in cancer patients. Although chemotherapy has been reported to be a risk factor for venous thrombosis in breast cancer (tamoxifen) and in Multiple Myeloma (thalidomide), no strong evidences are present in other neoplasms. Some markers of thrombin activation, such as D-dimer (D-d) test has been found increased after chemotherapy for breast and lung cancer (Weitz et al., Thromb Haemost 2002;88:213–220), however, whether or not this marker can identify patients who develop venous thromboembolism (VTE), and therefore who may need appropriate antithrombotic prophylaxis, is still unclear. Therefore, no definitive conclusions are present on the role of coagulation markers for predicting VTE complications during chemotherapy. **Study Objective:** In order to investigate whether D-d variations can identify patients at high risk for VTE, we have assessed, in an ongoing clinical trial, D-d levels at any time during a follow-up of 3 months after the last cycle of chemotherapy.

**Materials and Methods:** Consecutive patients receiving adjunctive chemotherapy for colon neoplasm were considered eligible. D-d test was tested at the beginning and at the end of any cycle of chemotherapy. Standard chemotherapy consists in Campto (CPT11) 180mg/mq, and Fluorouracil (400 and 600mg/mq) for 2 days. D-d was provided by Dade Behring (D-dimer PLUS, turbidimetric methods, normal value <192 μL). D-d variation before and after chemotherapy was assessed by Wilcoxon test. Compression Ultrasonography (CUS) of the lower limbs was performed at the baseline visit (before the beginning of the 1st cycle of chemotherapy). Symptomatic thromboembolism (deep vein thrombosis and/or pulmonary embolism) was objectively evaluated at any time during a follow-up of 3 months after the last cycle of chemotherapy. **Results:** At the interim analysis, 29 patients were evaluated; D-d levels did not significantly vary between the beginning and at the end of chemotherapy (1st cycle p = 0.8, 2nd cycle p = 0.9, 3rd cycle p = 0.67, 4th cycle p = 0.79). One patient only (3.4%) developed symptomatic DVT between the 2nd and 3rd cycle of chemotherapy; at that time, D-d was 1,298 μL. **Conclusions:** Our study do not show a significant increase of D-d levels in patients on chemotherapy for cancer of the colon; its association to VTE complications is under way.
Case 1: A 47-year old woman with k-chain BJ-MM. At diagnosis she had anemia, massive plasma cell infiltration of bone marrow, multiple bone lytic lesions and renal failure. Total homocysteine plasma level (tHcy) was 140 μM/L (NV < 15). She showed a DVT of right femoral vein at the beginning of hemodialysis treatment. Chemotherapy, low-molecular-weight heparin in addition to hemodialysis induced a good partial remission of BJ-MM and a resolution of thrombosis and renal failure with a decrease of tHcy to 12.5 μM/L. She assumed also folic acid at the dose of 5 mg p.d. during the whole treatment. Screening of mutations of MTHFR gene identified a homozygous C677T mutation. Case 2: A 55-year old woman with BJ-MM light chain k. At the diagnosis she had a creatinine value of 5.9 mg/dl, BUN 169 mg/dl, 24h proteinuria 3.5 g. Treated with chemotherapy, she was unresponsive and she could not undergo an autologous transplantation for a bilateral wide obstruction of subclavian veins up to brachiocephalic trunks that did not allow the insertion of central venous catheter. The patient started the hemodialysis for a worsening of the renal function with a tHcy value of 124 μM/L which remained high during hemodialysis in spite of assumption of folic acid at the dose of 15 mg p.d. Two heterozygous mutations C677T and A1298C were identified in the MTHFR gene. Both patients had normal plasma value of folate and vitamin B12. Our cases seems to demonstrate that mutations of MTHFR gene may be considered as a genetic predisposition for venous thrombosis but that probably a single cause responsible for this condition does not exist and that it should be thought as a complex multifactorial trait not yet completely understood.

P 31
Factor V Leiden Mutation and the Risk of Subclavian Vein Thrombosis in Patients with Breast Cancer and a Central Venous Catheter
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Background: One of the major complications of central venous catheters (CVC) is thrombosis of the subclavian vein and, in approximately one-third of DVT cases, pulmonary embolism. Even though the association between cancer and hypercoagulability is well established, the pathogenesis of VTE in these patients is not entirely elucidated. The aim of this study was to analyze the influence of the prothrombotic gene mutation factor V G1691A (factor V Leiden) and prothrombin G20210A on the risk of a first episode of catheter-related deep vein thrombosis (DVT) in a group of patients with breast cancer treated with chemotherapy. Methods: Between January 1999 and February 2001, the occurrence of a first symptomatic DVT was investigated in a cohort of 300 consecutive patients with locally advanced or metastatic breast cancer treated at a single institution with fluorouracil-based chemotherapy administered continuously through a totally implanted access port. A nested case-control study included 25 women (cases) with catheter-related DVT and 50 controls without DVT matched with cases for age, identical chemotherapy, stage of disease and prognostic features. The G1691A factor V and G20210A prothrombin mutation genotypes were analyzed. Results: Five cases (20% [95% CI: 9–39%]) and 2 controls (4% [95% CI: 1–14%]) were heterozygous carriers of G1691A factor V (p = 0.04). The age-adjusted odds ratio for catheter-related DVT was 6.1 (95% CI = 1.1–34.3). Only one patient (case) had the G20210A prothrombin gene mutation. Time from start of chemotherapy infusion to DVT was not significantly different between patients with (median 31 days) and without G1691A factor V mutation (median 43 days, p = 0.6). Conclusion: Factor V Leiden carriers with locally advanced or metastatic breast cancer have an increased risk of developing catheter-related DVT during chemotherapy.
Thrombosis is the most frequent complication and the second cause of death in patients with malignant disease. Primary central nervous system non-Hodgkin’s lymphoma represent a rare pathology. Recently, a new mechanism of hereditary thrombophilia characterized by a poor response to activated protein C (APC-resistance) has been identified. Resistance to APC is usually linked to a factor V (FV) gene mutation changing an Arg 506 to a Gln in the APC cleavage site. In our study, we aimed at investigating the presence of APC-resistance and other markers of hypercoagulability in 25 selected patients with a diagnosis of primitive cerebral lymphoma who had suffered from an ischemic episode of TIA and/or stroke. Fifty healthy subjects acted as a control group. We measured APC resistance, natural clotting inhibitors, (F1+2) and PAI-1 according to international guidelines. Genomic DNA was extracted from peripheral white blood cells and a PCR was performed to amplify genomic exon 10/intron 10 region of FV gene to search for Arg 506 to Gln point mutation. Our results showed that 11 out of 25 patients had a poor response to APC (<0.70, which represents the cut-off point in our general population) without deficiencies in natural clotting inhibitors. All patients had high plasma levels of F1+2 and PAI-1 compared to those found in healthy subjects (2.65 ± 0.75 nM vs. 0.40 ± 0.35 nM; 67.5 ± 18.5 ng/mL vs. 17 ± 11.5 ng/mL, respectively). In 9 patients resistance to APC was not associated to a FV gene defect demonstrating that such phenomenon may occur also as an acquired condition. However, the patients with resistance to APC showed the highest plasma values in F1+2 and PAI-1. In cerebral lymphoma with hypercoagulability the resistance to APC is not caused by the FV Arg 506 → Gln mutation (82%). APC resistance not caused by this FV gene defect may be an additional risk factor for thrombophilia in this selected population.

Malignancies of various types may be associated with venous thromboembolism, and there are evidences showing that a significant subset of patients who suffer from malignancy-associated thrombosis have elevated aPL. Aim of this study is to investigate a possible association between aPL [lupus anticoagulant (LA), anticardiolipin antibodies (aCL), anti β2-glycoprotein I antibodies (aβ2gpI) and anti protrombin antibodies (aPT)] and thromboembolic events in patients with cancer. Fifty-four patients with solid tumors or hematological malignancies have been enrolled in this study. Plasma and sera samples from 27 subjects with objectively diagnosed venous thrombosis (group 1) [19 females and 8 males (mean age: 62 ± 11)], and 27 sex and age matched cancer patients without a symptomatic thrombotic event (group 2) were screened for: LA, IgG and IgM aCL, IgG and IgM aβ2gpI, IgG and IgM aPT. Eleven out of 27 (40.7%) patients of group 1 were found to be positive for LA vs 6 out of 27 (22.2%) subjects of group 2 (p = n.s.). Prevalence of aCL, aβ2gpI and aPT were found to be not statistically different in the two groups of patients investigated. In conclusion we observed a higher prevalence of LA in cancer patients with venous thrombosis than in control subjects even if the prevalence was not statistically significant. These findings suggest a possible association between LA and venous thrombosis in cancer, future prospective studies are needed to address the clinical relevance of LA and to analyze its role in predicting thrombosis in cancer patients.
a marker of thrombin generation. Statistical analysis was performed with Student’s t-test for paired data. Differences were considered to be significant if p < 0.05. Seventeen patients of the study group showed a hypercoagulable state testified by increased levels of F1 + 2 compared to only two patients of the control group (3.25 ± 1.01 vs 0.78 ± 0.23 nM). Among coagulation inhibitors, the levels of free protein S were strongly decreased in cancer patients compared to the control group (68 ± 10 vs 107 ± 24%, p: 0.01). All the patients had normal liver function. This study suggests that the hypercoagulable state of these patients receiving a chemo-hormone therapy may be at least in part explained by the reduced levels of protein S. The mechanism by which this abnormality is produced needs to be further investigated.

**Study of Genetic Risk Factors in Oncology Associated Thrombophilia**

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**Introduction:** Since tumour cells activate in a number of ways the haemostatic system, cancer is a pro-thrombotic condition. In addition to the neoplasia, therapeutic procedures per se are also risk factors predisposing to thrombosis. Despite the extensive studies involving numerous coagulation activation markers, none of these markers has shown to have predictive value for clinical expression of thrombosis in oncology patients. The numerous genetic risk factors identified in recent years constitute potentially useful markers whose value needs to be clarified. **Aim:** To establish the prevalence of genetic risk factors in a set of 97 oncology patients and evaluate its usefulness as a predictive risk factor for thrombosis. 

**Materials and Methods:** Of the 97 randomly selected oncology patients studied, 48 originated from the hospital hipocoaagulation clinic and had a history of at least one thrombotic event. The remaining 49 patients (control group) had no history of thrombosis. Prothrombin G20210A, Factor V Leiden, PAI-1 4G/5G, MTHFR C677T and HPA 1a/1b were assayed by Real-Time PCR. Results were assessed by SPSS statistical software package.

**Results:** Patients’ age average was 59 years (26-78 years) in the thrombosis group, and 56 years (16-88 years) in the control group. Of the patients studied 50% were females (24 in the thrombosis and 25 in the control group). Significantly more solid tumour cases were found in the thrombosis group in comparison with the controls. None of the individual mutations was found to be more frequent in either the thrombosis or the control group. However, after calculating a relative genetic risk score, incorporating all mutations studied, we observed a significantly (p < 0.034) higher frequency of increased scores in the thrombosis group. Only two patients with high scores without thrombosis were found and both had liquid tumours.

**Conclusion:** Genetic risk factors, if considered as a panel of genetic assays may have a predictive value for thrombosis in oncology patients, particularly if considered in the context of the remaining risk factors.

**Risk Assessment of Thrombosis in Cancer (2)**

**P 37 Evaluation of Asymptomatic Deep Vein Thrombosis by Doppler Sonography in Lung Cancer and Lymphoma Patients Undergoing Chemotherapy**

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**Background:** Cancer patients undergoing chemotherapy are at increased risk for developing venous thromboembolism. The purpose of this prospective study was to determine the frequency of asymptomatic deep vein thrombosis (DVT) in lung cancer and lymphoma patients undergoing chemotherapy, using Doppler sonography of lower extremities. An attempt was also made to correlate between the presence of asymptomatic DVT to activated protein C (APC) resistance and D-dimer levels. **Patients and Methods:** Patients with diagnosis of lung cancer [small cell (SCLC) or non-small cell (NSCLC)] or lymphoma [Hodgkin’s disease (HD) or non-Hodgkin’s lymphoma (NHL)] and active disease, undergoing chemotherapy, were eligible if they fulfilled the following criteria: age <75 years, WHO performance status 0–2, no evidence for current or prior venous or arterial thromboembolism and no anticoagulant therapy. All patients underwent Ultrasound Doppler of the lower limbs. Coagulation tests included evaluation of D-dimer levels and APC-sensitivity ratio in 61 patients compared to 30 controls without previous history of malignancy or thromboembolism. **Results:** Thirty-two patients with lung cancer (median age 56 years, 28 with NSCLC and 4 with SCLC) and 30 with lymphoma (median age 59 years, 22 with NHL and 8 with HD) were investigated. Sonographic evidence of lower limb DVT was not found in any of these patients. Forty-four patients (75%) had D-dimer levels higher than cut-off value of 0.5 mg/l (median: 0.72, range: 0.22–20 mg/l). Mean APC-sensitivity ratio was lower in patients compared to controls (2.03 ± 0.28 vs. 2.82 ± 0.55, p < 0.001). **Conclusion:** Although lung cancer and lymphoma patients undergoing chemotherapy demonstrated a prothrombotic state, asymptomatic DVT of the lower extremities was not detected by Doppler sonography in this population.
Risk of Thrombosis in Lung Cancer
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Introduction: Previous data showed an increase in the prevalence of Deep Venous Thrombosis (DVT) in cancer patients, especially when they underwent a chemotherapy regimen. Aim of our study was to evaluate the role of prothrombotic factors that could explain the occurrence of DVT in lung cancer patients. Patients and Methods: We have studied 186 patients (173 males, 13 females; mean age 63 ± 10.2, range 37–89 years), affected by lung cancer (Non Small Cell 159, Small Cell 27), admitted in the Internal Medicine Department from January 2000 to May 2003, investigated before they were treated with a standard chemotherapy regimen, containing platin. DVT was found in 11 patients (5.91%). In these patients, Vascular US and a complete hemocoagulative assessment were performed (PT, aPTT, lupus anti-coagulant, Ab anti-cardiolipin, homocysteine, anti-thrombin, protein C and S). They were also submitted to a genetic study of some polymorphism (factor V Leiden, FII G-A20210, MTHFR genotype). Statistical analysis was performed by SPSS package v.11. Parametric and non-parametric test were used when appropriated. A p level less than 5% was accepted as significant. Results and Conclusions: No statistical difference was found for protein C and S levels, ATIII, genetic polymorphism (factor V Leiden, FII G-A20210 and MTHFR) between normals and DVT patients. The role of prothrombotic factors in DVT prevalence among cancer patient, before they were submitted to chemotherapy, is still questionable. Further studies will be necessary to better define the role of procoagulative pattern in these patients.

Incidence of Thrombosis in Gastro-Oesophageal Cancer: A Cohort Study of 649 Patients
LUMC Department of Clinical Oncology, Leiden, The Netherlands

Background: Patients with cancer have a 3- to 7-fold higher risk of developing venous thrombosis (VT). Of the various histological types of cancer, adenocarcinoma is believed to be associated with the highest risk of developing VT. However, limited data are available on the incidence of thrombosis in the different histological types of malignancy. To study the difference in incidence of VT between adenoc- and squamous carcinoma, a retrospective study was undertaken in patients with these two histological cancer types originating from the upper digestive tract. Oesophageal carcinoma comprises both aden- and squamous carcinoma, whereas stomach carcinoma only comprises adenocarcinoma. Patients and Methods: A total of 471 consecutive oesophageal cancer patients (178 aden- and 293 squamous carcinoma patients treated between 1980 and 1998) and 178 consecutive gastric adenocarcinoma patients (treated between 1990 and 1998) were studied. Information about thrombotic events occurring at the time of diagnosis was collected from medical records and the regional anticoagulation clinics. Results: The median follow-up from time of diagnosis in 649 patients until death or last follow-up was 9 months (range 1 wk to 18 yr). Thirty (6%) of the 471 oesophageal carcinoma patients and 23 (13%) of 178 patients with gastric adenocarcinoma developed thrombosis. In patients with oesophageal cancer, the risk to develop VT was higher in patients with aden- compared to squamous cancer (OR = 1.6; 95% CI 0.8–3.4). An increased risk for VT was also found in patients with adenocarcinoma of the stomach compared to patients with adenocarcinoma of the oesophagus (OR = 1.6; 95% CI 0.8–3.2). The risk for VT in gastric adenocarcinoma patients was increased when compared to all oesophageal cancers patients (OR = 2.1; 95% CI 1.2–3.8). Conclusions: Our data indicate that the risk for VT is indeed increased in patients with adenocarcinoma. The increased risk for VT in gastric versus oesophageal adenocarcinoma patients indicates that in addition to histological cancer type, other factors, e.g. (chemo)therapy, play a role in the development of VT.

Incidence of Thrombosis in Gastro-Oesophageal Cancer: A Cohort Study of 649 Patients
LUMC Department of Clinical Oncology, Leiden, The Netherlands

Introduction: A link between thrombosis and solid tumors is well documented as well as between thrombosis and chemoradiotherapy for some of them. Less data are available about thrombosis, non-Hodgkin’s lymphomas and chemotherapy for such diseases. Methods: We report on a 44-year-old woman who arrived to our attention for a mediastinal syndrome evident since February 2003. Oedema of the face and bilateral mild laterocervical lymphadenomegaly were present. Emocromocytometric values were as follows: WBC 11.800/μL, N 80%; Hb 11.8 g/dL, Plts 465,000/μL; moreover PT was 110%, PTT 411, fibrinogen 478 mg%, ESR 39 mm, and plasma proteins, plasmatic LDH, serum β2-microglobulin values were normal. CT scan performed at presentation, on March 7, evidenced a 7-cm lymph nodal enlargement of the antero-superior mediastinum, with superior vena cava compression. CT scan of the abdomen was normal. After two fruitless laterocervical lymph nodes biopsies, mediastinoscopy was performed and diagnosis was made of mediastinal B large cell lymphoma with sclerosis on April 9th. Chemotherapy was suddenly started, according to the weekly VACOP-B schedule. Results: After four weeks, together with an initial reduction of the facial oedema, the patient experienced a superficial thrombophlebitis of the left arm. LMWH at therapeutic dosage was given and a new CT scan of the thorax was performed on May 15, documenting the disappearance of the mediastinal mass but
the presence of a right internal jugular vein thrombosis extending to both brachiocephalic trunks and distally to the azygos vein. Routine coagulative tests confirmed the presence of a hypercoagulative state (PT 120%, PTT 38%); evaluation for an acquired or congenital abnormality was performed (in progress). Moreover, search for the presence of anti-heparin antibodies was done, although a normal platelet count and above all the timing of symptoms related to thrombosis, which pre-existed to heparin administration, made HIT type 2 unlikely. The negative result of test strengthened us to continuing full heparinization. **Conclusion:** This case offers matter of discussion about the clinical management of patients affected by non-Hodgkin’s lymphoma undergoing weekly chemotherapy; maybe an effort to correctly assess the risk for venous thromboembolism in these patients is needed.

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**P 41**

The Incidence and Time Course of Venous Thromboembolism Among Californians with Pancreatic Cancer

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**Background:** It is widely accepted that venous thromboembolism (VTE), which includes both deep venous thrombosis (DVT) and pulmonary embolism (PE), is a frequent complication of pancreatic adenocarcinoma (PCa). However, little data exist regarding the incidence, time course, and risk factors associated with VTE in these patients. **Methods:** Data from the California Cancer Registry and the California Hospital Discharge Data Set were merged. We used previously validated ICD-9-CM codes for lower extremity DVT and PE to identify cases of VTE. VTE were categorized as occurring prior to, concurrent or after the diagnosis of PCa. Cases were identified from 1991–1995, a period in which most patients with VTE were hospitalized. The expected number of VTE cases in 1 year was calculated based on the incidence of VTE among Californians in 1996, adjusted for ethnicity, sex and age. **Results:** 8,246 cases were identified. The median age was 72 (range 3–101); 50.1% female; 74.5% Caucasian, 8% African-American, 11.1% Hispanic, and 6.2% Asian. The staging was 7.7% localized, 28.3% regional, and 43.2% with metastatic disease (the remainder were not abstracted). The incidence of VTE within 1 year prior to the diagnosis of pancreas cancer was 0.3%, with a standardized incidence ratio = 0.81 (CI = 0.61–1.0). However, in 1.5% (CI = 1.2–1.8%) the cancer diagnosis was concurrent with VTE. The 1-year survival was 17.5%. During the year after diagnosis the Kaplan-Meier cumulative incidence of VTE (concurrent and after) was 9.3%. In a proportional hazards model, development of VTE within 1 year after diagnosis was significantly associated with age, HR = 0.96 for each 5 year age increment (CI = 0.93–0.99); Asian ethnicity, HR = 0.5 (CI = 0.3–0.8) versus Caucasian; and metastatic disease, HR = 3.0 (CI = 2.5–3.6) versus regional. **Conclusions:** The incidence of acute VTE in the year prior to the diagnosis of pancreatic CA was not significantly different than expected in people without pancreatic CA. The risk of VTE in the year after diagnosis of pancreas cancer was 9.3%. Higher stage was associated with a greater risk of developing VTE, whereas Asian ethnicity was associated with significantly lower odds.

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Cancer is a well known acquired thrombophilic condition and thrombosis can develop in early as well as late stages of the disease. The upper extremity deep vein thrombosis (UEDVT) is less frequent than the lower extremity DVT (LEDVT), however in the last years anti-cancer therapies and their routes and modalities of administration have apparently increased UEDVT. Implantation of central venous catheter (CVC) or port-a-cath (PAC), a common practice during prolonged chemotherapy, can also contribute to increase UEDVT. However, jugular thrombosis (JT) remains very rare. Moreover, usually JT could be divided in spontaneous and non-spontaneous and overall in localized JT and extended JT (from axillo-subclavian vein). We collected 12 cases of JT diagnosed at our Institute. In all patients JT had a relation with cancer, in particular two patients had a newly diagnosed cancer and 10 patients were undergoing anti-cancer therapy. Of the two patients with new cancer diagnosis, one had a spontaneous, localized JT, and one had extended JT (one as a first sign of lung cancer and one as a first sign of non-Hodgkin’s lymphoma (NHL). All of the 10 patients with JT during cancer therapy were bearing CVC or PAC (2 multiple myeloma, 1 Waldenström’s disease, 1 peritoneal metastasis from ovarian cancer, 1 ganglioneuroblastoma, 2 gastric cancer, 3 NHL). Additional thrombotic risk factors in the patients described were: bacteremia, prolonged bed rest, surgical treatment. Inherited thrombophilia was searched only in 4 patients, which resulted negative. CVC or PAC were in place for a variable period from 1 week to 3 months. JT was confirmed in all cases by ultrasound examination and in most cases also by CT scan. Pharmacological therapy was immediately started with LMWH followed by oral anticoagulation. Surgical treatment was needed only for one patient. No pulmonary embolism was recorded. Only one patient had a major bleeding (melena) during antithrombotic therapy. In conclusion, JT can be often observed in patients with cancer, particularly if CVC or PAC are placed for prolonged chemotherapy. This condition seems to be related to artificial surface of CVC or PAC. Additional thrombotic risk factors may be present (i.e. prolonged bed rest, surgery, bacteremia) and favor the thrombotic manifestation. Patients with UEDVT are at high risk of pulmonary embolism as reported by several authors, although this did not occur to our patients, who all had a good outcome, probably due to early diagnosis and management of JT. Further studies are warranted to improve our knowledge of diagnosis and management of JT in cancer, and understand the best modalities of thromboprophylaxis in patients with CVC or PAC.
P 43
Leiomyosarcoma of the Inferior Cava Vein: Description of New Case
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Introduction: The primary leiomyosarcoma of the cava vein is rare. Fewer than 100 cases were registered until today. It develops in the muscle tissue of the blood vessel. Clinical Case: 50-year-old female patient acute epigastric pain and oedema of the lower limbs confirmed by physical examination, in August 1995. An echography, abdominal TAC and cavangiography are performed suspecting a retroperitoneal tumor with a thrombosis of the inferior cava vein. Anticoagulant treatment is started with low molecular weight heparin (calcic nadroparin 15,000 UAXa each/12h) until the surgical resection of the tumor and of a fragment of the cava vein, which was affected above the renal hilum; it was a capsulated tumor of 11.2 x 5.7 x 6.4 cm, located in the retrohepatic vein. Post surgical radiotherapy is applied. 4,000 rads, 4 weeks, 200 rads per day in 2 fields anterior and posterior. Patient died by bone dissemination 12 months later. Conclusions: (1) It is an unfrequent neoplasia difficult to diagnose with a thrombotic component; (2) The quick metastatic dissemination described above occurs in more than 35% of the cases with local recurrences.

P 44
Short Term Assessment of Haemostatic Factors after Chemotherapy for Lung Cancer
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Thromboembolism is a known complication of cancer and there is general agreement that chemotherapy (CHT) potentiates the risk. No specific alteration in haemostatic components has been firmly demonstrated to play a role in predicting thrombosis in cancer patients. We prospectively assessed whether early haemostatic alterations may represent risk factors for thrombosis in patients undergoing CHT for lung cancer. Forty-nine patients (41 males, 8 females; mean age 63 yr, range 43–79 yr) with unresectable locally advanced or metastatic lung cancer (8 small-cell carcinomas; 41 non-small-cell carcinomas) were included. Patients were treated with platinum-based doublets (n = 41) or gemcitabine alone (n = 8) for 6 cycles. Common acquired thrombotic risk factors were recorded and genetic analysis for FVL and FII A20210G mutations was performed in all cases. A concomitant APS syndrome was excluded. The following parameters were measured at day 0, +7, +15, +21 of first CHT cycle: blood cells count, PT, PTT, fibrinogen (Fg), ATIII, D-dimers, PC, PS, homocysteine, folates, vitamin B12, APC-resistance (APCR). Patients were followed prospectively for thrombotic events during all CHT treatment. FVL and FII A20210G heterozygotes were 8.1% and 6.1% respectively. Average basal levels of PT, PTT, ATIII, D-dimers, PC, PS, folates, B12, APCR were in normal range and remained stable during CHT. Homocysteine and Fg basal levels were high (22 ± 12 μM/L and 547 ± 193 mg/dL respectively) but remained constant following CHT. An average reduction of platelet count was recorded at day +14 (148 ± 107 x 10^9/L) followed by a striking increase at day +21 (518 ± 270 x 10^9/L) (p < 0.001). Three early thrombotic events (one in a FVL carrier) and a single late event, were recorded. All events occurred in patients treated with platinum; three patients had adenocarcinoma and one had squamous cell carcinoma. Our findings exclude alterations of coagulation inhibitors (or activation of DIC/fibrinolysis) as factors favouring early thrombosis after CHT for lung cancer. A potential role of the remarkable increase in platelets count observed before the second CHT cycle, is suggested.

P 45
Age but not Malignancy as Modulator of the Protein C Pathway Activity in Surgical Cancer Patients
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Introduction: Various reasons for tumor-associated hypercoagulability have been postulated, one being an acquired resistance to activated protein C (APC-R). Our aim was to investigate consecutive surgical cancer and non-cancer patients preoperatively for an impairment of the protein C pathway with respect to diagnosis, age and extent of fibrin formation. Patients and Methods: Patients with FV based APC-R were excluded. In 273 evaluable patients, the protein C pathway was evaluated through activation of the patients endogenous protein C (ProC Global on a BCS analyzer, Dade Behring; results (‘PCG’) indicate normalized ratio to known control, reference range >0.8). Fibrin generation was evaluated with a fibrin monomer assay (Enzymun FM, reference value <3.6 μg/L, on ESI300, Roche Diagnostics). Results: A PCG <0.8 was not more frequent in cancer than non-cancer patients (c2 = 0.042, p = 0.838) and median PCG was not different either (median 0.89 both groups, p = 0.342). Cancer patients were older than non-cancer patients (median age 64 vs. 57 years, p < 0.001); patients >60 years had a higher probability of having malignancy (OR 2.45, 95% CI 1.37–4.40). Patients with malignancy had increased FM (OR for FM >3.6 μg/L 3.49, 95% CI 1.44–8.44; median FM 4.58 vs. 3.13 μg/L, p = 0.004). In a multiple logistic regression analysis including age, gender and diagnosis of malignancy, only age >60 years was a significant predictor of a PCG < 0.8 (OR 2.45, 95% CI 1.37–4.40) or increased FM (OR 5.09, 95% CI 3.01–8.61). These results held also true when subgroup analysis between cancer patients and non-cancer patients with low comorbidity (hernia repairs) were performed. In a multiple regression analysis, an inflammatory response (CRP) was not a significant predictor of an abnormal PCG, neither in cancer patients nor in non-cancer patients. Discussion: It is well known that the coagulation system is progressively activated with increasing age. The overall cancer risk is also time and therefore age dependent and comorbidity is an important prognostic factor. We thus evaluated whether there was a relationship between PCG, fibrin generation and age comparing cancer patients and overall non-cancer patients as well as a subgroup of non-cancer patients with low comorbidity. Our results suggest that age is an important (and probably underestimated) modulator of

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fibrin generation in cancer patients. It seems that impairment of the protein C pathway in cancer patients is a function of age rather than the presence of a malignancy and that cancer patients per se have a similar function of the protein C pathway upon activation of endogenous protein C compared to non-cancer patients. This suggests that therapeutic interventions to the protein C pathway might be similarly suited in non-cancer as well as in cancer patients. Verification of these results by other investigators would be desirable.

P 46

Patients Undergoing Primary Resection of Gastrointestinal Cancers Show Increased Intraoperative Fibrin Generation when Compared to Patients with Low Risk of Postoperative Venous Thromboembolism

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Background: Patients with gastrointestinal (GI) cancers have high fibrinogen concentrations and a high incidence of postoperative venous thromboembolism. However, it is unknown whether these patients have increased intraoperative fibrin generation (which might contribute to the increased incidence of postoperative venous thromboembolism). Objectives: To evaluate whether GI cancer patients have increased perioperative fibrinogen levels; have higher intraoperative fibrinogen consumption; and have higher intraoperative fibrin formation in comparison to non-cancer patients with low risk for postoperative venous thromboembolism.

Patients/Methods: Case control study in 29 patients undergoing surgery for colon, stomach and esophageal cancer matched with 29 patients undergoing spinal fusion surgery (a patient population known to carry a low risk for postoperative venous thromboembolism). Conclusions: The study suggests that in cancer a pathogenesis of neoplastic thrombophilia.

P 47

Prevalence of Anticardiolipin and Anti\beta2-Glycoprotein-1 Antibodies in Patients with Solid Tumors

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Antiphospholipid antibodies (aPA) are IgG, IgM or IgA that occur as a result of autoimmune disease or as a reaction to infections or drugs. Moreover aPA has been found in a large variety of malignancies (anticardiolipin antibodies, aCLA), but estimates of their frequency vary considerably. Objective: To investigate patients with cancer for the frequency and the plasma levels of some aPA to define their role in the pathogenesis of neoplastic thrombophilia. Methods: AntiPA screen IgG and IgM, aCLA IgG, IgM and IgA, and anti-beta2-glycoprotein-1 antibodies (a\beta2-GP1A) IgG, IgM and IgA, were evaluated in 130 cancer patients (70 men and 60 women, mean age 68 years) with gastrointestinal or pelvic solid tumors and in 84 control subjects matched for age and sex. On the basis of TNM staging, all patients were included in T1, N0,2, M0 stages. All antiphospholipid antibodies were determined by ELISA (Orgentec Diagnostika GmbH-Bouty).

Results: Statistically higher mean levels, although within the normal range, of both IgM and IgA aCLA and IgA were found in patients compared to controls (p < 0.01). Statistically higher frequencies of positivity of all aPA screen, aCLA and a\beta2-GP1A isotypes in patients compared to controls were also found (p < 0.01).

Conclusions: In our group of cancer patients there is a significant increase in the frequency of all examined antiphospholipid antibodies isotypes, specifically of a\beta2-GP1A, which are associated with thrombotic events even if their levels are slightly above the normal range. The study suggests that in cancer a\beta2-GP1A can act as a risk factor for thrombosis in a thrombophilic context.

Hemostatic Imbalance in Solid Tumors and Leukemias

P 48

Acceleration of Tumor Growth and Peri-Tumoral Blood Clotting by Imatinib Mesylate (Gleevec)

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Imatinib mesylate (Gleevec) inhibits the BCR-ABL tyrosine kinase in chronic granulocytic leukemia. Previous studies have demonstrated
that Gleevec also inhibits the survival and functions of normal mast cells by interfering with the receptor tyrosine kinase for stem cell factor (SCF), c-kit, that is expressed by mast cells. Because mast cells extensively surround many types of cancer and contain powerful anticoagulants such as heparin, we investigated the effects of Gleevec on blood clotting and tumor growth within subcutaneous implants of a mammary adenocarcinoma cell line (4T1) in Balb/c mice. After five days of oral treatment with 10 mg/kg of the drug, the average mass of the tumors in treated mice (198 ± 42 mg, n = 5) was significantly (p < 0.05) greater than the average mass of the tumors from untreated (control) mice (60 ± 23 mg, n = 5). Moreover, the tumors in the treated mice were frequently surrounded by large lakes of clotted blood that were not evident in tumors from the control mice. Accelerated growth and blood clotting were also observed in tumor-bearing mice treated with heparinase I enzyme to destroy endogenous mast cell heparin and in NDST-2 knockout mice in which there is a targeted disruption in the gene coding for mast cell heparin synthesis. We conclude that Gleevec accelerated the growth and peri-tumoral blood clotting of implants of mammary adenocarcinoma in mice. These results suggest that Gleevec may have significant effects on mast cells infiltrating tumors, in addition to its other biological activities. Our results also indicate that the mechanism of this effect may be related to the anticoagulant properties of mast cell heparin.

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P 49  
Thrombotic and Hemorrhagic Complications of Prostate Cancer: Challenges in Diagnosis and Treatment  
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Thrombotic and hemorrhagic complications associated with prostatic adenocarcinoma have been observed for almost 3/4 of a century. The expression of tissue factor and other procoagulants by prostate cancer cells, estrogen therapies, prolonged immobilization secondary to painful bone metastases and the presence of pelvic/retroperitoneal lymphadenopathy may all contribute to thrombotic events seen in these patients. Hereditary and acquired hypercoagulable states such as Factor V Leiden and prothrombin gene mutations may also predispose to increased risk of clotting. Bleeding diatheses in patients with prostate cancer are also well known. Urinary tract bleeding from direct invasion of the bladder by prostate cancer is not unusual and can be difficult to manage when patients are receiving anticoagulation or when they are otherwise coagulopathic. Thrombocytopenia in patients with advanced prostate cancer is common as a result of marrow invasion, radiation therapy, chemotherapy and disseminated intravascular coagulation (DIC). DIC is usually subclinical but may present as a dramatic bleeding when there is an excessive fibrinolytic response. This coagulopathy more commonly occurs late in the course of the disease, often after invasive procedures or biopsies, but it also has been reported as the presenting symptom. Despite these clinical observations, the true incidence of thrombotic and hemorrhagic complications in this disease is not known. While the treatment of thrombotic events incident to this and other cancers remains under intense investigation, there are few studies to guide clinicians in the management of prostate cancer with bleeding due to DIC or fibrinolysis. Anecdotal reports, involving small numbers of patients, have advocated a variety of therapies including androgen deprivation, chemotherapy and systemic radioisotopes in addition to clotting factors, platelet transfusions and, where fibrinolysis predominates, epsilon aminocaproic acid. Further studies are clearly needed to define the best treatment for this devastating complication.

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P 50  
Hemostatic Balance on Leukemic Cells Surface: Role of TF and uPAR  
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Introduction and Aims: The prevalence of thrombotic complications is increased in acute leukemia. As coagulation processes take place on cell membranes, we hypothesized that expression of coagulation proteins on blasts membrane determine the hemostatic balance on leukemic cell surface and may correlate with thrombotic manifestations. Methods: The study included 51 consecutive patients enrolled between 1/2002 and 11/2002, with newly diagnosed acute leukemia (25 AML-M0–2, 11 AML-M2, 6 AML-M4–5, 9 ALL). After obtaining informed consent, peripheral blood and/or bone marrow aspirate were drawn. The buffy coat was detected by flow-cytometry and the blast cells were analyzed by size, granularity and specific monoclonal antibodies. Hemostatic proteins tested included: tissue factor (TF), protease-activated receptor 1 (PAR-1), tissue factor pathway inhibitor (TFPI), urokinase plasminogen activator receptor (uPAR) and thrombomodulin (TM). Results: Thrombotic manifestations were present in 13/51 (26%) patients: In AML-M0–2 6/25 (24%) — mild to moderate in severity. In AML-M2 7/11 (64%) — severe, and none in the patients with AML-M4–5 or ALL. Thrombotic complications neither correlated with prothrombin fragment 1+2 nor with D-Dimer levels at presentation. TF was predominantly present on leukemic blasts surface (mean 46 ± 4%) as compared to PAR-1, TFPI, uPAR, TM (mean 14 ± 2%, 7 ± 2%, 21 ± 3%, 9 ± 2%, respectively; p < 0.001). In AML-M2 and AML-M4–5 TF was significantly elevated (mean 63 ± 6%) as compared to that in AML-M0–2 and ALL (mean 37 ± 4%, p < 0.001). In AML-M4–5 uPAR was also significantly elevated (mean 49 ± 11%) as compared to that in AML-M0–2, M2 and ALL (mean 17 ± 3%; p < 0.001). Conclusions: TF predominates on leukemic blasts surface, particularly in the M1 and M4–5 subtypes and uPAR is increased on M4–5 blasts. Hemostatic balance is present on blasts surface and may predict thrombotic manifestations in leukemic patients.
P 51
The Use of ‘Protein C’ in Allogeneic Bone Marrow Transplantation Patients
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Background: The use of Protein C concentrate is effective in patients with purpura fulminans, septicemia and disseminated intravascular coagulopathy (DIC), as reported by several studies.

Materials and Methods: We tested the use of Protein C concentrate (Ceprotin, Baxter) after allogeneic bone marrow transplantation in two patients grafted for Fisher-Evans syndrome and non-Hodgkin’s Lymphoma who developed DIC syndrome and sepsis respectively.

Results: One patient developed a DIC syndrome after pneumonia and septic shock on day +200 after transplant, was treated with Protein C at the dose of 100 UI/Kg daily for 7 days, ATIII and heparin with complete resolution of coagulopathy, sepsis and pneumonia. The second patient developed pseudomonas aeruginosa sepsis with pneumonia, increased bilirubin levels (4 mg/dl), low levels of ATIII and Protein C, and normal value of APTT, fibrinogenemia and D-Dimer on day +10 after transplant. The patient was treated with Protein C at the dose of 50 UI/Kg daily for 5 days and ATIII at the same dose, with complete resolution of pneumonia (normal chest X ray in few days), sepsis, normalization of bilirubin level, and mild increase of platelets count. Conclusions: The use of Protein C can be useful in sepsis after allogeneic bone marrow transplantation, also during the neutropenic period. Based on these preliminary results we started monitoring the Protein C level during the conditioning regimen and the neutropenic period, as predictor of infections and transplant-related complications.

P 52
One Day Long-Term Subcutaneous Treatment with LMWH (Nadroparin) in 5-Year Child Affected by ALL and Cerebral Sagittal Venous Thrombosis
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Background: Venous thromboembolism (VTE) is very rare in children (incidence 1:100,000/year), but is becoming a recognized cause of morbidity and mortality. The majority of children with VTE have underlying conditions such as cancer, congenital heart disease, trauma or other conditions known to predispose to VTE. Cerebral VTE is a very rare event. Case: We describe here a 5-year girl, S.M., with acute lymphoblastic leukemia, in complete remission, treated with cytotoxic drugs second reinduction phase (dexamethasone, vincristine, adriblastine, cyclophoshamide, cytosine-arabinoside and intrathecal methotrextate), with cerebral sagittal venous thrombosis. The thrombotic event occurred after 1h the lumbar puncture execution, suddenly the child presented headache, vomiting, nuchal rigidity followed by loss of conscience. The cerebral MR imaging and the angio-MR showed a sagittal venous thrombosis. The patient was immediately treated with unfractionated sodium heparin 500 UI/kg/daily for two weeks. The patient recovered quickly, in less than a week. A second angio-RM showed a partial vein recanalization, than the therapy was changed with LMWH (nadroparin) subcutaneously at 175 UI/kg/daily, with weekly monitoring anti-Xa inhibitor assay, wich was maintained between 0.4–0.7 UI/ml.

Conclusion: After 2 months the child improved her condition, with the complete recanalization of cerebral vein. We think that this antithrombotic approach is appropriate because we could limit some side effects as bleeding, osteoporosis, heparin-induced thrombosis and the trauma of repeated venipunctures in case of warfarin use.

P 53
Hemostasis Imbalance in Children Affected by ALL
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The thromboembolic complications in patients affected by Acute Lymphatic Leukemia (ALL) are thought to have a multifactorial pathogenesis. We started this multicentric study in order to pinpoint the specific role of the disease and of its treatment. Aim of the Study: To evaluate the possible thrombophyllic hemostatic alterations at the onset and during the induction in children treated according to the AIEOP ALL protocols 2000. Materials and Methods: Longitudinal, observational study. Patients: Children affected by ALL treated according to the ALL protocols 2000 by the AIEOP centres in Bari, Catania, Genoa and Turin. Observation period: April–September 2002. Times of the Study: Onset of the disease, 24th, 36th, 52nd day of the protocol. Analyzed Material: Plasma. Parameters: Indicators of thrombinic activation: Thrombin-Antithrombin complexes (TAT); Indicators of endothelial and platelet activation: VWF and P-Selectin; Indicators of inflammatory activation with procoagulant activity: TNF-α and IL-6. Statistics: Friedman test and ANOVA test for repeated measures, used for ordinal and spaced measures respectively. Subgroup analysis based on the following factors: phenotype (T vs non-T); presence vs absence of central venous catheter. Results: 34 cases recruited out of 38 observed. Only 22 patients resulted evaluable (after having at least three blood samples taken). Results are reported in table 1.
Table 1. A pulmonary thromboembolic event occurred in a case on the 35th day of the protocol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>24th day</th>
<th>36th day</th>
<th>52nd day</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT (ng/L)</td>
<td>9.2 ± 12.3</td>
<td>3.9 ± 2.0</td>
<td>5.2 ± 8.8</td>
<td>2.3 ± 1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>VWF:Ag (%)</td>
<td>142.7 ± 55</td>
<td>100.8 ± 36.4</td>
<td>114.6 ± 41.3</td>
<td>99.6 ± 36.5</td>
<td>0.01</td>
</tr>
<tr>
<td>P-selectin (ng/ml)</td>
<td>45.1 ± 38.5</td>
<td>33.5 ± 24.9</td>
<td>42.9 ± 19.2</td>
<td>40.6 ± 26.4</td>
<td>NS</td>
</tr>
<tr>
<td>TAFI (%NHP)</td>
<td>16.9 ± 11.1</td>
<td>3.2 ± 3.8</td>
<td>5.7 ± 4.8</td>
<td>3.8 ± 3.6</td>
<td>0.001</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>19.2 ± 16.9</td>
<td>1.3 ± 1.0</td>
<td>5.4 ± 9.7</td>
<td>2.2 ± 1.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Subgroup Analysis: P-selectin resulted significantly higher in the T phenotype at the onset of the disease and during the therapy in patients carrying CVC. Conclusions: The data reported suggest an endothelial and platelet activation at the onset of the disease decreasing during the therapy with a slight upturn around the 36th day of the induction. Pro-inflammatory and procoagulant indicators follow the same pattern.

P 54
Genetic and Plasmatic Markers of Venous Thromboembolism (VTE) in Patients with Glioma

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Venous/pulmonary thromboembolism (VTE/PE) is a frequent complication in the course of cancer, particularly in brain tumors. We investigated genetic and plasmatic factors associated with risk of VTE/PE in patients with high grade glioma (anaplastic astrocytoma or oligoastrocytoma and glioblastoma, AA/GBM). Mutations and polymorphisms located in the genes F2 (coagulation factor II, mutation G20210A), F5 (coagulation factor V, mutation Leiden/G1691A), MTHFR (methylenetetrahydrofolate-reductase, polymorphism C677T), PLAT (tissue-type plasminogen activator (tPA), polymorphism ins/del), PAI-1 (plasminogen activator inhibitor 1, polymorphism 4G/5G) and VEGF (vascular endothelial growth factor, polymorphism C936T) were not more frequent in the AA/GBM patients compared to healthy matched population. Genetic risk factors alone did not explain the high incidence of VTE/PE observed in AA/GBM cohort. D-imer, lipoprotein (lp) (a), homocysteine, VEGF, tPA and PAI-1 plasma levels were significantly higher in AA/GBM patients than in healthy controls. Homocysteine, VEGF, tPA and PAI-1 were found at high plasmatic level even in patients carrying the corresponding ‘low producing’ genotypes. Furthermore, lp (a), VEGF, tPA and PAI-1 plasma levels were higher in AA/GBM patients that experienced VTE/PE than in AA/GBM patients without VTE/PE episodes. Higher plasma levels of factors involved in the coagulation pathway indicate that the tumor itself might confer an increased risk of VTE. In AA/GBM patients, homocysteine, lp (a), VEGF, tPA and PAI-1 look like good candidates to be evaluated as VTE/PE prognostic factors.

P 55
Polypathologic Manifestations of Malignancy Associated Venous Thrombosis: Results from the Baseline Profiling of Cancer Patients Recruited in a Study for Secondary Prevention of Venous Thrombosis with a Low Molecular Weight Heparin (ONCENOX)


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Venous thromboembolic events (VTE) are often associated with active malignancy. The mechanisms of pathogenesis behind this hypercoagulability remain unclear at this time. Patients with active malignancy and acute VTE requiring intervention provide a unique setting to investigate the mechanism of thrombogenesis in cancer patients. We profiled baseline hemostatic parameters of subjects enrolled in a randomized, open-label, multi-dose, active comparator, parallel design study of patients with active cancer and acute symptomatic DVT and/or PE who were allocated to treatment with enoxaparin alone versus initial enoxaparin followed by warfarin for a 180-day period. A total of 102 patients from 25 centers in the US were recruited. Samples were collected in appropriate settings and analyzed using standard methods. Results compared to controls are given in the following table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ONCENOX patients (N = 65–72)</th>
<th>Age-matched controls</th>
<th>Abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F VIII (%NHP)</td>
<td>104.4 ± 21.9</td>
<td>94 ± 7.8</td>
<td>35</td>
</tr>
<tr>
<td>VWF (%NHP)</td>
<td>229.7 ± 95.4</td>
<td>135 ± 23.8</td>
<td>97</td>
</tr>
<tr>
<td>Protein C (%NHP)</td>
<td>85.4 ± 42.3</td>
<td>96 ± 8.9</td>
<td>33</td>
</tr>
<tr>
<td>Protein S (%NHP)</td>
<td>70.0 ± 34.4</td>
<td>95 ± 8.3</td>
<td>57</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>46.1 ± 20.1</td>
<td>32.1 ± 9.6</td>
<td>42</td>
</tr>
<tr>
<td>TAFI (g/ml)</td>
<td>27.8 ± 11.4</td>
<td>28.6 ± 4.6</td>
<td>7</td>
</tr>
<tr>
<td>TAFI (%NHP)</td>
<td>97.8 ± 40</td>
<td>90 ± 4.7</td>
<td>7</td>
</tr>
<tr>
<td>AT (%NHP)</td>
<td>104.6 ± 21.9</td>
<td>95 ± 9.5</td>
<td>8</td>
</tr>
<tr>
<td>DRVVT (s)</td>
<td>51.0 ± 25.0</td>
<td>33.7 ± 8.6</td>
<td>65</td>
</tr>
<tr>
<td>TNP (pg/ml)</td>
<td>31.3 ± 86.4</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>CRP (g/ml)</td>
<td>20.5 ± 17.2</td>
<td>5.18 ± 1.3</td>
<td>60</td>
</tr>
</tbody>
</table>

These results clearly show the polycOMPONENT nature of cancer associated thrombosis. Forty-four patients showed significant abnormalities in more than 5 of the surrogate markers. A pronounced increase in the von Willebrand factor (VWF) was noted. Interestingly, the cancer groups also showed an increase in inflammatory markers such as C-reactive protein (CRP) and tumor necrosis factor (TNF). While Protein C and S were down-regulated, antithrombin (AT) levels were not strongly influenced. Plasminogen activator inhibitor (PAI-1) and Factor VIII (F VIII) levels were also increased. The abnormal dilute Russell’s viper venom time (DRVVT)’s indicate a significant prevalence of lupus-like inhibitors in cancer patients that may not only predispose to thrombosis but also thwart accurate anticoagulant management with warfarin. This data supports the multifactorial pathogenesis of cancer associated thrombosis. Furthermore, it
underscores the potential therapeutic role of heparins which may modulate thrombogenesis at both cellular and humoral sites.

PO 56
Malignancy-Related Thrombotic State is Independent of Defects in Factor V Leiden, Prothrombin 20210 and MTHFR Variants: Results from the Initial Profiling of Cancer Patients Entering in a Secondary Prevention Trial of Venous Thrombosis with Enoxaparin (ONCENOX)

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Molecular defects (MD) such as the Factor V Leiden (FVL), prothrombin 20210 (G20210) gene mutation and methylenetetrahydrofolate reductase MTHFR are known to contribute to thrombotic complications. Malignancy associated thrombotic disorders include vascular damage/dysfunction, activation of coagulation, fibrinolytic deficit and inflammatory responses. We investigated the molecular profile of the baseline blood samples of subjects enrolled in a randomized, open-label, multi-dose, active comparator, parallel design study of patients with active cancer and acute symptomatic DVT and/or PE who were allocated to treatment with enoxaparin alone versus initial enoxaparin followed by warfarin for a 180-day period. Of the 102 patients from 25 centers in the US, 67 consented for molecular profiling of thrombophilia genes. Buffy coats collected from citrated anticoagulated blood were used to isolate DNA and to profile FVL, G20210 and MTHFR, using standard probes. Citrated plasma samples from these patients were analyzed for Factor VIII, von Willebrand factor, Protein C and S, plasminogen activator-1 antigen, thrombin activatable fibrinolytic inhibitor functional and antigen activity, antiphospholipid antibody profile, tumor necrosis factor, C-reactive protein, anti-heparin platelet factor-4 antibodies and homocysteine. Of the 67 patients profiled, 3 patients were shown to have a heterozygous FVL defect (4%), only 1 patient showed G20210 heterozygous defect (1.5%), 20 patients exhibited MTHFR (677T) defect (14 heterozygous and 6 homozygous) and 38 patients were positive for MTHFR 1298 (29 heterozygous and 9 homozygous). Only 1 patient showed simultaneous heterozygous defects in MTHFR (677T) and FVL whereas 2 patients showed simultaneous MTHFR 1298 and FVL defects. The surrogate markers of hemostatic defects in these patients showed marked aberrations in the coagulation, fibrinolysis, endothelial and inflammatory process, indicating the involvement of multiple mechanisms in the pathogenesis of thrombosis. When surrogate markers of hemostatic activation were compared in patients with MD, in thrombophilia genes, a clear relationship was not evident in the 3 groups of patients. This data suggests that MD of coagulation may not be the primary determinants of the thrombotic complications observed in malignancy. Therefore, prophylactic anticoagulation with low molecular weight heparins in cancer patients may be effective in the secondary prevention of thrombotic disorders.

PO 57
Clothing Factors Activity in Cancer Patients
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Several prothrombotic risk factors act in concert to produce thrombosis in cancer. To further investigate the hypercoagulable cancer state we measured the clotting factors activity in a group of patients with solid tumors. Methods: Fibrinogen, prothrombin and V, VII, VIII, IX, X, XI and XII factor activity was determined (by Dade Behring assay) in 136 cancer patients (74 men, 62 women, mean age 68 years) with gastrointestinal and pelvic N0 solid tumors and in 50 control subjects matched for age and sex. Results: Compared to the controls in cancer group the activity of the following factors was significantly increased: fibrinogen (p < 0.001), factor VII (p < 0.05), factor VIII (p < 0.001), factor IX (p < 0.01) and factor X (p < 0.01). Factor II, V and XI activity was instead no different between the groups. Conclusions: In cancer patients the observed fibrinogen, factor VIII and factor VII increased activity could be related to acute phase reaction of cancer, these clotting factors in fact behave as acute-phase reactants. Moreover, although elevated factor VIII, IX and X have usually been assumed to be indicative of hypercoagulability, further studies need to know if they are associated to the thromboembolic events in cancer and then if they are to be added to the thrombophilic cancer screening.

PO 58
Inhibitors of Coagulation in Lung Cancer Patients
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University Hospital, Department of Haematology, Varna, Bulgaria

Thromboembolic and hemorrhagic complications in patients with malignant tumors are question of present interest for clinical oncology with a view to their diagnosis, evasion, and management. The incidence of disturbances in hemostasis in such patients is between 30% and 85% according to different authors. There has been a growing interest to investigate fibrinolytic system in the process of cancer genesis. The aim of our study was to estimate plasma levels and activity of natural inhibitors of coagulation antithrombin III, α2-antiplasmin, α2-macroglobulin, α1-antitrypsin and the so-called slow inhibitors of kallikrein and fast inhibitors of kallikrein, in patients with lung cancer. One-hundred and twelve patients with radiographically, endoscopically, and morphologically proved lung cancer (squamous cell, undifferentiated, small cell and adenocarcinoma) III and IV stage, were included in the study. We observed that the levels of AT III and α1-antitrypsin were diminished in 90.4%. The levels of α2-antiplasmin and α2-macroglobulin were in the confines of rate. The activities of slow and fast inhibitors of kallikrein were reliably decreased. Necessity of appraisal of inhibitory system of coagulation before starting treatment (operation, chemotherapy, radiotherapy) is opened up for discussion.
Background: Home treatment of deep vein thrombosis (DVT) is a common practice in many centers. Cancer is frequently associated with DVT and is often considered an exclusion criterion for outpatient treatment. However, when feasible, home treatment of cancer patients with DVT improves quality of life. We performed a prospective cohort study to assess the rate of cancer patients who can be deemed eligible for outpatient treatment of their DVT. Methods: Consecutive outpatients with objectively documented DVT and known cancer referred to the Thrombosis Unit of the Hospital of Varese, Italy from February 2000 to December 2002 were evaluated for the home treatment program. Patients with poor clinical conditions, active bleeding or high risk of bleeding, pain requiring parenteral narcotics were admitted to the hospital. The choice between home treatment and hospitalisation was offered to all patients who were clinically eligible for outpatient treatment. All patients received low molecular weight heparin (LMWH) and warfarin or LMWH alone if warfarin monitoring was unfeasible. Recurrent thrombosis, bleeding and death were recorded at a 3-month follow-up. Results: We included 75 patients, their mean age was 69.6, 32 (42.7%) were males. Most frequent sites of cancer were gastrointestinal in 18, genitourinary in 18, and breast in 15 patients. Thirty-nine (52%) had known metastases. Forty-six patients (61%) were entirely treated at home (mean age 70.9). All patients who were clinically eligible for home treatment refused hospitalisation. Sites of cancer were equally distributed between hospitalised patients and patients treated at home. Recurrent thrombosis occurred in 2 patients (4.3%) treated at home and in 5 hospitalised patients (17%), major bleeding in 1 (2.2%) and in 1 (3.4%), respectively, minor bleeding in 6 (13%) and in 4 (13.8%), respectively. Three outpatients (6.5%) and 5 (17.2%) hospitalised patients died during follow-up. Conclusions: Antithrombotic therapy in cancer patients is difficult because of an increased risk of both bleeding and recurrent thromboembolic events, but it can be acceptably administered at home to more than 60% of patients. When clinically feasible, home treatment of DVT is clearly well accepted by patients with cancer.
**P 61**

**Double-Blind, Prospective Randomized Trial of Very-Low Doses Warfarin versus Placebo for Prophylaxis of Catheter-Related Thromboembolism in Patient with Metastatic Cancer Receiving Chemotherapy**

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1Division of Medical Oncology, 2Pharmacy Service, 3Pain Unit, 4Division of Cardiology and 5Division of Nephrology, 6Statistical Unit, Salvatore Maugeri Foundation, Pavia, Italy

**Background:** Upper extremity thrombosis (UET) is a major complication of central venous catheters (CVC) implanted for chemotherapy in cancer patients. A double-blind, prospective randomized trial was performed to verify whether very-low doses of warfarin are effective in UET prophylaxis and safe in cancer patients with CVC undergoing repeated cycles of chemotherapy. **Patients and Methods:** From December 1999 to August 2002, 80 consecutive eligible patients undergoing chemotherapy through CVC for metastatic cancers (56 breast, 17 colon, 2 lung, 5 others) were randomized to receive warfarin 1 mg (40 pts) or placebo (40 pts), three days before the CVC insertion and subsequently for 3 months. Before study entry, at the onset of symptoms and after 3 months upper extremity veins evaluation was performed by ultrasound Doppler technique. Furthermore serial evaluation of coagulative parameters was obtained weekly during the first month and monthly thereafter. **Results:** Three patients withdrew from the study (1 for intervening surgery, 1 for inclusion in HDC + PBSC transplantation program, 1 for an increased INR > 3). All patients were evaluable for toxicity and no bleeding complication had developed. Analysis according to intention-to-treat showed 1 (2.5%) symptomatic and Doppler-proven subclavian thrombosis in warfarin-treated patients and 5 (12.5%) symptomatic thromboses in placebo-treated patients (Relative Risk 5.54; 95% CI: 0.62–50; p = 0.096). **Conclusions:** Warfarin effectiveness in UET prevention is clinically evident, even if not statistically significant. A tendency towards a lower prevalence of symptomatic thrombosis in patients receiving warfarin is observed. The administration of very-low doses of warfarin is safe in metastatic cancer patients, receiving chemotherapy through CVC.

**P 62**

**Anticoagulant Prophylaxis Usage in Cancer Patients Undergoing Treatment**

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**Aims:** Venous thromboembolism (VTE) in cancer is common, and this risk is exacerbated by the majority of treatment modalities. We assessed current thromboprophylaxis practice during cancer treatment in the North of England. **Methods:** A close-ended questionnaire was posted to all identified oncologists in the North of England, using tick-boxes, scoring systems and limited prose. Questions were aimed to specifically establish: speciality; type of tumours treated; frequency of treatment modalities used (chemotherapy, hormone therapy and radiotherapy); estimated risk of thromboembolism with such modalities; and current prophylaxis practice. **Results:** 106 of 166 appropriate responses were received. Chemotherapy was most commonly used by 38%, and radiotherapy by 41%. 27% felt venous thromboembolism was not a significant risk in cancer patients undergoing treatment. This was not influenced by speciality or tumour type treated. Hormone therapy, chemotherapy and radiotherapy were described as little or no risk by 67%, 79% and 90% respectively. Prophylaxis was routinely used by 4, 4 and 2 people respectively, with 12, 7 and 2 using prophylaxis occasionally. 37% believed less than 1% of their patients, and 62% believed less than 5% to be on prophylactic anticoagulation. **Conclusions:** 27% of oncologists do not recognise the significant thrombogenic effects of cancer treatments. This study demonstrates the need for a VTE prophylaxis protocol during cancer treatment.

**P 63**

**Low Molecular Weight Heparin for Deep Vein Thrombosis in Glioma Patients**

F. Schmidt, M. Weller

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The treatment and secondary prophylaxis of deep vein thrombosis (DVT) and pulmonary embolism, a common complication in patients with malignant glioma, has remained controversial. We treated 11 patients with malignant glioma and DVT prospectively with low molecular weight heparin (LMWH) (Tinzaparin-Natrium, 20,000 Anti-Xa IU/mL, Innohep, B. Braun Melsungen AG, LEO Pharma GmbH, Germany) at 175 IU/kg for 10 days and then for 3 months at 100 IU/kg. No patient developed bleeding complications or any other severe side effects of LMWH treatment. Two patients had a dose reduction of LMWH to 75 IU/kg because of chemotherapy-induced thrombocytopenia. One patient developed progressive DVT and nonlethal pulmonary embolism at day 14 of LMWH at 100 IU/kg. After increasing the dose to 175 IU/kg he had no further recurrence. One patient had recurrence of DVT after a fracture of the leg affected by DVT at 8 months after the diagnosis of DVT and 5 months after the end of LMWH therapy. LMWH therapy may be safe and effective in the treatment and secondary prophylaxis of DVT in patients with malignant glioma.
Treatment of Superficial Thrombophlebitis (ST) of the leg is still debated. Some studies have suggested the efficacy of unfractioned heparin for primary prophylaxis. We have retrospectively assessed the safety and feasibility of an extensive thromboprophylaxis protocol (.5.4.4) in 155 patients with metastatic breast cancer (Mbc) undergoing chemotherapy. The aim of the study was to retrospectively assess the safety and feasibility of an extensive thromboprophylaxis recommendation concerning the use of low molecular weight heparin (LMWH) as a primary thromboprophylaxis in Mbc patients undergoing chemotherapy. The primary end-point was the incidence of newly diagnosed, clinically significant thromboembolic events, and the secondary end-point, data were collected concerning LMWH-related adverse events. Patients and Methods: From April 2000 to April 2003, 101 patients with Mbc were hospitalized, median age 54 years (range 28–73). Six patients with previous thromboembolism history were not included in the analysis. Thus, 95 patients (94%) were available for retrospective analysis. Results: The patients had been selected according to an extensive thromboprophylaxis risk factors score as determined by combining multiple clinical and laboratory factors predicting for thromboembolic events, such as D-Dimer elevation, hyperfibrinogenemia, hypo-antithrombinemia, thrombocytosis, bed rest, central venous catheter. A total of 25/95 were assessed as higher risk patients for thromboembolic disease and were treated with calcium nadroparin at median dose level of 89 anti-Xa IU (range 80–90)/kg/daily subcutaneously, as long as the malignancy was active and/or the patients were receiving chemotherapy. Results: Median length of follow-up was 30 weeks (range 1–137). Median duration of thromboprophylaxis: 10.5 weeks (range 1–55). Newly diagnosed overt thromboembolic events: 1 out of 95 (1.05%), occurring in the not heparinized patients group. For the 25 patients group treated with nadroparin, the LMWH-related unfavorable event were 1 hypersensitivity reaction and 2 minor bleeding. No thromboembolic event occurred during hospital stay. Conclusion: This retrospective study indicates that an extensive nadroparin calcium based thromboprophylaxis was effective and well tolerated in the Mbc patients undergone chemotherapy. As oral anticoagulants are not easily manageable in this patients setting, it seems reasonable to explore LMWH suitability with regard to prolonged primary thromboprophylaxis in cancer patients.

**P 65**

**Primary Thromboprophylaxis with Calcium Nadroparin in Metastatic Breast Cancer (Mbc) Patients Undergoing Chemotherapy: A Retrospective Analysis**

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**Background:** It has been estimated that close 5–15% of patients with cancer will suffer from thromboembolic phenomena during their life. Increased risk is observed during hospital stay. Historically, epidemiologic studies have documented a higher rate of overt thromboembolic disease (15–17%) in Mbc patients treated with chemotherapy. Despite none of the laboratory and/or clinical single variable is able to predict thromboembolic event, primary prevention of thrombosis should be currently considered for cancer patients. **Purpose:** Aim of the study was to retrospectively assess the safety and feasibility of an extensive thromboprophylaxis recommendation concerning the use of low molecular weight heparin (LMWH) as a primary thromboprophylaxis in Mbc patients undergoing chemotherapy. The primary end-point was the incidence of newly diagnosed, clinically significant thromboembolic events, and the secondary end-point, data were collected concerning LMWH-related adverse events. Patients and Methods: From April 2000 to April 2003, 101 patients with Mbc were hospitalized, median age 54 years (range 28–73). Six patients with previous thromboembolism history were not included in the analysis. Thus, 95 patients (94%) were available for retrospective analysis. The patients had been selected according to an extensive thromboprophylaxis risk factors score as determined by combining multiple clinical and laboratory factors predicting for thromboembolic events, such as D-Dimer elevation, hyperfibrinogenemia, hypo-antithrombinemia, thrombocytosis, bed rest, central venous catheter. A total of 25/95 were assessed as higher risk patients for thromboembolic disease and were treated with calcium nadroparin at median dose level of 89 anti-Xa IU (range 80–90)/kg/daily subcutaneously, as long as the malignancy was active and/or the patients were receiving chemotherapy. Results: Median length of follow-up was 30 weeks (range 1–137). Median duration of thromboprophylaxis: 10.5 weeks (range 1–55). Newly diagnosed overt thromboembolic events: 1 out of 95 (1.05%), occurring in the not heparinized patients group. For the 25 patients group treated with nadroparin, the LMWH-related unfavorable event were 1 hypersensitivity reaction and 2 minor bleeding. No thromboembolic event occurred during hospital stay. Conclusion: This retrospective study indicates that an extensive nadroparin calcium based thromboprophylaxis was effective and well tolerated in the Mbc patients undergone chemotherapy. As oral anticoagulants are not easily manageable in this patients setting, it seems reasonable to explore LMWH suitability with regard to prolonged primary thromboprophylaxis in cancer patients.
neutropenia and asthenia. No significant bleeding or thrombotic events were observed. Eleven patients had elevated D-dimer plasma levels prior to therapy, and 7 of these patients with a response or stable disease had a significant decline of the D-dimer during therapy. There were no consistent changes of the plasma levels of the angiogenic factors, except for transforming growth factor-beta-1 (TGF-β1). The median baseline level of TGF-β1 prior to therapy was 34.867 pg/ml. Twelve out of 13 patients who achieved a response or stable disease had a significant reduction of the TGF-β1 levels during therapy. Enoxaparin in combination with chemotherapy was safe and well tolerated in patients with advanced NSCLC. This preliminary data suggests that enoxaparin may prolong the time to progression, and therefore justify the continuation of this trial.

**Rationale:** Since the introduction of *E. coli* asparaginase into the induction chemotherapy of childhood acute lymphoblastic leukemia (ALL) there has been an increase in the frequency of thromboembolic events; according to the literature the incidence is found to be as high as 11%. According to data recently published, there seems to be a considerable difference in the incidence of thrombosis between patients with and without hereditary thrombophilia. However, small studies suggest that prothrombotic risk factors believed to be congenital can be modulated secondary to the chemotherapy and therefore should not be assumed to be stable thrombogenic risk factors. Most thromboses occur in patients who have received a central venous catheter (CVC) early during induction therapy. This led the study chair for earlier BFM trials (ALL-BFM 95) to recommend that, wherever possible, central venous catheters be avoided during the first month of therapy, even though this significantly impairs the patient's quality of life. Acquired AT deficiency is believed to play an important role in the pathophysiology of thromboembolism in CVC bearing individuals undergoing L-asparaginase containing chemotherapy for ALL. Recent data (the PARKAA study) suggest that AT replacement therapy in this setting is feasible and safe; the study was not powered to provide reliable efficiency data, but there seemed to be a trend for efficacy with regard to thromboprophylaxis. **Research Plan:** To further evaluate the possibility for an efficacious thromboprophylaxis, the Thrombotect trial has been designed: Thrombotect is an add-on protocol to the ALL-BFM-2000 trial; it evaluates in a prospective, randomised, multicenter setting if low dose i.v. heparin, fixed dose enoxaparin or target value triggered AT replacement therapy are equally effective in preventing thromboembolism in catheter bearing patients undergoing L-asparaginase containing induction chemotherapy for ALL. Thrombotect's accrual target is 945 patients randomized into the 3 above mentioned treatment arms. interim analyses are planned after reaching 1/3 and 2/3 of the planned sample size. Randomization is performed through the ALL-BFM headquarter at the University of Hannover, Germany. Under the current conditions, an initial thrombophilia work-up is provided for patients included. International recruitment has started January 1 2003; for now, 26 centers in Germany and Switzerland are participating in the trial. **Safety:** Low dose i.v. unfractionated heparin via CVC is a commonly used procedure aimed to reduce thromboembolism. Prophylactic use of enoxaparin (Elhasid et al., Blood Coagulation & Fibrinolysis, 2001) and antithrombin (Mitchell et al., Thrombosis and Haemostasis, in press) have been shown to be safe in the planned setting. The study will be supervised by a safety board comprising Prof. Gadner, Vienna; Prof. Lämmle, Bern; Dr. Falanga, Bergamo; and Prof. Manco-Johnson, Denver. The Thrombotect Study Group is interested in collaboration with additional treatment centers that apply the ALL-BFM or similar treatment protocols. Collaboration with other research groups working in the field are also in our interest.

For any further information on the Thrombotect protocol, please contact jeannette.greiner@gd-kispi.sg.ch or wolfgang.korte@ikch.ch.

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**The Thrombotect Trial of the ALL-BFM Trial Group for Prevention of Thromboembolism in L-Asparaginase Containing Induction Chemotherapy in Childhood ALL – Rationale and Research Plan**

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**Background:** Venous thromboembolism (VTE) is a common complication following major abdominal surgery. Thromboprophylaxis (TP) with LMWH is well documented and is traditionally given for 1 week after surgery. However, the optimum duration of TP is controversial as there is evidence that activation of the coagulation system persists for up to 4 weeks after surgery. The FAME study was carried out to evaluate the efficacy and safety of 4 weeks, compared with 1 week, of TP with the LMWH dalteparin after major abdominal surgery. **Methods:** FAME was a large randomised study of 590 patients. Following major abdominal surgery, patients received dalteparin (5,000 IU sc, od) and wore graduated compression stockings for 1 week, and were then randomised to either no TP (short-term TP group) or a further 3 weeks of TP with dalteparin (5,000 IU sc, od) (prolonged TP group). All patients underwent assessor-blinded bilateral venography at 4 weeks. The primary efficacy endpoint was objectively-verified VTE occurring between 1 and 4 weeks after surgery. Any bleeding events during this period were recorded. Efficacy analysis was on an ITT basis. **Results:** Patients in the two treatment groups (ITT population, prolonged TP group, n = 165, short-term TP group, n = 178) were well matched for age, gender, weight, previous VTE, previous cancer and type of abdominal surgery. Over half the patients underwent surgery for malignancy, 60% and 56% in the short-term and prolonged TP groups, respectively. There was a significant reduction in the incidence of all VTE from 16.2 to 7.3% (55% RRR, 95% CI 15–76%, P = 0.01), proximal DVT from 8.0 to 1.8%

**Safety:** Low dose i.v. unfractionated heparin via CVC is a commonly used procedure aimed to reduce thromboembolism. Prophylactic use of enoxaparin (Elhasid et al., Blood Coagulation & Fibrinolysis, 2001) and antithrombin (Mitchell et al., Thrombosis and Haemostasis, in press) have been shown to be safe in the planned setting. The study will be supervised by a safety board comprising Prof. Gadner, Vienna; Prof. Lämmle, Bern; Dr. Falanga, Bergamo; and Prof. Manco-Johnson, Denver. The Thrombotect Study Group is interested in collaboration with additional treatment centers that apply the ALL-BFM or similar treatment protocols. Collaboration with other research groups working in the field are also in our interest.

For any further information on the Thrombotect protocol, please contact jeannette.greiner@gd-kispi.sg.ch or wolfgang.korte@ikch.ch.

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**Prolonged Thromboprophylaxis with Dalteparin Following Major Abdominal Surgery Prevents Late VTE: The FAME Study**

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**Background:** Venous thromboembolism (VTE) is a common complication following major abdominal surgery. Thromboprophylaxis (TP) with LMWH is well documented and is traditionally given for 1 week after surgery. However, the optimum duration of TP is controversial as there is evidence that activation of the coagulation system persists for up to 4 weeks after surgery. The FAME study was carried out to evaluate the efficacy and safety of 4 weeks, compared with 1 week, of TP with the LMWH dalteparin after major abdominal surgery. **Methods:** FAME was a large randomised study of 590 patients. Following major abdominal surgery, patients received dalteparin (5,000 IU sc, od) and wore graduated compression stockings for 1 week, and were then randomised to either no TP (short-term TP group) or a further 3 weeks of TP with dalteparin (5,000 IU sc, od) (prolonged TP group). All patients underwent assessor-blinded bilateral venography at 4 weeks. The primary efficacy endpoint was objectively-verified VTE occurring between 1 and 4 weeks after surgery. Any bleeding events during this period were recorded. Efficacy analysis was on an ITT basis. **Results:** Patients in the two treatment groups (ITT population, prolonged TP group, n = 165, short-term TP group, n = 178) were well matched for age, gender, weight, previous VTE, previous cancer and type of abdominal surgery. Over half the patients underwent surgery for malignancy, 60% and 56% in the short-term and prolonged TP groups, respectively. There was a significant reduction in the incidence of all VTE from 16.2 to 7.3% (55% RRR, 95% CI 15–76%, P = 0.01), proximal DVT from 8.0 to 1.8%
Late thromboembolic complications occur in 16% of patients following major abdominal surgery, despite short-term TP. Prolonged TP with LMWH (dalteparin 5,000IU sc, od) for 4 weeks compared with 1 week significantly reduces VTE after major abdominal surgery, with no increase in bleeding.

Conclusions: Late thromboembolic complications occur in 16% of patients following major abdominal surgery, despite short-term TP. Prolonged TP with LMWH (dalteparin 5,000IU sc, od) for 4 weeks compared with 1 week significantly reduces VTE after major abdominal surgery, with no increase in bleeding.