POSTERS

HYPERCOAGULABILITY

P001

COMBINED USE OF CLINICAL PRETEST PROBABILITY AND D-DIMER TEST IN CANCER PATIENTS WITH CLINICALLY SUSPECTED DEEP VENOUS THROMBOSIS

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Background. The value of the D-dimer (DD) test in combination with the clinical pretest probability (PTP) has not been evaluated in cancer patients with suspected deep vein thrombosis (DVT), whereas this group of patients usually accounts for 10-25% of clinically suspected DVT.

Aim. To determine the distribution of the various PTP categories in patients with cancer as well as the occurrence of DVT in each of these groups. Second, we evaluated the diagnostic accuracy of the DD test (SimpliRED DD test. Agen Biomedical Ltd Brisbane, Australia) in combination with a low PTP and with a low-moderate PTP in cancer patients versus non cancer patients.

Patients and methods. A cohort of 2,066 consecutive patients with clinically suspected DVT was investigated. Patients were judged to be positive or negative for DVT according to the outcomes of serial compression ultrasound and a 3-month period follow-up with imaging test verification of the symptomatic cases. Diagnostic accuracy indices of the DD test according to the PTP score were assessed in patients with and without cancer.

Results. Of the cohort, 244 (11%) were known to have cancer at presentation. Among the cancer patients, 17% were considered to have a low PTP, 35% a moderate and 41% a high PTP. A venous thromboembolic event was diagnosed in 41% of the patients with cancer and in 22% of the patients without malignancy. A DVT was confirmed in 10% (4/42) of the cancer patients with low PTP, in 27% (28/102) of those with moderate PTP, and in 68% (68/100) of those with high PTP.

The negative predictive value (NPV) of the DD test was 100% (95% CI, 85-100) and 97% (95% CI, 88-99) among cancer patients with low PTP or low-moderate PTP. In the absence of malignancy, the corresponding NPV were 98% (95% CI, 97-99) and 97% (95% CI, 96-98), respectively. The specificity of the DD test progressively decreased moving from the low to the higher PTP.

Conclusions. In cancer patients with clinically suspected DVT, a negative DD might be useful in excluding the diagnosis within the low or low-moderate PTP groups. More studies are warranted to confirm these findings.

P002

PROTEOMIC ANALYSIS OF ACUTE MYELOID LEUKEMIA SUBTYPES: IDENTIFICATION OF NEW EARLY BIOMARKERS RELATED TO THE TUMORAL PATHOPHYSIOLOGY

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Background. Leukemogenesis is a complex process developed through different pathways that involves multiple gene mutations, and finally leads to a great number of protein alterations. The multitude of possible mutations and their combinations results in a tremendous variability in clinical behavior even within the same disease. These pathological changes in haematologic neoplasias might be reflected in proteomic patterns in cells, and identification of these altered proteins may represent a way of discovering new tumoral markers.

Aim. To analyze the differential pattern of protein expression of blasts from acute myeloid leukaemia (AML) patients in comparison to normal white blood cells from matched healthy individuals, in order to identify potential marker proteins associated with some characteristics of the pathology related to these different subtypes.

Patients and Methods. A total of 15 patients with AML, diagnosed according to the French-American-British (FAB) committee classification, and ten healthy controls were entered into this study. Proteomic analysis of blasts from different AML subtypes was performed using 2D-electrophoresis and MALDI-TOF mass fingerprinting analysis.

Results. Proteins identified as more significantly altered between the different AML subtypes belonged to the group of suppressor genes (RhoGDI2 and annexin A10), metabolic enzymes (α-enolase, triosephosphate isomerase, fumarase hydratase or ATP synthase), antioxidants (catalase and peroxiredoxin 2), structural proteins (tropomyosin 3) and signal transduction mediators (disulfide isomerase, zinc finger proteins or lipocortin 1). Complementary molecular and protein studies were performed to analyze if blast samples showing altered expression of some of the identified proteins relevant to tumor progression (such as lipocortin 1 or tropomyosin 3) also displayed other molecular markers that might be related to the clinical behavior of this malignancy. We found a direct correlation between the expression of those proteins and that of a cell-surface receptor (Tissue Factor), angiogenic factors (such as Vascular Endothelial Growth Factor and Kinase insert Domain-containing Receptor) and intracellular pathways (such as MEK/ERK or NFÎB) directly related to the negative outcome of the disease.

Conclusions. Our overall data suggest that pathological

changes in AML may be reflected in proteomic patterns, and thus identification of those altered proteins represents a way of discovering new tumoral markers. Supported by FIS 02/0215, FIS 04/1291 and JA61/02

P003

IS THERE AN ALTERED HAEMOSTATIC RESPONSE TO CHEMOTHERAPY IN PATIENTS DEVELOPING CHEMOTHERAPY-INDUCED VENOUS THROMBOEMBOLISM?

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Background. VTE during breast cancer chemotherapy cancer is common (2-18%) and a frequent cause of death in advanced breast cancer (8%), however thromboprophylaxis is rarely used.

Aim. To identify predictive markers for chemotherapy-induced VTE in breast cancer.

Patients and methods. Procoagulants (tissue factor, cancer procoagulant, serum and plasma vascular endothelial growth factor (sVEGF and pVEGF)) and markers of haemostasis (APTT, PT, thrombin-antithrombin (TAT), fibrinogen, D-dimer, platelet count) were measured prior to chemotherapy and at one, four and eight days following commencement of chemotherapy in early (n=87) and advanced breast cancer (n=36) patients. Duplex ultrasound imaging was performed one month following commencement of chemotherapy or if symptoms developed.

Results.

	Fibrinogen* g/L	D-dimer ng/mL	Tissue factor µg/mL	pVEGF
VTE	4.7 (3.0-6.4){8}	1514 (936-2448){13}	261 (121-561) {12}	26 (14-48) {13}
No VTE	3.4 (3.2-3.7) {95}	714 (618-824) {119}	105 (84-132) {119}	16 (14-18) {117}
p	*0.01*	*0.01*	*0.03*	*0.05*

*Geometric mean (*mean) and CI, {n}. All the above predicted for VTE.*

Mean baseline platelet count in advanced breast cancer patients developing VTE was 434x10^9/L (normal range 150-400x10^9/L) compared to 313x10^9/L in VTE-free patients (p=0.1). Using a cut-off value of D-dimer >700 ng/mL and fibrinogen >3g/L, VTE could be predicted with a sensitivity of 67%, specificity 69%. This increased to 100% and 53% respectively in the advanced breast cancer group. In patients developing VTE compared to those without VTE, a marked reduction of APTT (p=0.007) and pVEGF (0.04), and increase in TAT (p=0.01) was identified within 24 hours of commencing chemotherapy.

Conclusions. Fibrinogen, D-dimer, tissue factor and pVEGF, at baseline, and the response of APTT, TAT and pVEGF to chemotherapy may allow a biochemical profile of patients at risk of VTE to be developed. Alterations in haemostasis occur with 24 hours of chemotherapy. Administration of a once-only anticoagulant with each chemotherapy cycle may abolish this procoagulant episode.

P004

A PROSPECTIVE EPIDEMIOLOGICAL STUDY ON THE PREVALENCE AND ROLE OF ANTIPHOSPHOLIPID ANTIBODIES IN CANCER PATIENTS

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Background. Antiphospholipid antibodies (aPL) have a relatively high prevalence in cancer patients, ranging from 28 to 68%, according to the literature.

Aims of the study 1. To define the prevalence of aPL in a cohort of consecutive patients admitted to Oncology and Haematology Departments with a new diagnosis of cancer, asymptomatic for venous or arterial thromboembolism. 2. To evaluate a possible role of aPL in this clinical setting, as risk factor for thromboembolic disease and overall survival. Two years of clinical follow-up is scheduled. Patients Inclusion Criteria were: 1. age >18 years, 2. were enrolled patients with: a) solid tumors: breast, colon-rectal, head-neck, lung or b) onco-haematological diseases : lymphoprolipherative diseases, myelodisplastic disease and acute leukemia, 3. Patients were all enrolled at diagnosis, 4. Life expectancy >1 year, 5. Informed consent.

Methods. All patients enrolled were screened for Lupus Anticoagulant (coagulative method according to recommended criteria from the International Society on Thrombosis and Haemostasis Subcommittee on Lupus Anticoagulant-phospholipid-dependent-antibodies); anticardiolipin antibodies (ACA) and antiβ2GlycoproteinI antibodies (IgG and IgM) were assayed by ELISA commercial kits. Laboratory tests were repeated after thrombotic events. Patients were visited or phoned every three months. If signs or symptoms suggested a venous occlusive disease, CUS or venous US, or Spiral TC were performed to objectively diagnose a vascular event.

Results. Enrolment was started in February 2004 and was stopped in February 2005. 137 patients were enrolled, 100 female and 37 male, median age 61 (range 29-83). 15 patients had haematological diseases, 21 colon-rectal tumors, 77 breast cancers, 16 head-neck tumors and 8 lung cancers. In February 2005 (the last follow up visit) mean follow up was 6,9 months/patient. aPLs were found positive in 19,7% of patients. Patients with colon-rectal disease had the higher prevalence of aPL: 26.25%. During the follow up 7 patients had a thromboembolic event, and 8 patients died (1 for pulmonary embolism). Among patients with a thrombotic event, 1 out of 7 was aPL positive; 1 out of 8 deaths was aPL positive.

Conclusions. Follow-up is still short and our data not conclusive. After a mean of 6 months of follow-up no relationship is found between thromboembolic events and aPL in oncological and onco-haematological patients. In about one more year of follow-up we will obtain definitive data to clarify or to rule out the role of aPL in this clinical setting.

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P005

HEMOSTATIC ALTERATIONS IN PEDIATRIC LEUKEMIAS

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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 evaluate the specific role of the disease and of its treatment.

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 2002-March 2004 Times of the Study Onset of the disease, 24th, 36th, 52nd day of the protocol. Parameters Thrombin-Antithrombin complexes (TAT); vWF-Ag and hight molecular weight multimers HMWM; TNF-α and IL-6.

Results. 104 cases recruited out of 112 observed. Only 84 patients resulted evaluable (after having at least three blood samples taken).

Results are reported in Table.

Parameter	t0	24th day	36th day	52nd day	p
TAT (μg/L)	5.1 (1.2-300)	4.2 (1.4-60)	2.6 (1.4–2)	2.4 (0.8–9)	<0.001
vWF: Ag (%)	117.5±37.3	109.2±34.8	110.2±34.2	108.2±24.4	NS
vWF -HMWM (%)	16.2±5.7	14.6±5.7	13.3 ± 5.9	14.8 ± 6.0	<0.05
TNF-α (pg/mL)	1.5 (0.3–71)	6.8 (0–22)	8.0 (0.3–22)	6.4 (0–15)	<0.001
IL-6 (pg/mL)	6.4 (0.3–272)	2.2 (0–94)	4.9 (0.4–40)	2.6 (0.1–66)	<0.001

[TAT, median (Min-Max), vWWF: Ag (M±SD), vWF:HMWM (M±SD), TNF-α, median (Min-Max), IL-6, median (Min-Max)].

A pulmonary thromboembolic event occurred in a case on the 35th day of the protocol, and a cerebral sinovenous thrombosis occurred in a case on the 22th day of the protocol.

Conclusions. The data reported suggest an endothelial activation at the onset of the disease. Pro-inflammatory and procoagulant indicators follow the same pattern.

P006

HAEMOSTATIC RISK FACTORS FOR CENTRAL VENOUS LINE-RELATED THROMBOSIS IN LEUKAEMIC PATIENTS

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Background. Central Venous-Lines (CVL) are frequently placed in cancer patients to deliver chemotherapy. Discordant data have been reported on the incidence of CVL-related thrombosis in these patients. However, recent reports suggest an incidence of about 5%.

Aim. Because this figure does not justify extended pharmacological prophylaxis it would be important the identification of patients at higher risk who might benefit from prophylaxis. Patients We report preliminary data on endothelial and blood clotting activation markers and thrombophilic factors obtained in 15 leukaemic patients (8 males, 7 females, mean age 55.4±9.5 yrs, range 44-71) in whom CVL position was planned.

Methods & Results. Plasma vWF, TFPI and D-Dimer levels were high in all the patients, suggesting both endothelial and clotting activation (vWF: 271.6±111.9 %; TFPI: 76.0±27.0 ng/mL; D-Dimer 703.1±480.9 ng/mL). Two patients developed a CVL-related thrombosis after one and three weeks respectively. Both were heterozygous for G20210A gene II mutation. No patient was positive for Factor V Leiden.

Conclusions. This anecdotical report underscores the potential role of hereditary thrombophilia as a risk factor for CVL-related thrombosis.

P007

CORRELATION BETWEEN PLASMA D-DIMER LEVELS AND AXILLARY LYMPH NODE STATUS IN OPERABLE BREAST CANCER PATIENTS

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In patients bearing solid tumours, several mechanisms may induce activation of the coagulation process. The extent of such activation has been reported as correlating with tumour stage and prognosis in some malignancies. D-dimer is a stable end-product of fibrin degradation, and levels of D-dimer are elevated by enhanced fibrin formation and fibrinolysis. Consistently, D-dimer levels are increased in patients with various solid tumours. Recently, some authors have suggested a tight correlation between early tumour metastasis, lymphovascular invasion, and plasma D-dimer levels in operable breast cancer patients. However, the usefulness of combining plasma D-dimer levels with sentinel lymph node biopsy (SNB) in primary breast cancer is still unclear. The aim of our study is to verify a possible correlation between quantitative D-dimer levels (Instrumentation Laboratory) and lymph node involvement, in particular focusing on sentinel node (SN) status. One hundred forty-two breast cancer patients were enrolled in the study. All patients (aged 29-84) underwent preoperative D-dimer plasma evaluation before surgery. Forty out of 142 patients (28 %) underwent axillary lymph node dissection, while 102/142 patients (72%) were eligible for SNB technique. The median D-dimer level of the whole series was 210 ng/mL (range 45-709). D-dimer levels resulted higher in patients with nodes involvement as compared with nodes negative patients. However this difference did not reach the statistical significance (p=0.37), both for patients with multiple metastatic axillary nodes (p=0.27) and for patients with single SN involvement (p=0.60). In conclusion, we found no statistical correlation between D-dimer plasma levels and clinical stage in breast cancer patients. Such lack of correlation does not support the hypothesis that D-dimer plasma might be related to lymphovascular invasion and clinical stage.

P008

HIGH INCIDENCE AND TRANSITORY NATURE OF ACQUIRED RESISTANCE TO ACTIVATED PROTEIN C IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Non factor V Leiden APC resistance (aAPCR) has been described in cancer patients and found to be associated with an increased risk of deep venous thrombosis (DVT).

Aim. We analyzed the incidence of APC resistance, clinical impact and possible correlations with disease markers in a large group of multiple myeloma patients.

Patients and methods. APC resistance was tested in 1178 myeloma patients using an aPTT-based resistance assay in the presence of excess of factor V-deficient plasma and the ratio with or without APC was calculated (≤2.00 was considered abnormal). PCR amplification of genomic DNA was used to detect Factor V Leiden.

Results. A total of 109 patients (9.3%) were found to have abnormal APC resistance, 83 of those were then tested for factor V Leiden, 31 had the mutation and 52 (63%) did not have it. The presence of APC resistance and in particular of aAPCR was associated with an increased risk for DVT ($p \leq 0.001$). In thirty-two patients with aAPCR, the test was repeated at least twice, with a median time of 87 days between the assessments. In only one patient we found persistent aAPCR (after 98 days), while all other patients showed a subsequent normal APC ratio. Levels of CRP, IL-6, B_2M, serum and urineM-component, and disease status were assessed in 26 patients. In this series, status of active disease emerged as the most important factor associated with aAPCR, as nineteen patients had a concomitant normalization of the APC ratio and clinical response to therapy. No correlation has been found between aAPCR and CRP, IL-6, B_2M, or levels of paraprotein. Baseline APC resistance was also tested in a subgroup of 254 chemotherapy naïve myeloma patients; in 11% of those patients the APC ratio was abnormal and two third of them were not carriers of factor V Leiden.

Conclusions. This study demonstrate that: a) aAPCR is common in myeloma patients and correlates with DVT; b) aAPCR can be transitory in nature.

P009

RELATIONSHIP BETWEEN HEMOSTATIC PARAMETERS AND PROGNOSTIC/ PREDICTIVE FACTORS IN BREAST CANCER

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Background. There is an interactively relation between hemostatic system and cancer. Activation of coagulation system occurs commonly in patients with malignancy. Elevated factor VIII, IX, V, and XI levels and diminished antithrombin III levels can be found in various solid tumors. This prothrombotic state may have prognostic importance.

Aim. In this study we investigate whether the relation between hemostatic parameters and prognostic/predictive factors.

Patients and methods. One hundred newly diagnosed breast cancer women were included. Patient did not receive systemic therapy or radiotherapy. The healty control group was include age matched 100 women. In the patient group, grade, axillary lymph node status, steroid receptor status, p53 and c-erbB-2 was eveluated. Plasma levels of factor VIII, factor IX, D-dimer, fibrinogen, protein C, protein S, von Willebrand Factor,and antithrombin III was measured in both groups. Two-sided $p<0.05$ was considered significant. All statistical analysis were performed with the SPSS 11.0. *Results.* The plasma levels of factor VIII, factor IX, vWF, and CRP in patients with breast cancer were higher than in controls. The protein S levels in patients were lower than in controls. Among all of these for only factor VIII there was a correlation with prognostic factors. There was no significant differance in D-dimer and fibrinogen levels between patient and control groups. There was a strong correlation between axillary lymph node status, the number of metastatic nodes and factor VIII levels(r_s=0,503; p<0,01and rs = 0,634; p<0,01, respectively).

Conclusions. The strongest prognostic factor for breast cancer is the presence of axillary lymph node metastasis. In our study there was a strong correlation between axillary lymph node status, the number of metastatic nodes and factor VIII levels. High factor VIII levels in cancer patients were not attributable solely to an acute-phase reaction. No correlation was found between factor VIII levels and levels of CRP, which is a sensitive indicator of an acute-phase reaction. Our study shows that plasma factor VIII levels associated with lymph node metastasis in breast cancer. Therefore,the determination of factor VIII levels might be helpful to identify patients with lymph node metastasis.

P010

ANTIPHOSPHOLIPID ANTIBODIES AND ACUTE PHASE RESPONSE IN CANCER PATIENTS

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Background. The acute phase response is activated in cancer patients, the antiphospholipid antibodies have been found in a large variety of malignancies. The aim of our study was addressed to investigate the relations between the generation of the antiphospholipid antibodies and some serological markers of inflammation in cancer patients. *Methods.* Eighty-one cancer patients with non metastatic gastrointestinal solid tumors and fifty-two control subjects were tested for the presence of the anticardiolipin (aCL) of the anti-ß2-glicoprotein-1 ($a\beta_2GP1$) IgG, IgM and Ig A isotypes, and of some acute phase reactants, such as the fibrinogen (antigen and coagulative), the factor VIII:C, and the C4b-binding protein (C4BP).

Results. In the cancer patients the fibrinogen (antigen and coagulative), the factor VIII:C and the C4BP plasma mean levels were significantly higher than in the control group ($p<0.01$) as were the isotype IgA of both the aCL ($p<0.05$) and the anti-β_2GP1 ($p<0.01$) antibodies; moreover the C4BP concentrations were significantly correlated with the aCL IgA ($p<0.01$), the aCL IgG ($p<0.05$) and the $a\beta_2GP1$ IgG ($p<0.01$) antibodies. In both groups the aCL antibodies IgA were significantly correlated with the fibrinogen antigen ($p<0.05$). In addition, in the cancer group, using the principal component analysis separately applied to the set of serological markers of inflammation variables (fibrinogen

functional and antigen, Factor VIII: C and C4BP) and to the set of the antiphospholipid antibodies (aCL and $a\beta_2$GP1 IgG, IgM and IgA), the two principal components resulted significantly and positively correlated ($p<0.01$). The prevalence of the presence of the aCL and of the $a\beta_2$GP1 IgG, IgM and IgA antibodies was instead not statistically different between the groups.

Conclusions. Our data suggest that in cancer patients the finding of the antiphospholipid antibodies that is associated with the acute phase proteins, could be an indirect index of the inflammatory reaction induced in the host by the neoplastic disease. The pathogenetic mechanism involved in the antiphospholipid antibodies development is nevertheless incompletely understood.

P011

ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH CANCER: PREVALENCE AND CLINICAL SIGNIFICANCE

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Background. The association between cancer and venous thromboembolism (TE) has been established even though the exact mechanisms are still a matter of debate and investigation. The role of acquired thrombophilic factors in the development of venous thrombosis in patients with cancer remains unclear. Lupus anticoagulant (LA) and anticardiolipin antibodies (aCL) are acquired antibodies to phospholipids (aPL). Typically the aPL transiently found in association to infectious diseases belong to the infectious type or also named β_2Glycoprotein I (β_2 GpI)-independent as apposed to the autoimmune type (β_2 GpI-dependent) which are associated with an increase thrombotic risk.

Aim. To evaluate the prevalence and clinical significance of LA and aCL β_2 GpI dependent and independent antibodies in a population with different types of cancer and stages of the disease.

Patients and methods. IgG and IgM aCL antibodies in serum from 58 patients with cancer of different localization and stages of disease (I-II/III-IV): 13 with lung cancer (2/11), 12 with genitourinary cancer (5/7), 13 with digestive cancer (3/10), and 20 with breast cancer (7/12) were studied by standardized enzyme-linked immunosorbent assay in the presence (bovine serum) and absence (ovalbumin) protein cofactor (mainly β_2 GpI). The LA was tested in plasma of these patients according to SSC-ISTH criteria.

Results. Five of 13 patients with lung cancer (38.4%); 4 of 12 patients with genitourinary cancer (33.3%); 6 of 13 patients with digestive cancer (46%), and 7 of 20 patients with breast cancer (35%) showed a positive aCL titre for IgG and/or IgM isotypes (>20 GPL/MPL units). Most of these antibodies (80, 100, 83.3 and 71.4%) were mainly cofactor independent in the patients with lung, genitourinary, digestive and breast cancer, respectively. Only one patient with lung cancer tested positive for LA.

Conclusions. We did not find any association between the presence of aPL and the development of thrombosis, when the patients were followed up over period of 14 months, as well as for the localization and stages of the disease. This agrees with the hypothesis that there is no clinical thrombotic symptom associated with aCL antibodies because these are cofactor-independent despite to the high prevalence of them in cancer patients.

P012

DIRECT CORRELATION BETWEEN PROTEASE-ACTIVATED RECEPTOR-1 EXPRESSION AND MALIGNANT PHENOTYPE IN HUMAN MELANOMA

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Background. The protease-activated receptor-1 (PAR-1) is a unique G-protein-coupled receptor, which belongs to the protease-activated receptor family. Activation of PAR-1 involves proteolytic cleavage of the extracellular amino terminal domain by thrombin to unmask a new amino terminus, the tethered ligand for the receptor, which leads to downstream signalling events that evoke a variety of cellular responses. Overexpression of PAR-1 has been detected in numerous human cancers and increasing evidence implicates PAR-1 as a contributor to tumor invasion and metastasis of human melanoma.

Aim. The aim of the current study was to investigate the expression of PAR-1 in human melanoma cell lines, characterized by different malignant phenotype and to correlate it with the expression of other receptors associated with melanoma progression, such as FLT-1 and TIE-1. Benign and malignant melanocytic lesions from patients were also investigated.

Materials and methods. PAR-1 expression was evaluated by Ribonuclease Protection Assay (RPA) in several human melanoma cell lines, including WM35 (early non metastatic) and WM983A (primary metastatic advanced). In addition, PAR-1 expression was investigated in paraffin-embedded tissues derived from common melanocytic nevi and melanomas using a paraffin block RNA isolation procedure and RPA. Total RNA was hybridized to RNA probes, synthesized from supplied template set (hAngio-1, RiboQuant multiprobe set; BD Biosciences). PAR-1, FLT-1 and TIE-1 expression was quantified using L32 as housekeeping gene using Sigma Gel analysis software. The expression of PAR-1 in paraffin embedded tissues was analyzed by immunohistochemistry, using anti-PAR-1 monoclonal antibody raised against aminoacids 42-45 of human PAR-1 (ATAP-2, Santa Cruz Biotech).

Results. PAR-1 was significantly overexpressed in WM983A in comparison with WM35. We also observed a direct correlation between the levels of expression of PAR-1 and FLT-1 and TIE-1 in human melanoma cells lines. In line with these findings, PAR-1 expression was significantly higher in melanomas in comparison with common nevi and PAR-1 overexpression was confirmed by immunohistochemistry.

Conclusions. Our results provide strong evidence for a direct correlation between overexpression of PAR-1, as well as of FLT-1 and TIE-1, and the malignant phenotype of human melanoma.

P013

PERSISTENT HYPERCOAGULABILITY AND RISK OF CANCER IN OLDER MEN

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Background. In the second Northwick Park Heart Study (NPHS-II), persistent plasma hypercoagulability characteri-

sed by increased thrombin generation and activity was documented in a higher than expected proportion of healthy men. During median 10 years follow-up, cancer incidence and mortality were higher in men with persistent hypercoagulability, suggesting coagulation plays a role in preclinical cancer.

Aim. The aims of the current study were to further characterise the haemostatic state in cases who developed cancer compared to controls (matched for age, practice location and year of sample) who did not.

Methods. Stored NPHS-II plasma samples were assayed in 203 cases and 394 controls using commercial ELISAs for tissue factor (TF), thrombin-antithrombin complex (TAT), thrombomodulin (TM), urokinase-type plasminogen activator (u-PA), u-PA-plasminogen activator inhibitor type 1 (PAI-1) complex and D-dimer.

Results. Mean u-PA-PAI-1 complex was higher in cases (OR 1.64 (0.99-2.71), p=0.055). There were no significant differences in other variables between cases and controls. Based on prothrombin fragment F1.2 and fibrinopeptide A results, 569 men were categorised as persistently hypercoagulable (n=25) or non-hypercoagulable (n=544). In univariate analysis, both u-PA-PAI-1 complex and D-dimer were significantly higher in the hypercoagulable group. Following adjustment, the differences between the groups remained significant only for D-dimer (p=0.02). Categorised according to both cancer status and hypercoagulability, levels of u-PA-PAI-1 complex were significantly higher in cases with than without hypercoagulability (p=0.003).

Conclusions. Samples collected years before the diagnosis of cancer show an increase in levels of u-PA-PAI-1 complex in cases compared to controls. Both D-dimer and u-PA-PAI-1 complex were higher in men with persistent hypercoagulability, although only D-dimer remained associated after adjustment. After categorisation by both cancer status and hypercoagulability, u-PA-PAI-1 complex was significantly higher in those with hypercoagulability who subsequently developed cancer than in those without hypercoagulability who developed cancer, consistent with this marker being a feature of the biochemical state that is linked to the hypercoagulability of cancer.

P014

CORRELATION BETWEEN SELECTED CLOTTING SYSTEM PARAMETERS AND APOPTOSIS OF PERIPHERAL CD3$^+$ T CELLS IN NSCLC

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Background. The relationship between thrombogenity and apoptosis was studied in various cell lines and a significant correlation between thrombin generation and degree of apoptosis was found.

Aim. The aim of this study was to investigate a possible relation between selected clotting system parameters involved in thrombin generation and apoptosis of circulating T lymphocytes in NSCLC.

Materials and methods. The study group consisted of 15 healthy donors and 15 NSCLC patients, aged from 47 to 78 years (with a mean of 63 years), who underwent radical pulmonary resection (<IIIB stage of disease). Concentration of selected clotting parameters including TF - *tissue factor*, TFPI - *tissue factor pathway inhibitor*, F1+F2 - *fragments of prothrombin*, AT – antithrombin, TAT - *thrombin-antithrombin complex*, protein C, protein S and APCR - *resistance to activated protein C* were assessed with the use of ELISA technique or standard laboratory procedure. Apoptosis of peripheral T lymphocytes was assessed with the use of JC-1 probe (showing the early stage of apoptosis, associated with mitochondrial membrane depolarization) and multicolor flow cytometry, respectively.

Results. The percentage of apoptotic peripheral T lymphocytes was significantly higher in NSCLC patients than in control group (2.45±1.75 % vs 0.88±0.46%, respectively, p=0.03). In NSCLC patients and in control group we found that apoptosis of peripheral T lymphocytes was not associated with TF (p=0.66 and p=0.83, respectively), TFPI (p=0.49 and p=0.08, respectively), F1+F2 (p=0.19 and p=0.74, respectively), AT (p=0.83 and p=0.43, respectively), TAT (p=0.91 and p=0.92, respectively), protein C (p=0.25 and p=0.55, respectively), protein S (p=0.46 and p=0.94, respectively) and APCR (p=0.36 and p=0.31, respectively). *Conclusions.* We conclude that thrombin generation correlated with TF, TFPI, F1+F2, AT, TAT, protein C, protein S and APCR is not associated with apoptosis of peripheral T lymphocytes.

P015

THROMBIN GENERATION AND EXPRESSION OF THE DEATH RECEPTOR FAS (CD95) ON PERIPHERAL T LYMPHOCYTES IN NSCLC

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Background. A correlation between thrombin generation and apoptosis was found in various human tumor- and non-tumor cell lines. Expression of the death receptor Fas on peripheral lymphocytes could lead to apoptosis of these cells.

Aim. Therefore in this study we try to assess a possible correlation between selected clotting system parameters connected with thrombin generation and expression of the death receptor Fas (CD95) on circulating T lymphocytes in NSCLC.

Materials and methods. The study group consisted of 20 healthy donors and 20 NSCLC patients, aged from 47 to 78 years (with a mean of 63 years), who underwent radical pulmonary resection (<IIIB stage of disease). Concentration of clotting parameters including TF - *tissue factor*, TFPI - *tissue factor pathway inhibitor*, F1+F2 - *fragments of prothrombin*, *AT – antithrombin, TAT* - thrombin-antithrombin complex, protein C, protein S and APCR - *resistance to activated protein C* were assessed with the use of ELISA technique or standard laboratory procedure. Expression of the death receptor Fas (CD 95) on peripheral T lymphocytes (CD3$^+$, CD4$^+$ and CD8$^+$) was assessed with the use of multicolor flow cytometry.

Results. In NSCLC group altered APCR was correlated with increased percentage of CD4$^+$/CD95$^+$ T lymphocytes (p=0.01), whereas in control group lower APCR (however still normal) was correlated with increased percentage of CD4+ T cells (p=0.04). There was no correlation between expression of the death receptor Fas (CD95) on all subpopulations of T cells (CD3$^+$, CD4$^+$, CD8$^+$) and the level of TF, TFPI, F1+F2, AT, TAT, protein C and S neither in control nor in NSCLC patients.

Conclusions. These results could suggest that in NSCLC patients, expression of the death receptor Fas on CD4$^+$T cells correlates with altered APCR. The mechanism leading to this phenomenon could be associated with thrombin generation, but does not depend on TF, TFPI, F1+F2, AT, TAT, protein C and protein S level.

P016

TAFI, FIBRINOGEN AND D-DIMERS IN RADICALLY OPERATED NSCLC

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Background. Alterations of fibrinolysis are frequently observed in patients with malignancy. Thrombin Activatable Fibrinolytic Inhibitor (TAFI) may play a central role in thrombosis and fibrinolysis due to its ability to retard fibrin clot lysis.

Aim. The aim of this study was to determine whether NSCLC patients (with no symptoms of thromboembolism) show any alteration in TAFI, fibrinogen and d-dimers concentration.

Materials and methods. The study group consisted of 70 NSCLC patients, aged from 47 to 78 years (with a mean of 63 years), who underwent radical pulmonary resection. All patients were under IIIb stage of disease and remained active smokers. Control group consisted of 20 healthy donors, similar to patients group according to sex, age and smoking habit. In study group blood samples were collected preoperatively. Concentration of TAFI was measured in blood serum using ELISA technique, whereas fibrinogen and d-dimers – with thw use of standard laboratory procedure.

Results. In NSCLC the mean level of fibrinogen and d-dimers concentration was significantly higher in compression to control group (6.3±1.9 g/L vs 3.6±0.7 g/L, respectively, $p<0.001$ for fibrinogen and 260±193 ug/L vs 144±65 ug/l, respectively, $p=0.01$ for d-dimers). There was no differences between the mean value of TAFI concentration between NSCLC patients and control group (0.96±0.2 ug/L vs 1.0±0.2 ug/L, respectively, $p=0.53$). Neither in NSCLC patients nor in control group, there was no correlation between concentrations of TAFI and fibrinogen ($p=0.14$ and $p=0.96$, respectively) or TAFI and d-dimers ($p=0.93$ and $p=0.25$, respectively).

Conclusions. Our results suggest that in the group of radically operated NSCLC patients (with no symptoms of thromboembolism) thrombosis and fibrinolysis are activated, however probably with no influence of TAFI.

P017

AT, TAT AND F1+F2 FRAGMENTS OF PROTHROMBIN IN RADICALLY OPERATED NSCLC

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Background. Increased thrombin generation is frequently observed in patients with malignancy and in many cancer patients, the coagulation system is activated without clinical signs of thromboembolism.

Aim. The aim of this study was to determine whether NSCLC patients (with no symptoms of thromboembolism) show any alterations in activity of Antithrombin (AT), Thrombin-Antithrombin Complexes (TAT) and F1+F2 fragments of prothrombin concentrations.

Patients and methods. The study group consisted of 70 NSCLC patients, aged from 47 to 78 years (with a mean of 63 years), who underwent radical pulmonary resection. All patients were under IIIb stage of disease and remained active smokers. Control group consisted of 20 healthy donors, similar to patients group according to sex, age and smoking habit. In study group blood samples were collected preoperatively.

Results. Activity of AT, TAT and F1+F2 concentrations were measured in blood serum using ELISA technique. The mean value of AT activity was significantly higher in control group in comparison to NSCLC patients (108±9% vs 101±10%, respectively, $p=0.04$), whereas there were no differences in mean concentrations of TAT and F1+F2 between control and cancer group (6.34±7 ug/L vs 3.7±3 ug/L, respectively, $p=0.16$ for TAT and 1.53±1.6 ng/mL vs 1.13±1 ng/mL, respectively, $p=0.64$ for F1+F2). In NSCLC patients, but not in control group, a positive correlation between mean concentrations of TAT and F1+F2 ($p<0.001$) was found.

Conclusions. Our results suggest that in the group of radically operated NSCLC patients with no symptoms of thromboembolism, the clotting system might be activated due to decries of AT activity and that concentrations of TAT is positively correlated with F1+F2.

P018

TF AND TFPI IN SURGICALLY TREATED NSCLC

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Background. Coagulopathies are frequently observed in patients with malignancy and in many cancer patients, the coagulation system is activated without clinical signs of thromboembolism. Tissue factor (TF) and tissue factor pathway inhibitor (TFPI) influence the extrinsic pathway of coagulation and are believed to have a critical function in homeostasis, activation and inhibition of thrombogenesis.

Aim. The *aim.* of this study was to determine whether NSCLC patients (with no symptoms of thromboembolism) show any alteration in TF and TFPI concentration.

*Patients and methods.*The study group consisted of 70 NSCLC patients, aged from 47 to 78 years (with a mean of 63 years), who underwent radical pulmonary resection. All patients were under IIIb stage of disease and remained active smokers. Control group consisted of 20 healthy donors, similar to patients group according to sex, age and smoking habit. In study group blood samples were collected preoperatively. Concentration of TF and TFPI were measured in blood serum using ELISA technique.

Results. There were no statistically significant differences between control group and NSCLC patients in mean concentrations of TF (36.6±24 pg/mL vs 30.8±11 pg/mL, respectively, $p=0.35$) and TFPI (185.1±71 ng/mL vs 190.2±60 ng/ml, respectively, $p=0.81$). There was also no correlation between TF and TFPI concentrations neither in control ($p=0.86$) nor in cancer group ($p=0.23$).

Conclusions. Our results suggest that in the group of radically operated NSCLC patients with no symptoms of thromboembolism, there is no correlation between TF and TFPI generation, and that there is no difference in concentrations of TF and TFPI between cancer and control group.

P019

DETERMINATION OF KNOWN RISK FACTORS OF THROMBOEMBOLISM IN LARYNGEAL AND NON-SMALL CELL LUNG CANCER PATIENTS

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Background. In many cancer patients, the coagulation system is activated without clinical signs of thromboembolism.

Aim. The aim of this study was to determine whether cancer patients with no symptoms of thromboembolism, show any alteration in known risk factor of thrombophilia.

Materials and methods. The study group consisted of 40 patients (38 men and 2 women), aged from 47 to 78 years (mean 63 years), including 20 patients with laryngeal cancer and 20 patients with non-small cell lung cancer (NSCLC), who underwent radical tumor resection. Control group consisted of 20 healthy donors, similar to patients group. Activity of antithrombin (AT), protein C and S, APCR, concentration of fibrinogen, d-dimer and homocystein were assessed with the use of commercially available tests.

Results. In cancer patients the mean activities of AT and protein S were significantly lower (108±9% vs 101±9%, respectively, p=0.02 for AT and 70±24% vs 56±23%, respectively, p=0.04 for protein S), whereas the mean fibrinogen and d-dimers concentrations were significantly higher in comparison to control group (5.9±1.9g/L vs 3.6±0.7g/L, respectively, p<0.001 for fibrinogen and 253±161ug/l vs 144±66 ug/L, respectively, p=0.01 for d-dimer). There were no differences in mean concentration of homocystein, activity of protein C and APCR between analyzed groups. When patients were divided according to type of cancer we found that only in laryngeal cancer patients the mean activity of AT was significantly lower than in control group (p=0.02) whereas only in NSCLC patients the mean activity of protein S was significantly lower (p=0.01). Additionally in NSCLC patients the mean APCR was significantly lower than in control group (1.9±0.4 vs 2.3±0.3, respectively, p=0.01).
Conclusions. Our results suggest that in cancer patients alteration of known risk factors of thrombophilia occur in spite of no symptoms of thromboembolism and that these alterations seem to be different in laryngeal and NSCLC cancer patients.

P020

PROGNOSTIC ROLE OF PROTEASE-ACTIVATED RECEPTOR 1 (PAR-1) AND 4 (PAR-4) IN STAGE IB NON-SMALL CELL LUNG CANCER (NSCLC)

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Background. PAR-1 and PAR-4 play a major role in both tumor invasion and in the neoangiogenesis process.

Aim. To assess PAR-1 and PAR-4 expression in NSCLC and to correlate these data with microvessel density (MVD) and disease prognostic factors.

Patients and methods. We analyzed 60 cases of stage IB (pT2N0) NSCLC [30 adenocarcinomas (AC) and 30 squamous cell carcinomas (SCC)]. Median age: 67 (range 50-82); 54 males, 6 females. All patients underwent radical resection in our institution. The percentage of PAR-1 and PAR-4 positive cells in each tumor sample was assessed following immunohistochemistry (IHC) staining with anti-PAR-1 and anti-PAR-4 antibodies. MVD was quantified by CD34 staining in the 3 most vascularized sample areas. *Results.* PAR-1 and PAR-4 expression were detected in 25 (42%) and in 39 patients (65%) respectively. The percentage of PAR-1 and PAR-4 positive cases, as well as PAR-1 and PAR-4 expression intensity in each sample (% of positive cells), were significantly higher among AC compared to SCC samples. In 18 cases PAR-1 and PAR-4 were expressed on the same sample. Average MVD (microvessel/m^2) was higher in AC samples compared to SCC samples (p<0.001). No correlation was found between PAR-1 and PAR-4 expression and MVD levels. Median follow up was 38 months. Actuarial overall 3-year survival was 43%. At univariate analysis, overall 3-year survival was significantly shorter in patients expressing PAR-4 compared to patients with no PAR-4 expression (p=0.002). Similarly, 3-year survival was significantly shorter in patients with PAR-1 and PAR-4 coexpression or positivity for either one receptor, compared to patients with no PAR expression (p=0.002). A trend toward a shorter 3-year survival was also seen in PAR-1 positive patients compared to PAR-1 negative cases. Multivariate analysis identified expression of PAR-1 and/or PAR-4 as independent prognostic factors for reduced survival. No difference in survival was observed according to histology, sex, age, tumor size, type of surgery and MVD.

Conclusions. Expression of PAR-1 and/or PAR-4 in both AC and SCC tumor samples emerged as a negative prognostic factor in surgically resected stage IB NSCLC, and therefore could be helpful in selecting subgroups of patients at higher risk of recurrence.

PLATELET AND PROTEOLYTIC ENZYMES

P021

HETEROGENEITY OF TISSUE-DERIVED ANTICOAGULANT EFFECTS ON PLASMA THROMBIN GENERATION

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Introduction. The blood coagulation system serves a critical role in maintaining a balance between pro- and anticoagulant forces. Reduced expression of anticoagulant molecules may, under conditions of inflammation or atherosclerosis, lead to intravascular fibrin formation or overt thrombosis. Besides alterations in major vessels, organs containing an extensive endothelium surface, provide a major pool of anticoagulant reserve. Tissue dependent heterogeneity in pro- and anti-coagulant forces may be relevant for thrombogenicity but the functional expression of its determinants is unknown.

Aim. In order to investigate potential thrombogenic activity in tissue homogenates we established a method to determine the endogenous thrombin potential on mouse organs.

Methods and Results. The contribution of murine tissues to the thrombin generation in human plasma was analyzed by the calibrated automated thrombogram (Thrombinoscope, Maastricht, The Netherlands). Addition of liver and brain homogenates had no influence on thrombin generation in normal human plasma (1847±38 nM/min). In contrast, the addition of lung, heart, or kidney homogenates caused a substantial inhibition of thrombin generation in human plasma. Kidney homogenates inhibited thrombin formation in human plasma up to 80% (1478±332 nM/min; n=3), whereas addition of heart and lung homogenates reduced thrombin formation to respectively 5% (86±20 nM/min) and 1% (14±25 nM/min). The tissue-derived anticoagulant effects on plasma thrombin generation were completely abolished in protein C-deficient plasma. No inhibition on thrombin generation in human plasma was observed after the addition of any of the tissue homogenates derived from TM pro/pro animals. Kidney (2425±22 nM/min), heart (1625±468 nM/min), and lung (2000±175 nM/min) from TMpro/pro animals restored thrombin generation to respectively 131%, 88%, and 108% compared to homogenates from wild-type animals.

Conclusions. Normal mouse tissues, except brain and liver, have a substantial anticoagulant effect in a thrombin generation assay. This anticoagulant effect appears to be due to tissue-derived thrombomodulin, mediating the activation of plasma protein C. Murine lung and heart contain most thrombomodulin activity, whereas kidney posses moderate levels. In conclusion, the calibrated automated thrombogram method is a powerful tool for determination of altered anticoagulant reserve of different organs in pathophysiological conditions.

P022

ROLE OF DISCOIDIN DOMAIN RECEPTORS IN BLOOD COAGULATION

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Background. Discoidin domain receptors (DDR1 and DDR2) are widely-expressed receptor tyrosine kinases found upregulated in several malignant cell lines. DDRs play a critical role in cell proliferation, migration and adhesion and in regulation of the extra-cellular matrix protein, collagen. Although collagen has been identified as a ligand for DDRs, the nature of DDR-collagen interaction is less understood. It is important to examine the expression and role of such collagen-binding proteins in bleeding disorders as they may regulate the native structure and content of collagen which is crucial for platelet aggregation and blood coagulation. *Aim.* To elucidate a novel pathway by which the coagulation cascade may be affected via upregulation of DDRs in normal and malignant cells, we examined the nature of DDR-collagen interactions and their resulting effect on platelet aggregation. Further, we assessed the expression of DDRs in acute promyelocytic leukemia (APL) cells. Notably, APL is characterized by disseminated intravascular coagulation (DIC) leading to frequent bleeding and/or thrombotic events in untreated patients.

Materials and methods. Purified DDR-Fc fusion protein, *in vitro* biophysical techniques and high resolution microscopy were used to investigate DDR-collagen interaction. *In vitro* platelet aggregation assays on blood from healthy donors were performed to examine how the collagen morphology present with DDRs alters platelet aggregation. The expression of DDRs in the APL cell line NB4 was examined using PCR and Western Blotting.

Results. We established that by binding to collagen type 1, DDRs affect the fibrillogenesis of collagen type 1 and alter the morphology and structure of collagen fibers. Platelet aggregration assays with collagen fibers formed in presence and absence of DDRs suggest that DDRs can affect platelet aggregation in a concentration dependent manner. Further, we have established that DDR1 is highly overexpressed in the APL cell line NB4.

Conclusions. Our results indicate that DDRs may play a crucial role in blood coagulation by disrupting the initial step of platelet aggregation. Indeed we found that in APL which is characterized by DIC, DDR1 is highly upregulated. Our further work is targeted to examine the role of DDR overexpressing cancer cells in affecting platelet aggregation. Based on these results DDRs may serve as a potential therapeutic target for bleeding disorders in a variety of cancers.

P023

MORPHOFUNCTIONAL PECULIARITIES OF PLATELETS AND MEGAKARYOCYTES IN PATIENTS WITH CHRONIC MYELOGENEOUS LEUKEMIA TREATED WITH IMATINIB

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Background. As far as cytogenetic disorders in patients with chronic myelogeneous leukaemia (CML) take place not only in granulocytes but in thrombocytes as well and becau-

se of described in literature thrombocytopenia during imatinib treatment we became interested in imatinib influence on platelet component of hemostasis. Aim To determine the efficacy of imatinib on megakaryocytes and platelets, to find prognostic criteria of hemorrhagic syndrome among patients with CML. Materials & Methods We enrolled 11 patients with CML in chronic phase (28–45 year old) who received treatment with imatinib 400 mg/day and were followed for a 10±2.3 months. All the patients were examined on Ph-gene, rates of primary and secondary hemostasis and enzyme activity in platelets and megakaryocytes (glycogen content and activity of α-naphthizilacetatesterase (ANAE)). *Results.* A major cytogenic response was achieved in 4 patients. In 3 patients we observed complete cytogenetic remission. Four patients didn't achieve major cytogenic response. Nine patients had normal initial platelet count (220±15×10^9/L). In 27% of patients treated with imatinib we observed loss of platelet count up to 120±12×10^9/L, and in one patient up to 60×10^9/L. Loss of megakaryocyte count in bone marrow up to 10×10^9/L was observed in every examined patient. And only in 3 patients (who achieved complete cytogenetic remission) we revealed a tendency to normalization of megakaryocyte count. That correlated with loss of Ph^+ cells in bone marrow. Cytochemical research revealed initial loss of glycogen content (index Kaplow (IK) 1,87±0,16) and ANAE activity (IK 2.01±0.22). These changes was aggravated during imatinib treatment. Initial glycogen content in platelets was normal (IK 2.02±0.22) and ANAE activity was increased (IK 1.94±0.18). Subsequently we observed loss of glycogen content (IK 1.68±0.14) in 46% of patients (mostly in those who achieved complete cytogenetic remission) and normalization of ANAE activity was in 56% of patients.

Conclusions. Imatinib undoubtedly influences both on megakaryocyte formation and granulocytopoiesis precursors. We suppose that imatinib induced thrombocytopenia is a result of apoptosis stimulation in megakaryocytes with Philadelphia translocation and can be considered as manifestation of cytogenetic response.

P024

CHEMOTHERAPY-INDUCED CHANGES IN PLATELET FUNCTION IN EARLY AND ADVANCED BREAST CANCER

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Background. Platelet activation is increased in cancer patients. Platelet-tumour cell interactions are important in tumour growth, metastases and angiogenesis. Chemotherapy may have an altered effect on platelet function in patients with high and low tumour load. Aim To establish alterations in platelet function (inferred by platelet content of vascular endothelial growth factor (VEGF)) in response to chemotherapy in advanced breast cancer (ABC) and early breast cancer (EBC) patients. Patients and Methods Platelet content of VEGF ({serum (sVEGF) – plasma VEGF (pVEGF)}/platelet count) was measured prior to chemotherapy (baseline) and at one, four and eight days, and three and six months following commencement of chemotherapy in EBC (n=87) and ABC (n=36) patients. Clinical and radiological follow-up was at three, six, 12 and 24 months. *Results.* Prior to chemotherapy, pVEGF, sVEGF and platelet content of VEGF were increased in ABC (p=0.02, p<0.001 and p<0.001 respectively). Platelet VEGF decreased in EBC but not ABC in the eight days following chemotherapy (p=0.007), however, there was a more marked decrease in platelet count and sVEGF in ABC compared to EBC at this time (p=0.01 and 0.01). Both platelet count and content of VEGF increased in all cancer patients by three months compared to baseline. Platelet content of VEGF was increased at baseline in ABC who progressed within six months compared to ABC with stable disease (geometric mean (CI) 1.1 (0.9-1.4) μg/ml platelet ×10^9, 0.7 (0.3-1.8) μg/mL platelet ×10^9, n=28 and 7 respectively, p=0.1). Platelet content of VEGF was decreased at baseline in EBC who developed metastases within two years compared to EBC in remission (geometric mean (CI) 0.3 (0.1-0.9) μg/mL/platelet ×10^9, 0.6 (0.5-0.7) μg/mL platelet x109, n=5 and 77 respectively, p=0.03). Conclusions Platelets, in the presence of a large tumour load (ABC), have an altered response to chemotherapy. Platelet function, prior to chemotherapy, may act as a surrogate marker for response to chemotherapy.

P025

THE EFFECT OF SURFACE MODIFIED LIPOSOMES ON AGGREGATION BETWEEN TUMOR CELLS AND PLATELETS

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Background. Metastasis is still the most serious reason for the high mortality of cancer patients. The successful prevention of metastatic tumour growth is therefore still a serious challenge in cancer therapy. Metastasis is a very complex process in which platelets play a crucial role. Several attempts have been performed to inhibit the metastatic process, some of these using modified liposomes. Aim Investigation of the aggregation behaviour of human platelets and HT29 colon carcinoma cells in the presence of liposomes with a modified surface.

Materials and methods. Liposomes with a basic composition of PC/CH/DMPE were prepared unmodified, sterically stabilized by polyethylene glycol (PEG-DSPE), or equipped with the carbohydrate ligand sialyl-$Lewis^X$ (conjugated to PEG-DMPE or DMPE as anchor) by the lipid film method in combination with extrusion to obtain small vesicles. The effect of these vesicles on complex formation of HT29 colon carcinoma cells in the presence of platelets and liposomes was quantified *in vitro* and characterised by fluorescence microscopy. In addition, the influence of these vesicles on metastatic growth of HT29 colon carcinoma in mice was tested *in vivo*.

Results. The presence of surface modified liposomes caused an up to 2.9-fold increase in aggregation of human platelets in plasma. In addition, when HT29 tumour cells were mixed with platelets and surface modified liposomes, the number of tumour cells found in aggregates increased significantly from 8.3% (only tumour cells) to 30.2%. This result was supported by fluorescence micrographs demonstrating a strong association of platelets and liposomes around the tumour cells. In addition, a clear decrease in number and a change in the distribution of metastases after intravenous injection of HT29 cells in combination with liposomes was observed *in vivo*. While in control mice metastases in lung, liver and in intestine were prevailing, liposomal treatment resulted in a new localization of metastases in muscles. *Conclusions.* We suggest that a time-dependent formation of aggregates comprising tumour cells, platelets and surface modified liposomes *results.* in the development of

microthrombi which then enhance the metastatic process. The capability of vesicles to interfere with the metastatic process might have implications for the use of such liposomes for therapeutic applications.

P026

ACQUIRED COMBINED α-DELTA STORAGE POOL DEFICIENCY IN PATIENT WITH THYROID TUMOR: COINCIDENCE OR COMMON PATHOGENETIC PATHWAY? A CASE REPORT

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Background. Storage pool deficiency is a heterogeneous group of diseases, usually congenital, in which there is an impaired ability to store appropriate products within platelet α or delta granules. Few cases, described as acquired, were reported in myeloproliferative and rheumatologic disorders. The case of combined α and delta storage pool disease is very rare and there is no evidence in current literature of acquired forms. We report a case of acquired combined α and delta storage pool disease.

Patient, methods, results. A 67 y.o. woman underwent to our observation for slight thrombocytopenia (130,000/ mmc). Physical examination was normal but in clinical history was present radical thyroidectomy 2 years before for thyroid cancer. No major or minor or surgical bleeding were reported. Viral serologies and autoimmunity tests were normal. Patient tooks levothyroxine as only drug. Bleeding time was 19 minutes (Ivy). Other coagulation tests were normal. Morphology of platelets on peripheral blood smear, stained with May-Grunwald Giemsa, was normal. A cytofluorimetric study on patient platelets was performed. Expression of GPIIb, GPIIIa, GPIb, GPIX, were normal, excluding Glanzmann thromboasthenia and Bernard-Soulier syndrome. α granules content showed reduced fibrinogen (30%) and Von Willebrand Factor (40%). Platelets P-selectin and fibrinogen expression were normal, showing that α granules content was reduced not for platelets activation and degranulation. Patient PRP was tested for primary and secondary response in aggregometric analysis. Primary response to ADP 5 μM, ADP 2.5 μM, ADR 0.1 mcg/mL, ADR 10 mcg/mL was normal in aggregometric test. Secondary response to ADP 5 μM, ADP 2.5 μM, ADR 0.1 mcg/mL, ADR 10 mcg/mL, collagen 1 mcg/mL, arachidonic acid 0.5 mM, was reduced. The normal response to TRAP and Ristocetin excluded alteration of thrombin receptor and Bernard-Soulier syndrome. These results suggested the storage pool deficiency diagnosis. *Conclusions.* The combined α and delta storage pool disease is usually due to Rabs gene mutations. Rabs are small GTPases which split GTP to provide energy for membrane fusion events and modifications of cytoskeleton. Rabs gene mutations are, sometimes, involved in tumor genesis and progression. In this case the same genetic mutation could be at origin of platelet disease and neoplastic event.

P027

PLATELET FUNCTION INVESTIGATION IN THROMBOPHILIA DIAGNOSTICS IN CANCER PATIENTS

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Background. Development of algorithm of analysis haemostasis state will allow decreasing risk of VTE complications. Aim To determine necessary range of laboratory tests for a high-grade estimation of hemostasis state in cancer patients.

Patients and methods. Group of inspection: 167 women was divided on 7 groups: 27 have ovarian cancer I-II stages (OvC I-II); 24 have ovarian cancer III-IV stage (OvC III-IV); 17 have vaginal and vulva cancer (VVC); 27 have uterine carcinoma I-II stage (UtC I-II); 21 have uterine carcinoma III stage (UtC III); 27 have cervical carcinoma I-II stage (CC I-II), 25 have cervical carcinoma III stage (CC III). Group of comparison – 64 women with benign tumor: 24 have uterus myoma (UtM); 19 have ovarian cyst (OvCy) and 21 have combination uterus myoma and ovarian cyst (M&Cy). Laboratory tests: Platelet aggregation tests with following stimulators: Adrenaline 10-4 M, Ristocetin and ADP in various concentrations (10-3, 10-5, 10-7 M) to determine degree of their activation. Platelet activation marker test – platelet factor 4 (PF4). Disseminated intravascular coagulation (DIC) and thrombophilia marker tests: D-dimer and TAT complexes.

Results. Before operative intervention the rate of subcompensated chronical DIC was 40.7% in patients with OvC I-II; in OvC III-IV – 50%; in VVC - in 23.5%; in UtC I-II – 22.2%; in UtC III – 38.1%; in CC I-II – 18.5% and in patients with CC III - 36%. In the group of comparison the rate of subcompensated chronical DIC was 33.3% in patients with UtM, in OvCy – 26.3% and in M&Cy – 38.1%. In postoperative period the rate of subcompensated chronical DIC have been considerably increased and patients with decompensated chronical DIC also have been found: in patients with OvC I-II – 55.6%; in OvC III-IV – 75%; in VVC - in 58.8%; in UtC I-II – 55.5%; in UtC III – 76.2%; in CC I-II – 51.9% and in patients with CC III – 72%. In patient with benign tumor the rate of subcompensated and decompensated chronical DIC was 41.7% in UtM, in OvCy – 31.6% and in M&Cy – 42.9%.

Conclusions. The most informative methods of detection latent forms of thrombophilia and cronic DIC are definition of level PF4 - a marker of platelet activation, which indicate the rate of thrombocytopathy and thrombophilia. The second most informative method is platelet aggregation test with ADP in various concentrations to define the degree of platelets hyper- and hypofunction.

P028

SEMISYNTHETIC P-SELECTIN-ANTAGONISTS: *IN VITRO*- AND *IN VIVO* INVESTIGATIONS OF STRUCTURE-ACTIVITY RELATIONSHIPS

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Background. P-selectin is stored in platelets and endothelial cells and expressed on their surface after activation. By binding to mucin ligands it mediates interactions with leukocytes and certain types of tumor cells. P-selectin plays an important role in inflammatory and thrombotic processes including atherosclerosis and restenosis as well as in tumor metastasis. Therefore, targeting P-selectin represents an interesting therapeutic option.

Aim. The aim of the present study was to examine the structural requirements on structurally defined glucan sulfates (GS) for the inhibition of cell-binding to P-selectin and to compare their activities with those of various heparins.

Materials and methods. Series of GS differing in their degree of sulfation (DS), molecular weight (MW), sulfation pattern or type of glycosidic binding, resp., and four heparin fractions were included in the study. First, their blocking effect on the adhesion of U937 cells to P-selectin coated on microplates was determined. Next, their inhibitory potency on the cell rolling was investigated in a parallel plate flow chamber by evaluating the rolling fraction and - velocity of U937 cells on a P-selectin layer. Finally, by using intravital microscopy the influence on P-selectin-dependent rolling of activated platelets along endothelial surface in murine skin vasculature was monitored.

Results. In all three test systems, congruent structure-activity relationships were found. Depending on their structure, GS turned out to represent much more potent inhibitors than heparins. The activity clearly increases with increasing DS and is additionally modulated by the MW. Moreover, both the sulfation pattern and the glycosidic binding influence the activity, e.g. α-1,4/1,6-GS are more active than β-1,3-GS.

Conclusions. In conclusion, structurally defined semisynthetic glucan sulfates are potent P-selectin antagonists with several advantages over heparins and thus represent promising candidates for the development of new drugs.

P029

MYELODYSPLASTIC SYNDROMES: PLATELET MEMBRANE GLYCOPROTEINS

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The receptors of platelet membrane are significant for their function. Most of them are glycoproteins (GP) and play a major part in the adhesion and aggregation of platelets, in a way that any change of their quantity or quality to lead in functional defects of the second.

The *Aim* of this study was to determine the quantitative expression of platelet membrane glycoproteins (percentage of platelets that express a particular glycoprotein on their surface) among patients with MDS, as a reflection of the variable defects that characterize those syndromes.

Material and methods. Twenty-four patients were studied, 15 male and 9 female with mean age 73.2 years, who suffered from all of the types of MDS (FAB-classification). Specifically, 9 patients (pts) presented RA, 2 pts RARS, 7 pts RAEB, 2 pts RAEB-t and 4 CMML. All of the pts, so as 16 normal subjects (8 male and 8 female with similar mean age) who were used as controls, were investigated using flow cytometry and certain monoclonal antibodies (CD42a and CD42b for GPIb-V-IX, CD41 and CD61 for glycoproteins GPIIb-IIIa and CD62P for P-selectin). The method of statistical analysis, which was used, was the comparison of mean values.

Results.

Patients	RA	RARS	RAEB	CMML	RAEB-t	Total
N	9	2	7	4	2	24
CD42a%	97.6±3.2	80.5±17.5	89.4±14.1	97.3±2.0	95.3±2.1	92±20.1
CD42b%	49.5±25.2	52.8±42.8	58.9±29.9	37.8±20.7	32.8±4.9	46.3±27.9
CD41%	60±32.4	36±35.8	58.8±29	70.3±25.7	44.1±9.1	53.8±29.8
CD61%	91.8±17.2	72±26	77.6±28.2	95.8±2.7	93.9±1.85	86.2±21.8
CD62%	8.2±7.4	6.7±3.3	6.3±4.5	2±0.5	7.2±6.7	6±6

Controls	
N	16
CD42a%	95.9±3.4
CD42b%	78.23±17.5
CD41%	85.5±8.5
CD61%	95.36±3.0
CD62%	14.1±10.6

Conclusions. As shown in the table above, GPIb (CD42b), P-selectin (CD62P) and GPIIb (CD41) are reduced in all of the categories of pts. As long as GpIIIa (CD61) is concerned, it is reduced only among pts with RARS and RAEB. On the contrary, CD42a (GpIX) seems not to have been affected by the disorders of hemopoiesis. It is possible that the reduction of those receptors a) reflects changes in the production of normal platelets and b) interprets functional disorders of platelets.

P030

THE EFFECT OF THE NOVEL SYNTHETIC DERIVATIVE OF PROSTAGLANDIN E1 ON THE PLATELET AGGREGATION AND THE PLATELET COUNT

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Background. Clinical practice has revealed that prostaglandin E1 (PGE1) and its synthetic analogs prevent platelet aggregation at several pathological states.

Introduction. Of potential NO-donors (such as dinitroglycerin) to PGE1 molecules seems to be promising for development of new active agents.

Aim. To study the influence of the PGE1 novel synthetic dinitroglycerin ester, prostanit, on the platelet aggregation and on the platelet count.

Materials. 1) Blood was taken from healthy subjects into sodium citrate at 9:1 volume ratio. Aorta was removed from white rats narcotized by inhalation of ethyl ether, washed in 0.05M tris-HCL buffer (pH=7.5) and cut longitudinally. Aorta fragment was incubated with acetylsalicylic acid (10 mg/mL) and washed in 0.05M tris-HCL buffer. Platelets were incubated with aorta fragment and the substance for 5 min at 37°C 2) Prostanit was administrated to rabbits intrave-

nously at the dose 0.2 mg/kg. Blood was taken at 2, 15, 30 and 60 min after injection. Platelet aggregation studies were performed according to G. Born's method. ADP (final concentration 10^{-5} M), collagen (final concentration 50 μg/mL) and arachidonic acid (final concentration 10^{-3} M) being used as an aggregation agents. The platelet count was measured using B. Walkowiak's method.

Results. Prostanit reduced the human platelet aggregation *in vitro* induced by arachidonic acid at concentrations 1-1μ×10^{-6} μg/mL. The platelet aggregation decreased from 41±3% (control) to 29±1% at prostanit concentration 10-6 μg/mL. In the case of the ADP-induced aggregation prostanit was effective at concentrations 1-1×10^{-4} μg/mL. Under suppression of endothelial cyclooxygenase by acetylsalicylic acid prostanit inhibited the human platelet aggregation at concentrations 10-3–10^{-7} μg/mL. Under these conditions prostanit at concentrations 10-6 μg/mL and 10^{-7} μg/mL was 1.6 and 1.25 times more effective than prostanit *per se* (arachidonat-induced aggregation). Being administrated to rabbits prosranit decreased the ADP- and collagen-induced aggregation *ex vivo* from 50±3% (control) to 32±4% and 30±3%, respectively. The effect was observed for 2 min after injection. Prostanit reduced the platelet count 1.2 times at 30 min after administration.

Conclusions. Prostanit inhibited the platelet aggregation both *in vitro* and *ex vivo*. The anti-aggregation effect enhanced in the presence of the vessel wall.

P031

ALTERED FIBRINOLYTIC SYSTEM IN NIGERIANS WITH BENIGN PROSTATE HYPERTROPHY

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Background. Haemorrhage may be a major complication in post prostatectomy. it has been shown that fibrinogen and fibrin occur in both benign and malignant lymphoid tissue and that the transformation of fibrinogen to fibrin is attributed to macrophages, which initiated thrombin formation.

Aim. The aim of this study was to investigate possible fobrinolytic activity during prostatectomy despite its complicated fibrin formation. Materials and Methods A total of thirty-five subjects comprising of fifteen patients with benign prostate hyperplasia (BPH) attending the out patient clinic of the University of Benin Teaching Hospital, Benin City, Nigeria and twenty apparently healthy, age and sex matched subjects as controls. Platelet count (PC), Euglobulin lysis time (ELT), plasma fibrinogen concentration (PFC), packed cell volume (PCV) and relative plasma viscosity (RPV) were determined in both groups.

Results. Platelet count, PCV and PFC were significantly lower (p<0.05 respectively) compared with controls. There was a significant decrease in ELT in post operative stage in patients with B.P.H compared with the pre value and controls. Platelet count, PCV and RPV were significantly lower (p<0.001) in B.P.H. patients compared to post prostatectomy state. Conclusion We conclude thrombocytopenia coupled with hypofibrinogenemia with an enhanced fibrin clearing mechanism are major consequences of BPH and could be determinants of bleeding tendencies in the patients. The reduced plasma viscosity could be due to low levels of Fibrinogen and could be regarded as a positive rheologic index.

P032

VON WILLEBRAND FACTOR, FACTOR VIII IN COLORECTAL CANCER

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Background and Aim. High plasma levels of vWf have been reported in patients with colorectal cancer and tend to increase in a stage-dependent manner. Aim was to evaluate the levels of vWf and factor VIII in patients with colorectal cancer and correlation of these values with primary tumor localization, tumor stage, histology, obstruction, perforation, plasma levels of lactate dehydrogenase,carcinoembryonic antigen.

Materials and methods. This study is consisted of 65 newly diagnosed patients with histologically confirmed colorectal carcinoma, 37 healthy controls. None of the patients included no prior cytotoxic therapy. The patients were categorized in to stages according to Modifiye Aster Coller Classification. None of the controls suffered from diabetes mellitus, coronary artery disease and no history of orderly medication causing coagulation disorders. Plasma levels of of vWF were measured by immuno-turbidimetric assay of Dade Behring, reference interval was %17.5-37.5. Plasma levels of factor VIII were measured by coagulometric assay of Dade Behring, reference interval was 70-150% of the norm. Statistical analysis was carried out using the SPSS 11,0 for WINDOWS statistical software package. Nonparametric tests were employed since none of the parameters elicited normal distrubution despite logarithmic transformation. Mann Whitney U-test was applied for statistical comparison of the patients and controls.

Results. Patients with colorectal cancer (%113±42.4, n=65) have significantly higher plasma levels of vWF than controls (%52.4±27.6, n=37) (p=0.0002). There was not statistical significant correlation between plasma levels of vWf and gender, tumor staging, pathology,plasma levels of CEA and LDH, bowel obstruction and perforation. Statistical significance was not reached for factor VIII.

Conclusions. Our data indicates that plasma levels of vWF were elevated in patients with colorectal cancer comparing with healthy controls, but not a stage-dependent manner in contrast to literature. At the other hand, there was not statistical significant correlation between plasma levels of factor VIII and gender, tumor staging, pathology, plasma levels of CEA and LDH, bowel obstruction, perforation.

P033

THE COW-BRAIN THROMBOPLASTIN AS AN ALTERNATIVE SOURCE OF TISSUE THROMBOPLASTIN FOR PROTHROMBIN TIME TESTS

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Our objective is to prepare an in-house Thromboplastin from Cow-brain tissue for PT and to assess its reproducibility, efficiency and usage in detecting coagulation disorders.

We carried out Prothrombin Time (PT) tests using an in-house preparation of cow brain Thromboplastin at the Department of Haematology, A.B.U Teaching Hospital Zaria-Nigeria, and tested this on citrated plasma from 50 healthy blood donors. As a control, commercial rabbit brain Thromboplastin was used and the tests were carried out in duplicates at 37°C.

The average PT was found to be 26.70±1.75 as against the control of 15.02±0.81. This was significantly higher ($p<0.05$) and correlated well with the commercial reagent [r=0.9150, t=15.7162, $p<0.05$]. There was a close range between the reference values of the cow brain Thromboplastin demonstrating an acceptable level of precision.New product was efficient in detecting established cases of prolonged PT; we thus, suggest that cow brain Thromboplastin could be an alternative reagent for Prothrombin Time tests.

P034

THROMBOCYTIC RESPONSE IN NIGERIANS WITH BENIGN PROSTATIC HYPERTROPHY

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Background. During open prostatectomy, the adenomatous zone of the prostate gland is removed leaving behind the carcinomatous zone, which is a large raw vascular surface from which haemorrhage is likely to continue after surgery for sometime regardless of any degree of haemostasis at surgery. It is therefore necessary to investigate platelet counts which is of primary haemostatic importance with benign prostate hypertrophy (B.P.H) before and after transvesical prostatectomy.

Aim. This study was therefore designed to study thromcocytic response before and after prostactectomy.

Methods. Twelve (12) histopathologically proven BPH patients from surgical out patient clinic that had prostatectomy were included in the study. Platelet counts (PLC) were estimated intensively for a period of 3 weeks. Twenty-five age and sex matched apparently healthy elderly men with no evidence of neoplastic or haemostatic disorders were used as controls.

Results. There were no significant difference between controls and pre-surgical values of PLC but a graded statistically significant increases were recorded after surgery. ($p<0.05$ respectively).

Conclusions. We conclude that continuous rise in PLC after prostactectomy could be a haemostatic advantage at the onset but a predisposing factor to thromboembolic complications. Post-surgery monitoring especially in the first 3 weeks is hereby emphasized.

EPIDEMIOLOGY OF THROMBOSIS

P035

PROGNOSIS OF OVARIAN CANCER ASSOCIATED WITH VENOUS THROMBOEMBOLISM: A NATIONWIDE DANISH COHORT STUDY

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Background. Few data exist on the prognosis of ovarian cancer discovered during or after an episode of venous thromboembolism.

Aim. Our aim was to estimate the impact of venous thromboembolism on survival of ovarian cancer.

Materials and methods. We identified all ovarian cancer patients registered in the Danish Cancer Registry (1980-2003), with a hospital discharge diagnosis of venous thromboembolism (VTE) in the Danish National Registry prior to or at the same time as the cancer diagnosis. Survival of patients who received a diagnosis of ovarian cancer at the same time as or after an episode of venous thromboembolism were compared with that of all other Danish ovarian cancer patients without VTE (control cohort). The patients were followed at least one year (range 1-24 years). Cox-regression analyses were used to adjust the mortality rate ratios for age, stage of disease and calendar period.

Results. 240 ovarian cancer patients were registered with a diagnosis of venous thromboembolism at the same time as or before the cancer diagnosis. Of 88 patients who had cancer at the same time as an episode of venous thromboembolism, 38.6 % of those with data on stage of the disease (83 patients) had stage IV disease, as compared with 32.1 % of 12,778 ovarian cancer patients (all ovarian cancer patients without VTE) with data on stage (prevalence ratio, 1.2; 95% confidence interval (CI), 0.9 to 1.6). We found an adjusted mortality ratio of 1.9 (95% CI, 1.5-2.4) for the patients diagnosed with VTE and ovarian cancer at the same time during the first month after the cancer diagnosis. Patients in whom ovarian cancer was diagnosed within one year after an episode of venous thromboembolism also had an increased risk of having stage IV disease at the time of diagnosis (prevalence ratio, 1.4; 95% CI, 1.0 to 1.8), and adjusted mortality ratio was 1.1 (95% CI, 0.8-1.4).

Conclusions. The prognosis of ovarian cancer is associated with the complication VTE. Ovarian cancer discovered at the same time as venous thromboembolism tends to be at an advanced stage with a poor prognosis.

P036

LOW RATE OF SYMPTOMATIC THROMBOSIS FOLLOWING ECOGUIDED CENTRAL VENOUS CATHETERISATION IN CANCER PATIENTS

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Background. The incidence of Central Venous Catheter (CVC)-related thrombosis in cancer patients has been recently reported to be lower compared to previous data; improved catheter characteristics and advances in insertion techniques may explain this difference.

Aim. We evaluated the incidence of symptomatic CVC-associated thrombosis following a new procedure of central venous line insertion in cancer patients.

Patients and methods. 550 consecutive cancer patients were enrolled (260 males, 290 females) median age 65 (range 20-85): 344 patients had solid tumors (186 colon carcinoma; 64 gastric carcinoma; 94 others) and 206 haematological malignancies (70 non-Hodgkin's lymphoma, 46 acute myeloid leukaemia, 31 acute lymphoblastic leukaemia, 59 multiple myeloma). In 82 patients CVC was inserted prior to high dose chemotherapy and bone marrow transplantation. We choose ecoguided approach, using a 7.5-Mega-Hertz probe placed upon sternocleidomastoid muscle insertion onto the clavicula. Ultrasound technique allowed us a real time control of the needle hip while it was inserted in the last portion of internal jugular vein. Sekalon Seldy, a 16 Gauge single lumen catheter of Becton Dickinson, was employed in 478 patients. In 72 patients a 14-gauge Arrow double lumen was employed in order to perform 72 peripheral blood stem cell collection. No specific antithrombotic pharmacologic prevention was scheduled for any patients.

Results. This route of insertion so far was applied 641 times in 550 patients: 4 times in 3 patients, three in 11 and twice in 60. Among 641 catheterisation attempts, only five failed; no pneumotorax was registered. 26 catheterisations were succesfully performed without complications when platelets were under 20,000/μL. Mean time of catheter permanence was 151 days (range from 30 to 701 days); symptomatic thrombosis of internal jugular vein was diagnosed in four patients (0.7%) and in all of them catheters were removed because of malfunction. Conclusions Our study shows a 0.7% rate of symptomatic trombosis in absence of thromboprophylaxis following CVC placement in cancer patients. The new US guided insertion technique and the small diameter of the used in our trial may have contributed to the low incidence of CVC-associated thrombosis.

P037

THE RISK OF A SECOND DIAGNOSIS OF CANCER AFTER HOSPITALISATION FOR VENOUS THROMBOEMBOLISM IN CANCER PATIENTS

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Background. Venous thromboembolism is common in patients with cancer and may be a marker of undiagnosed cancer. However, it is not known if it venous thromboembolism is a marker of a new malignancy among patients already diagnosed with cancer.

Aim. Our aim was to examine if venous thromboembolism in patients with known cancer is associated with risk of a new malignancy.

Materials and methods. Using the Danish Cancer Registry and National Registry of Patients, we studied a population-based cohort of 6,285 patients with cancer who had an episode of venous thromboembolism. The risk of a new cancer diagnosis was compared with that among 30,713 cancer patients without venous thromboembolism, matched for age, sex, cancer site and year of diagnosis.

Results. Overall, the relative risk for a new cancer diagnosis was 1.3 (95% confidence interval (CI), 1.1-1.4). However, the excess risk varied with the time from the initial cancer diagnosis to the thrombotic event. If the thrombotic episode occurred within the first year, the relative risk was 1.0 (95% CI, 0.9-1.3), but if the venous thromboembolism occurred more than one year after the initial cancer, the overall relative risk for a new cancer was 1.4 (95% CI, 1.2-1.7). Strong associations were found for cancers of the digestive organs, ovary, and prostate during the first year of follow-up.

Conclusions. The association between venous thromboembolism and subsequent incident cancer extends to patients who already have a cancer diagnosis.

P038

PREVALENCE OF G20210A AND MTHFR MUTATION IN IRANIAN PATIENTS WITH VENOUS THROMBOEMBOLISM DISORDER

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Background. Several inherited defects of coagulation disorder, including recently identified G20210A mutation in prothrombin gene and C677T mutation in methylene tetrahydrofolate reductase gene, are associated with increased risk of thrombosis and recurrent thrombosis. However, the rate of these mutations is controversial in increasing the risk of recurrent venous thrombo-embolism (VTE).

*Aim*s. In this cross-sectional descriptive study we investigated the prevalence of this mutation in Iranian Patients with a VTE disorder history.

Material and methods. Using PCR-RFLP method a total of 299 patients (38.8% male and 61.2% female) with clinically approved VTE were genotyped for prothrombin G20210A and MTHFR C677T mutations. Complete evaluation for Protein C&S, anti-thrombin III and APC-R, have been done using clotting and colorimetric methods (Diagnostica Stago, France).

Results. A total of 5 patients (1.7%) harbored heterozygote genotype for G20210A mutation, 40% of patients had evidence of DVT in lower extremities, 20% had CVA history and 40% had experienced idiopathic abortions. In contrast, the mutation frequency for MTHFR C677T was 30.8%, from which 23.2% were heterozygotes and 7.6% were homozygotes. A meaningful relationship (p=0.024) was investigated between this mutation and thrombosis at lower extremities and thrombosis at unusual sites.

Conclusions. Here we report allelic frequencies for sequence variation of two genes which their presence is correlated with an increased risk of thrombosis. According to our study for VTE patients the prevalence of G20210A mutation is very low comparing to MTHFR C677T mutation. The hall mark of the study is site-specific association of this mutation in the absence of other genetic predisposing factors (e.g; Proteins C&S).

P039

HIGH INCIDENCE OF VENOUS THROMBOEMBOLISM EVENTS IN PATIENTS TREATED WITH THALIDOMIDE, TEMOZOLOMIDE AND CISPLATIN FOR GLIOBLASTOMA. *RESULTS.* FROM A PHASE I STUDY

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Background. Th's anti-angiogenetic effect has an emerging role in the haematological and oncological practice. Combining standard chemotherapy and Th is one of the challenge for present and future clinical studies. Side effects of Th single agent are well known but some new issues are emerging combining Th with standard chemotherapy. In Pts with multiple myeloma there is evidence of increased VTE risk when Th is combined with doxorubicin-containing regimens (16 Vs 2.5 %, Zangari *et al.,* Blood, 2002).

Materials and methods. We conducted a phase I study valuating the combination of T and C every three weeks, without and with Th, in Pts with glioblastoma (maximal tolerated dose of Th 100 mg daily, Cisplatin 75 mg/m^2 day 1 every 21days and T 150 mg/m^2 days 1 to 5 every 21days).

Results. We reported symptomatic VTE in 3 Pts out of 5 (2 DVT and 1 PE) in the group of patient treated with full combination (C+T+Th) - overall rate of 60% - and none case of VTE in 6 Pts treated with C+T only (Zustovich F, Proc ASCO 2005, #1554). Overall VTE incidence in high-grade malignant gliomas pts is usually about 20% (Dhami MS *et al.* Thromb Haemost, 1993). Moreover the combination of Th (median daily dose of 200 mg) and T (200 mg/m^2 for 5 every 28 days) does not seem to increase the VTE risk in HGMT with an overall VTE rate of 25% (Susan M *et al.* 2004).

Conclusions. Our series is too small to perform any statistical analysis and/or valuation of known risk factor as presence of paresis. Nevertheless these data suggest that C+Th, as doxorubicin, may enhance the risk of VTE. To our knowledge there are no other reports on this matter in the worldwide literature.

P040

VENOUS THROMBOEMBOLISM DURING BREAST CANCER CHEMOTHERAPY: THE IMPACT ON SURVIVAL

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Background. Venous thromboembolism (VTE) in cancer chemotherapy is common, occurring in 2-10% of early breast cancer patients (EBC) and up to 17.6% of advanced breast cancer (ABC) patients. VTE in cancer patients may be a surrogate marker for aggressive disease and poor outcome.

Aim. To assess the incidence and risk factors for VTE during breast cancer chemotherapy, and the relationship of VTE to cancer progression and survival.

Patients and methods. EBC (n=87), ABC (n=36), and neoadjuvant breast cancer (NBC, n=11) patients undergoing chemotherapy were prospectively followed for two years. Duplex ultrasound imaging was performed one month following commencement of chemotherapy. Clinical and radiological follow-up was at three, six, 12 and 24 months.

Results. Six of 36 (17%) ABC and seven of 87 (8%) EBC, but none of 11 NBC developed VTE. The increased rate of VTE in ABC compared to EBC approached significance (p=0.06).

Three (8.3%) ABC patients, each of whom developed VTE within 3 months, died from VTE. Nine of the 13 (69%) patients developing VTE did so within three months of commencing chemotherapy. Two of seven EBC with VTE developed metastases within two years, compared with four of 80 EBC without VTE. All 6 ABC with VTE progressed or died within six months, compared with 16 of 30 ABC without VTE. VTE within three months of commencing chemotherapy suggested a poorer outcome (p=0.07). Age, menopausal status, hormone receptor status and recent surgery were not associated with VTE (p=1.0-0.2).

Conclusions. This study demonstrates a similar rate of chemotherapy-induced VTE to other published studies. The presence of a large cancer load (ABC) is the only identified risk factor for VTE. VTE may be a surrogate marker for aggressive cancer however VTE also occurs in patients with a good cancer prognosis. Thromboprophylaxis to avoid such morbidity and mortality should be considered.

P041

PROSPECTIVE COMPARATIVE TRIAL OF PATIENTS WITH AND WITHOUT CANCER SUFFERING FROM ACUTE VENOUS THROMBOEMBOLISM: IS THERE A DIFFERENCE?

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Objective to investigate and compare the epidemiology, clinical and laboratory characteristics of cancer and non-cancer patients diagnosed with venous thromboembolism (VTE) in a large referral medical center in Israel.

Patients and methods. Between February, 2002 and February, 2003 all patients, diagnosed in Rambam medical center as suffering from VTE event (deep vein thrombosis) and/or pulmonary embolism), based on diagnostic findings on Doppler ultrasonography, pulmonary scan or diagnostic spiral CT scan for pulmonary embolism, were prospectively identified and evaluated. In addition, at the conclusion of the study period, the results. of all spiral chest CT scans performed during the aforementioned period in this hospital were retrospectively reviewed to minimize the number of unidentified cases. Blood samples were drawn for evaluation of coagulation profile.

Results. 147 patients (79 men and 68 women) with 153 events were identified. The annual incidence of venous thromboembolism in this hospital was approximately 0.25%. Cancer group included 63 patients (43%), most of them had an advanced disease (63%). The most common malignancies were cancer of lung, breast, colon and pancreas (in a descending order). Among 121 events of venous thrombosis (with or without pulmonary embolism) there were 14 events of upper extremity thrombosis (11.5%). The most common risk factors for venous thromboembolism, except malignancy, were immobilization, surgery/trauma and congestive heart failure (in a descending order). There was no difference in the prevalence of various risk factors between cancer and non-cancer patients. During an acute VTE event, D-dimer levels were higher in cancer patients, [4.04 ($\pm$4.27)], than in non-cancer patients, [2.58 ($\pm$1.83), p=0.055]. There was no difference in APC-SR or GLPC values. *Conclusions.* In large Israeli referral medical center, which includes cancer treating center, the proportion of cancer patients among patients suffering from venous thromboembolism was high, while their demographic, clinical and laboratory characteristics (during an acute event) were not different from those of non-cancer patients, except higher D-dimer levels.

P042

VENOUS THROMBOEMBOLISM IN PATIENTS WITH PRIMARY BONE OR SOFT TISSUE SARCOMAS

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Background. The association between malignancy and venous thromboembolism (VTE) has long been recognised. Equally, orthopaedic surgery is known to be a risk factor for VTE. Patients with bone or soft tissue tumours who undergo orthopaedic surgery are likely to be at even higher risk for thromboembolic events, however there is a paucity of data in the literature about this group of patients.

Aim. The aim of this retrospective study was to determine the rate of clinically detected deep venous thrombosis and pulmonary embolism in patients with trunk or extremity bone or soft tissue sarcomas.

Patients and methods. The clinical records of patients with a confirmed diagnosis of primary bone or soft tissue sarcoma presenting between 1998 and 2003 were reviewed. Data relating to patient demographics, diagnoses and surgical interventions, risk factors for thromboembolism, thromboembolic prophylaxis and clinical thromboembolic events were retrieved.

Results. 252 patients were identified. 94 had a diagnosis of primary bone sarcoma and 158 a diagnosis of primary soft tissue sarcoma. 137 (54%) were male of mean age 53 (range 15 to 94). Thirty-seven patients were suspected clinically of having a deep venous thrombosis, 10 of which were confirmed radiologically, giving a rate of 4%. Nine patients had a suspected pulmonary embolism, 2 of which were confirmed radiologically and one of whom died of pulmonary embolism, giving an overall rate of fatal pulmonary embolism of 0.4%. Overall, 70% of the cohort received chemical or mechanical prophylaxis with 60% of patients receiving LMWH. All patients with thromboembolic events had large thigh tumours, with the majority of events (6 of 10 deep venous thromboses and 2 of 3 pulmonary embolisms) occurring prior to definitive surgery.

Conclusions. The risk of a clinically apparent thromboembolic event in patients with bone or soft tissue sarcomas is comparable to that in other orthopaedic patients. Risk factors for venous thromboembolism include lower extremity sarcomas and mechanical obstruction of the venous system. Consideration should be given to excluding deep venous thrombosis before surgery.

P043

DIAGNOSIS AND MONITORING OF CENTRAL VENOUS LINES RELATED THROMBOSIS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA: PROSPECTIVE STUDY DURING CVL PLACEMENT AND AFTER CVL REMOVAL

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Background. Children with ALL have a significant risk of CVL-related VT during induction treatment with L-asparaginase. The reported long term outcome of symptomatic and asymptomatic CVL-related VT are post-phlebitic syndro-

me, recurrent VT and rupture. Bilateral venography is the gold standard to investigate intrathoracic veins, as ultrasound (US) may detect VT mainly in cervical veins. In children a non invasive and valid alternative to venography may be spiral computed tomography (CT).

Aim. To detect and monitor outcome of symptomatic and asymptomatic CVL-related VT, by using spiral CT (Siemens Somaton Plus and Magic View software) and ultrasound (US) at the end of induction (after 8 doses of L-asparaginase) and after CVL removal.

Materials and methods. 50 (age range 1-14 years) out of 70 patients with ALL consecutively diagnosed between January 2000 and December 2002, obtained a complete follow-up. Patients were treated according to AIEOP ALL 95 and 2000 protocols (13 and 37 respectively). In ALL 2000 protocols patients were randomized for prednisone or dexamethasone during induction. CVL were inserted within 15 days from diagnosis by venous cut-down technique in 39 cases (in 61% via right jugular vein) and percutaneous (subclavean vein) in 11 patients. Patients were screened for FV Leiden, G20210A, MTHFR, Lp(a), basal homocysteine, LAC. *Results.* The first spiral CT showed abnormalities in 38/50 (76%) patients: 20/50 (40%) abnormalities were CVL thrombosis and 18/50 (36%) defined the borderline group. Ultrasound performed at the same time of CT was able to demonstrate neck veins thrombosis in all cases. No correlation was found between pro-thrombotic markers and CVL-VT. Only three patients, all in the induction phase, had physical findings of symptomatic thrombosis with an incidence of 6%. After CVL removal CT scan was normal or showed reduction in 68% of patients; so the overall incidence of CVL VT in this population after CVL removal is 24%. No patient experienced post-phlebitic syndrome.

Conclusions. Incidence of early asymptomatic CVL-related VT in children with ALL is significant and spiral CT is sensitive in detecting occlusion and monitoring outcome. Prospective study are needed to provide effective strategies to prevent early positioned CVL related VT in children with ALL.

P044

INCIDENCE OF DEEP VENOUS THROMBOSIS ASSOCIATED WITH CENTRAL VENOUS CATHETERS IN CANCER PATIENTS: A MULTICENTER STUDY

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Background. The reported incidence of DVT-CVC, obtained mostly from old series, is extremely variable (0.3-28.3% for symptomatic thrombosis and 27-66% for asymptomatic thrombosis). Data in regarding new catheter materials and insertion techniques is very limited. Likewise, the use of antithrombotic prophylaxis in cancer patients with a CVC remains debatable.

Aim. The objective of this study is to document the incidence of DVT-CVC in patients with cancer and identify the possible associated risk factors, as a previous step for initiating a clinical trial with antithrombotic prophylaxis in these patients.

Patients and methods. A prospective epidemiologic study was performed, which included adult patients with active cancer and no history of venous thromboembolism, in whom a long-term CVC was inserted (minimum time expected of catheter maintenance, 3 months). Patients were followed-up at 90 days, during which a phone interview took place every 15 days and a bilateral upper extremity doppler-ultrasound was performed on days 45±5 and 90±5. Likewise, basal, 45 day, and 90 day blood samples were taken and will be analyzed for hypercoagulability markers and prothrombotic factors.

Results. inclusion period lasted from july 2004 until may 2005. 132 patients have completed the follow-up, with a median age of 52.1 years (range 21-87). The cumulative incidence of DVT-CVC was 15.2% (20/132): 6.8% were symptomatic (9/132) and 9.6% were asymptomatic (11/115). All thrombotic events, excluding 4 cases, occurred before day 45. Neither age, gender, type of tumor (solid or haematological cancer), tumor stage, type of CVC (Port or tunnelled), number of lumens, side of insertion, platelet count or use of antithrombotic prophylaxis were significantly associated with the incidence of DVT-CVC. Patients with prior central catheters tended to have a greater incidence of DVT-CVC (p=0.10). The cumulative incidence of other complications related to the CVC were: occlusion 9.1%; infection 3.8%; premature removal 6.0%.

Conclusions. DVT-CVC is a frequent complication in cancer patients, as with solid tumors so with haematologic malignancies. The incidence of DVT-CVC seems to be specially high the first few weeks following catheter placement. New studies must be performed to allow identification of at risk patients who might benefit from antithrombotic prophylaxis.

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P045

INHERITED AND ACQUIRED THROMBOPHILIA IN ADULTS WITH ACUTE LEUKEMIA AND THROMBOEMBOLIC EVENTS

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Background. Thrombotic events (TE) are poorly investigated complications of adults with acute leukemia (AL).

Aim. of this study was to determine the prevalence of TE and to detect any association of TE with the presence of congenital or acquired prothrombotic factors in patients affected by AL.

Methods. From January 2000 to June 2005 we evaluated the prevalence of genetic and acquired risk factors of thrombosis in a consecutive series of 114 adult patients with AL at diagnosis. Forty-four patients had acute lymphoblastic leukemia (ALL) and 70 acute myeloblastic leukemia (AML) (AML-M3 in 14 cases). Median age was 39 years (range 16- 62). They were treated according to the established GIMEMA protocols. No patients received heparin or any other anticoagulants. Factor V G1691A Leiden mutation (FV-L), prothrombin G20210A (FII20210) gene variant, MTHFR C677T variant, protein C, protein S and antithrombin (AT) deficiency and the presence of Lupus Anticoagulant (LA) and anticardiolipin antibodies (aCL) were evaluated. *Results.* FV-L was observed in 3 patients (2.6 %), FIIA20210 mutation in 5 patients (4.4%) and MTHFR C677T variant in 15 (15.4%) patients. The prevalence of these mutations was not statistically different from that observed in the general population from the same ethnic background. Forty-six (45%) patients had hyperhomocysteinemia (>95% above reference values), partially related to the presence of TT MTHFR homozygosity. As far as natural anticoagulants are concerned, 12 patients (14.8%) showed low levels of pro-

tein S, 19 patient (23.4%) showed low levels of protein C and four patients (3.8%) showed AT deficiency. Low titer of aCL was found in 5 cases and LA positivity in thirteen patients. Forty-four patients (8.6%) had a single defect, thirty-three (28.9 %) had; ≥2 defects. Eleven patients (9.6%) (2 ALL, 5 AML and 4 AML-M3) developed TE, 9 out of them in the first month of follow up. The OR for TE in patients with one or double thrombophilic defect was 2.04 (95% CI=0.43-9.64, p=0.5) and 6.11. (95% CI=1.21-30.94, p=0.035) respectively.

Conclusions. The prevalence of TE is exceedingly high in adults with AL. Patients with thrombophilic defects appear to be at high risk for the development of early TE.

P046

VENOUS THROMBO-EMBOLISM: A COMPLICATION OF ACUTE LEUKEMIA AND CHEMOTHERAPY; A CLINICAL EVALUATION OF RISK FACTORS ON A COHORT OF 42 AML PATIENTS

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Background. VTE appears to be a common complication of A.M.L., mostly during chemotherapy, often related to the use of VCC and to genetic factors.

Aim. To assess the risk factors dependent on AML and chemotherapy and distinguish them from those that appear related to genetic, molecular, and *local* special conditions. *Patients and methods.* 42 patients were evaluated between November 2001 and November 2004. We excluded *Frail* patients and older than 70 year, PML, full-blown DIC, and patients treated with Gemtuzumab Ozogamicin. We adopted the following chemoterapeutic regimens: LAM99P, GIMEMA0594, AML03, FLAG; FLAD, Ara C monotherapy. VTE was detected performing D-Dimer, regional ultrasonography, echocardiogram, routinely blood tests for A.L., blood count chiefly, PT, aPTT, Fibrinogen assays, assiduous clinical examinations. On VTE patients we evaluated the following: a) general factors of risk related: - to leukaemia, - to chemotherapy, - to neutropenia, - to long confinement to bed, - to infections; b) individual factors of risk - mutations of FactorV, of Prothrombin, of MTFHR, c) VCC-related risk *Results.* In the 42 AML patients we found: M_0=7, M_1=8, M_2=9, $M_4$9 M_5=8, M_7=1; 26 males, 16 females; 2 had abnormal transcripts leukaemia-related; 8 VTE patients: DVT, PE, blocked VCC, 2 VTE occurred at admission, 4 over chemotherapy, 2 during the aplasia and simultaneous with severe bacterial infections. Among the 42 patients, 30 had VCC. In 6 the removal was needed because of thrombotic occurrences. 2 had previous IMA. Six VTE patients had a thrombofilic genetic risk. Two VTE patients had <40,000 PLTs/mL and significant haemorrhages, two pts had >40,000 PLTs/mL, and four pts had >80,000 PLTs/mL.

Conclusions. The incidence of VTE on our cohort of patients is quite higher than on general population, it is higher than on internistic patients and almost similar to the orthopaedic patients. The *results.* support the evidence that VTE is a significant risk during leukaemia and chemotherapy. They present us with the necessity to distinguish the *general* risk from those due to individual genetic frame or to proteosomic trouble induced by leukaemic occurrence .

P047

THE CLINICAL COURSE OF DEEP VEIN THROMBOSIS IN PATIENTS WITH GYNECOLOGIC CANCER

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Background. Multiple clinical, pathologic and laboratory studies confirmed the association between venous thrombosis and malignancy. Epidemiological studies have demonstrated an increased risk of subsequent cancer in patients diagnosed with idiopathic venous thrombosis or thromboembolism. An extensive workup of cancer is generally not indicated in these patients because in most cases the cancer is widespread and the prognosis is poor.

Aim. The aim of this study was to evaluate the prevalence of deep vein thrombosis in gynecologic cancer patients distributed between ovarian, uterine, cervix, vulva, vagina, diagnosed by Doppler.

Methods. We retrospectively reviewed the charts of patients admitted to our Institution with gynecologic malignancy who had suspicious of deep vein thrombosis (DVT) from July to December 2004. Patients' data were collected by the register of the Doppler of veins. A total of 111 patients with clinical suspicious of deep vein thrombosis (DVT) were submitted to exam.We analyzed regarding site, stage, histology, treatment, and proximity of DVT to treatment with surgery, chemotherapy and radiotherapy. This study was limited to cases of ovarian, uterine, vagina, vulva and cervical cancer

Results. A total of 50 cases were identified. They were distributed between ovarian (23), uterine (6), cervix (17), vulva (2) and vagina (2).

Conclusions. The development of DVT in conjunction with a gynecologic malignancy connotes a poor prognosis. It is possible that this poor prognosis is related to the pathophysiology that results. in venous thrombosis and not just the presence of cancer. The major prevalence in ours patients were in tumor of ovarian followed by cervical cancer.

P048

UNEXPECTED PULMONARY EMBOLI ON ROUTINE CANCER STAGING CT SCANS

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Background. Multi-detector row CT (MDCT) scanning for routine cancer staging has led to an increase in the detection of pulmonary emboli (PE) in patients whose referring physicians were not suspecting the diagnosis.

Aim. We evaluated the risk factors, malignancy types and stages, cancer treatments, and PE characteristics among a large group of patients with unsuspected PE. We searched for signs or symptoms characteristic of venous thromboembolism (VTE) which were overlooked or attributed to other etiologies.

Materials and methods. We performed a retrospective chart review of 60 patients found to have unsuspected PE on routine cancer staging MDCT scans between May, 2003 and December, 2004. We recorded patient demographics, risk factors, primary malignancies, cancer therapies and signs/symptoms reported in the medical record within 2 weeks of diagnosis with PE. The location and number of PE were also documented.

Results. Seven patients were excluded from the final analysis due to known VTE within 1 year. Of the 53 patients included in the analysis, there were 21 females and 32 males

with a median age of 64.5 years. Twenty (37.7%) patients had a central line and 11 (20.7%) patients had prior history of VTE. The most common primary malignancies were colorectal adenocarcinoma (n=18), bladder cancer (n=5), and gynecologic malignancies (n=4). Forty-five (84.9%) had Stage IV malignancies. Forty-two (79.2%) had received systemic cancer therapy; of those, 28 (66.7%) had received therapy within 30 days of diagnosis of PE. A total of 106 PE were identified in the following regions: main pulmonary artery (n=9), lobar (n=29), segmental (n=38) and subsegmental (n=30). Twelve (22.6%) patients had isolated subsegmental PE. Signs/symptoms included: tachycardia (n=10), limb pain or swelling (n=9), chest pain (n=3) and shortness of breath (n=12). In all, 23 (43.4%) patients had any sign or symptom of PE.

Conclusions. We are the first authors, to our knowledge, to document and characterize a large number of cancer patients unexpectedly found to have PE on routine cancer staging. Most were receiving cancer therapy and many actually had signs or symptoms attributable to VTE. We also report the highest incidence yet of isolated subsegmental PE.

P049

CLINICAL SIGNIFICANCE OF REVEALING OF GENETIC FORMS OF A THROMBOPHILIA IN CANCER PATIENTS WITH RECURRENT VTE

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Background. High rate of the recurrent venous thromboembolism (VTE) as often complication in cancer patients has induced us to carry out association between congenital defects of a hemostasis system and the recurrent VTE in oncogynaecological patients.

Aim. Determination of the rate and structure of genetic forms of a thrombophilia in cancer patients with recurrent VTE.

Patients and methods. We have examined 31 patient with trombotic episodes in the past: 17 patient has ovarian cancer, 8 uterine carcinoma, 6 cervical carcinoma. Medical history: 2 patient has a myocardial infarction, 6 patients has stroke, 9 patients has deep vein thrombosis (DVT), 6 patients has pulmonary embolism (PE), a combination of 2 and more forms of VTE has 5 patients, 3 patients has a thrombosis of retina vessels. 11 patients has occult cancer then was initial episode of VTE, 20 patients have VTE after malignancy was determined. 24 patients have family history of VTE. The control group includes 30 women with gynaecological cancer without any VTE episodes and has not family history of VTE. Laboratory tests: Detection of FV Leiden mutation, prothrombin G20210A mutaton, gene PAI-1 G4/G5 polymorphism, gene MTHFR C677T mutation, genes of platelets glycoproteins polymorphism: GP IIb/IIIa, GP Ia/IIa, GPIbα, GP ADP.

Results. We have detected the incidence of FV Leiden mutation is 6 (19.4%); of homozygous gene MTHFR mutation is 13 (41.9%); of heterozygous gene MTHFR mutation is 16 (51.6%); of prothrombin mutaton is 5 (16.1%); of gene PAI-1 polymorphism is 9 (29%); of platelets glycoproteins polymorphism is 14 (45.2%). In control we have detected FV Leiden mutation in 3 patients (10%); homozygous gene MTHFR in 2 (6.7%), heterozygous gene MTHFR mutation in 5 cases (16.6%); gene PAI-1 polymorphism in 3 (10%) and platelets glycoproteins polymorphism is 3 patients (10%). *Conclusions.* Higher frequency of genetic forms of a thrombophilia in cancer patients with recurrent VTE allows to conclude that presence of multigenic thrombophilia is the expressed trigger of VTE. So patients with multigenic thrombophilia should be permanently treated by anticoagulats (Low-Molecular-Weight Heparin (LMWH) (under the control of thrombophilia markers, such as D-dimer, TAT complexes and platelet factor 4.

P050

THE PREVALENCE OF FACTOR V LEIDEN AND PROTHROMBIN G20210A POLYMORPHISM IN PATIENTS WITH GASTRIC CANCER

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Background. The pathogenesis of haemostatic disorders in cancer is complex and namely on acquired basis. Nevertheless there is some evidence that prothrombin G20210A mutation may be involved in gastrointestinal cancer pathogenesis.

Aim. We performed a prospective case-control study to analyze the prevalence of the two commonest thrombophilic mutations, factor V Leiden and prothrombin G20210A polymorphism, in patients with gastric cancer.

Methods. 121 patients with primary gastric carcinoma and 130 healthy subjects, comparable for age and sex, were investigated. The factor V Leiden was detected by polymerase chain reaction and restriction enzyme digestion, the prothrombin gene mutation G20210A by allele-specific PCR. Statistical analysis was performed by χ^2 test for group comparison frequencies.

Results. In the cancer group the frequency of prothrombotic polymorphisms was the following: factor V Leiden mutation 3.3 % (GA genotype: 4 cases), prothrombin G20210A substitution 8.3 % (GA genotype: 10 cases). In the control group, the frequency of the prothrombotic polimorphysms was the following: factor V Leiden mutation 4.6 % (GA genotype: 6 cases), prothrombin G20210A substitution 6.1 % (GA genotype: 8 cases). We did not find any patient which was heterozygous to more than one locus. There was not statistical difference between the prevalence of the two polymorphisms in the two groups.

Conclusions. this study suggests that in gastric cancer the risk factors of the thrombophilic cancer state are on acquired basis rather than on genetic reasons, and thus prothrombin G20210A doesn't seem to be a cofactor in gastric cancer pathogenesis.

P051

PREVALENCE OF CANCER AND THROMBOPHILIA IN PATIENTS WITH ACUTE VENOUS THROMBOEMBOLISM

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Background. Relationship between symptomatic venous thromboembolism and occult malignant disease is generally accepted but still controversial and there is no consensual recommendations for thrombophilia screening. *Objectives.* To determine the prevalence of congenital and acquired thrombophilia and the frequency of malignant disease (known and occult) in patients hospitalized with acute VTE.

Methods. We studied 360 consecutive patients, [168 women, 192 men, mean age 58±19; patients <40 y.o. n=72 (20%) or >40 y.o n=288 (80%)] with VTE. They were investigated for thrombophilia [Antithrombin (AT), Protein C (PC), Protein S (PS), antiphospholipid antibodies syndrome (APA), Factor V Leiden (FVL) and Prothrombin G20210A (FII)] and occult cancer.

Results. A known cancer was present in 60 patients (16%). We discovered 23 occult cancers (7%) all of them in patients >40 y.o. Thrombophilia was present in 70 patients (20%) among them 16 were previously known: 10 AT (3.15%), 8 PC (2.5%) and 12 PS (3.8%) deficiency, and 6 (1.9%) APA, 28 inherited APC resistance due to FVL mutation (11.3%), heterozygous in 26 and homozygous in 2, and 16 (7.4%) FII mutation. A combined thrombophilia was present in 10 patients (2.7%). Thrombophilia had been detected before the present episode in 4 patients for AT, in 5 patients for PC, in 7 patients for PS, in 4 patients for APA and in 6 patients for FVL. Only one patient with previously known cancer had FVL. Among 69 patients <40 y.o thrombophilia was present in 33 (48%), 16 with FVL or FII mutation. Among 249 patients >40 y.o thrombophilia was present in 37 (15%), 24 with FVL or FII mutation. Moreover, thrombophilia was significantly more frequent in patients with pulmonary embolism (PE) and deep vein thrombosis (DVT) 20.5% (33/161, 19 with FVL or FII mutation), or DVT alone 28% (33/118, 21 with FVL or FII mutation) than in patients with PE alone 10.5% (4/38, 3 with FVL).

In conclusion, hereditary thrombophilia was 3 times more frequent in patients < 40 y.o. than in those > 40 y.o while cancer was absent in patients <40 y.o. However, in patients with FVL and / or FII mutation, acute VTE was more frequently observed after 40 years of age than in patients with AT, PC and PS deficiency, suggesting that all thrombophilias are not the same. The usefulness of screening patients for congenital thrombophilia is suggested in view of its high prevalence. The frequency of occult cancer was relatively high in patients >40 years of age, suggesting that screening for occult cancer seems justified in this group of patients.

P052

IS THERE A ROLE FOR ACQUIRED AND GENETIC THROMBOPHILIC FACTORS IN THE PATHOGENESIS OF THROMBOEMBOLISM IN GASTROINTESTINAL CANCERS?

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Background. TE is a well known and feared complication in cancer even though its relationship with acquired and genetic thrombophilic factors is still not clearly understood.

Aim. To evaluate the levels of physiological coagulation inhibitors and some acquired and genetic thrombophilic factors, and to assess their impact on the development of TE in patients with gastrointestinal cancers.

Patients and methods. From October 2002 to June 2004, 102 consecutive patients (60 males and 42 females) aged >18 years (median:67; range: 28-92) with a newly diagnosed gastro-intestinal cancer observed at the University Hospital *Campus Bio-Medico* have been included in this study. PC, PS, ATIII, LAC, ACA IgG, homocysteine, annexin V and activated PC resistance were tested in all patients before and after surgery and chemotherapy. Moreover, the first 48 patients receiving chemotherapy were also investigated for the presence of Factor V Leiden, prothrombin G20210A gene mutation and the C677T mutation in the MTHFR gene.

Results. A significant modification in the values of LAC of DRVVT type, ACA IgG, PS, ATIII has been observed in the samples taken after surgery ($p<.001$) and before chemotherapy ($p<.004$). Moreover, PC levels were significantly reduced only after surgery ($p<.000$); LAC of KCT type was significantly prolonged before starting chemotherapy ($p<.003$) while homocysteine levels were significantly reduced ($p<.001$) just after the end of the chemotherapeutic protocols. A DVT was diagnosed in 4/20 patients with metastatic disease (20%) and in 4/82 patients (4.8%) with a surgically treatable disease ($p<.02$) with a TE prevalence of 7,8% (8/102). Of the 48 patients tested for thrombophilic genetic mutations 7 (14.5%) had a DVT, even though none had Factor V Leiden or G20210A Factor II mutation, while 4 had a C677T MTHFR mutation (p=NS). Of these 48 patients, 39 received a 5-Fluorouracil (FU) based chemotherapy, and a grade III-IV hematological or gastrointestinal toxicity was present in 25% (7/28) of patients with the C677T polymorphism of the MTHFR while none of the 11 patients without mutation had any toxicity (p=0.06).

Conclusions. The main responsible of DVT in our study is an advanced disease, whereas thrombophilic genetic and acquired factors seem to have no effect on its development. Moreover, patients carrier of the C677T mutation in the MTHFR gene subjected to antimetabolites-based chemotherapy have a tendency to a higher grade (III-IV WHO) hematological or gastrointestinal toxicity (OR: 1.4; C.I. 95%: 0.88-2.24; p=0.06).

P053

EPIDEMIOLOGICAL STUDY OF CANCER AND THROMBOEMBOLIC EVENTS IN AN INDUSTRIAL REGION

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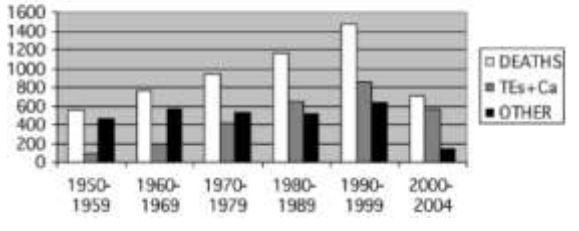
According to previous studies we found out profound change in the causes of death between eastern and western regions of Thessaloniki outside the industrial zone which was developed mainly in the 1950-1960s (the construction of refineries, petrochemical manufactories and tanneries have lead to a significant air and water pollution).

Aim. In order to investigate the changes in the causes of death in the same area we made an epidemiological research by reviewing the death certificates of the last 55 years. Material and methods. We checked 5603 official death certificates and we divided them in three categories: 1-thromboembolic diseases, 2-all type of cancers and 3-others.

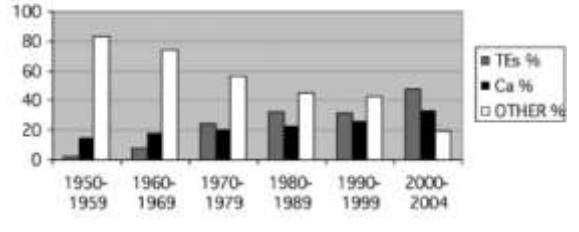
Results. according to graphs 1 and 2 a total reverse of the causes of death between 1950-and 2004 can be seen.

Conclusions. This study confirms our previous ones made by Thrombosis and Haemostasis Unit during the last years (for the east zone the corresponding frequencies are 1 out of 4 for TEs and less than 1 out of 5 for cancer, indicating a potential correlation of death causes with the environmental factors). Our recent study finds a rather significant increase in the percentage of thromboembolic events which raised from 2.6 in the 1950s to 47.5 in our decade. The most strenuous explanation of our findings is related to the air pollution caused by micro particles emitted by the related industries.

Graph 1. Comparison of the total of deaths to the sum of TEs + Ca and Others



Graph 2. Comparison of the total deaths to the sum of TEs, Ca and Other



MANAGEMENT OF THROMBOSIS/BLEEDING AND SYSTEMIC SYNDROMES

P054

DIC IN GASTRIC CANCER: A CASE REPORT

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Background. Disseminated intravascular coagulation (DIC) is a rare paraneoplastic syndrome which can occur in several malignancies. Unusually it occurs as isolated clinical entity.

Case Report. A female patient 55 years old was admitted to our Hospital Unit for thrombocytopenia and multiple ecchymosis. Laboratory findings, i.e. Plts= 21,000/mmc, Prothrombin activity= 71%, Partial Thromboplastin Time (PTT)= 32"8, Fibrinogen=119, Antithrombin III= 96%, D/D= 1024 ng/ml, proved a subclinical DIC. Bone marrow morphologic examination showed 4-5% of cells with 8-10µ size, basophilic cytoplasma and eccentric nucleus. In addition high serum levels of bio-chemical neoplastic markers were found: AFP=2.11, CEA=16.6, CA19-9=93.29, CA125=28.68, CA15-3=32.31, Ferritin=1,179, CA50=37.3, CA72-4=11. Gastric biopsy revealed: *focal infiltration by adenocarcinoma with mucinosis features.*

Conclusions. Abnormal mucina secreted by gastric cancer cells is indicated as DIC's responsible in gastric cancer. Rarely DIC is associated with solid tunors. In several reports it occurs in advanced gastric cancer with methastasis frequently with severe symptoms and under these circumstances antineoplastic chemotherapy starting is the therapeutic approach of choice.

P055

COX-2 INFLUENCES VASCULAR REGULATION AND CORRELATES WITH CLINICAL OUTCOME IN PATIENTS WITH RELAPSED PULMONARY OSTEOSARCOMA

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Background. The prostaglandin pathway is crucial in inflammatory processes, and plays an important role in regulating tumor growth. The most important mechanisms by which COX-2 stimulates tumor proliferation include angiogenesis and inhibition of apoptosis. COX-2 upregulates VEGF expression in malignant cells and therefore contributes to new blood vessel formation. Furthermore, overexpression of COX-2 in adult solid tumors has been shown to correlate with poor prognosis.

Aim. To determine if COX-2 expression in osteosarcoma pulmonary metastases may be used as a prognostic marker. *Patients and methods.* Thirty-six patients initially diagnosed with osteosarcoma between 1990 and 2001 were included in this study. All patients received combination chemotherapy pre- and post-operatively. Chemotherapeutic agents administered included cisplatin, doxorubicin, moderate dose ifosfamide, and high dose methotrexate. Eleven of 36 (30%) patients studied, presented initial metastases to the lungs, the most common site of metastatic involvement. The remaining 25 (70%) patients had localized disease at diagnosis, but subsequently developed osteosarcoma pulmonary metastases either during or after chemotherapy. Archived, paraffin-embedded osteosarcoma lung metastases specimens from these 36 patients were analyzed for COX-2 expression by immunohistochemistry. Grading was assigned according to intensity of COX-2 staining. Patients with negative (0) or very weak (1) COX-2 expression were classified under Group I, whereas those with weak (2), moderate (3), or strong (4) staining were classified under Group II. Clinical data was correlated with immunohistochemical staining results, and cure rates were determined. Cure was defined as having no evidence of disease 5 years after diagnosis.

Results. A total of 11 patients had evidence of initial pulmonary metastases. Five of those 11 (45 %) patients, were classified under Group I. The remaining 6 of 11 (55 %) patients met criteria for Group II. A total of 25 patients presented localized osteosarcoma without pulmonary metastases at diagnosis. Thirteen of those 25 (52%) were classified under Group I, whereas 12 of 25 (48%) met criteria for Group II. Patients without initial metastatic disease achieved a cure rate of 46 % (6/13) if classified as Group I, but only 16 % (2/12) cure rate was observed if patients were classified as Group II. Patients with evidence of initial metastases presented similar cure rates in both groups (25% Group I vs. 20 % Group II). Other variables analyzed including age, gender, race, histologic classification, tumor location, and extent of necrosis, did not affect the outcome of patients with either metastatic or non-metastatic disease. *Conclusions.* Negative or very weak COX-2 expression in osteosarcoma pulmonary metastases established after initial diagnosis is associated with a better outcome. No difference in cure rate is identified for patients with evidence of metastatic disease at diagnosis. COX-2 expression may serve as a prognostic marker for patients with osteosarcoma lung relapse.

P056

FIBRINOGEN PROPERTIES IN HEMATOLOGICAL MALIGNANCIES WITH HIGH FIBRINOGEN LEVELS

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Background. Elevated fibrinogen levels have consistently been found in hematooncological patients. Very little is known, however, about the structure and function of the fibrinogen in these patients. It remains unknown whether elevated fibrinogen has any pathophysiological role or is only a reflection of disease pathogenesis.

Aim. Characterization of fibrinogen structure and function in oncohematological patients with markedly elevated levels of fibrinogen.

Materials and methods. The structure and function of fibrinogen was studied in well characterized groups of oncohematological patients with very high fibrinogen levels using standard coagulation tests, kinetics of fibrinopeptide release, time-course of turbidimetric changes during fibrin formation, SDS PAGE, HPLC and highly accurate and real time optical methods - surface plasmon resonance, atomic force microscopy and MALDI-TOF (matrix assisted laser desorption ionization – time of flight). Normal platelet adhesion in patients plasma on well-characterized fibrin deposits formed on the surface in micro-well plates was

also investigated since platelet adhesion promoted by deposition of fibrinogen might contribute to the development of inflammatory response. We have previously shown that using stepwise synthesis fibrin mesh can be created on the surface starting from adsorbed fibrinogen.

Results. In majority of patients we have not found any signs of structural changes, translational or additional post-translational modifications, changes in fibrinopeptide release, fibrin formation or fibrin structure. Very high fibrinogen level in itself, however, markedly influenced platelet adhesion to fibrin deposits. In some patients, but far from all of them, we have found oxidatively modified fibrinogen which had different ability in binding platelets and in initiating platelet aggregation of washed (or gel-filtered) platelets by ADP. Using a sensitive ELISA method and immunoblotting for nitrotyrosine, nitration of fibrinogen could be shown. Oxidation of fibrinogen influenced the rate of fibrinopeptides release by thrombin, fibrin structure, and also very significantly adhesion of platelets to adsorbed modified fibrinogen.

Conclusions. Very high fibrinogen level in oncohematological patients influences mainly platelet adhesion. Since high fibrinogen concentration predisposes to formation of fibrin(ogen) deposits the finding has its important pathophysiological impact. The oxidative damage of fibrinogen in some patients is also important since nitration and oxidation of fibrinogen considerably change fibrin structure.

P057

SIGNIFICANCE OF REVEALING OF THROMBOPHILIA MARKERS IN CANCER PATIENTS DURING CHEMOTHERAPY AND RADIATION THERAPY

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Background. The chemo- and radiation therapy are significant contributed the risk of venous thromboembolism (VTE) and hemorrhage complications in cancer patients, especial with widespread malignant process. Chemotherapy stipulates for endothelium damage, direct platelet activation and reduction of fibrinolytic activity.

Patients and methods. 180 patients were divides on 3 groups: 56 have ovarian cancer; 70 have cervical carcinoma; 54 have uterine carcinoma. Laboratory tests: Platelet aggregation tests with following stimulators: Adrenaline 1×10^{-4} M, Ristocetin and ADP 1×10^{-3} M. Platelet activation marker test – platelet factor 4 (PF4). Disseminated intravascular coagulation (DIC) and thrombophilia marker tests: D-dimer, TAT complexes, F1+2 prothrombin fragments. Fibrinolytic activity tests: determine PAI level, Protein C and S levels.

Results. We have detected the sign of thrombofilia and DIC in more than 90% patient during chemotherapy and radiation therapy. The rate of thrombotic and hemorrhage complications was about 8%. Patient with uterus and cervix carcinoma radiation therapy has been conducted. It was detected that in 19% concentration of TAT complexes, D-dimer and F1+2 prothrombin fragments was significant higher on normal values, and were not registered spontaneous normalisation of this markers level without anticoagulation therapy by anticoagulats (Low-Molecular-Weight Heparin (LMWH)). It was observed consumption coagulopathy in 15.6 % patients during radiation therapy. During course of chemotherapy in ovarian cancer patients was detected slight reduction in levels of thrombophilia molecular markers. However it was observed damage of fibrinolytic activity due to iatrogenic effects of chemotherapy: reduction in proteins C and S levels, increase PAI concentration, platelets hyperaggregation in ristocetin presence. *Conclusions.* Monitoring of haemostasis state in cancer patient during chemo- and radiation therapy should determine an individual approach to medication application of anticoagulants (LMWH or Warfarin) and allows to lower frequency of VTE and hemorrhage complications.

P058

THE EXPERIENCE OF AN INTERNAL MEDICINE UNITIN DIAGNOSIS AND THERAPY OF THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background. Thrombotic thombocytopenic purpura (TTP) is a rare disease whose annual incidence is about 1/200,000-300,000. A rapid diagnosis and an adequate treatment based on plasma-exchange (PE) and immunosuppression is crucial in abating mortality rate.

Aim. To verify the real prevalence of the disease in the Province of Pordenone, Italy, and to evaluate the outcome of the patients.

Patients. Between 1997 and 2005 seven patients with TTP were treated in our Medical Unit. M/F was 2/5 and mean age was 42.1 years (range 22-67). At presentation mean Hb level was 8.7 g/dl (5.9-11.7), mean platelet count was 22.300/μL (5,000-84,000), mean creatinine 1.75 mg/dL (0.7-5.6 , mean LDH 1,028 U/L (523-2,005); neurological signs were present in 5 and fever in 4 patients. Diagnosis was made on the basis of clinical data and the relief of hemolytic microangiopatic anemia. In four cases ADAMTS13 was found in normal range (determinations after the acute phase); one patient had used ticlopidine for one month before and four patients presented a mild infection some days before the onset of the disease; no one had a history of familial disease. All patients were treated with metil-prednisolone (1-2 mg/Kg/die), fresh plasma infusions and PE, the mean number of procedures was 13 (3-58). Two patients relapsed, one underwent splenectomy, two patients had severe skin rash with fever attributed to plasma so PE was stopped; two were treated with vincristine and could reach complete remission (CR) but it didn't prevent relapses. *Results.* Incidence: The finding of seven patients in 8 years balances the expected number of patients in the population of our Province (276,000). Outcome: the treatment was efficacious and all the patients are still alive and in CR (4-105 months of observation).

Conclusions. The treatment of TTP is based upon PE; corticosteroids are often used as part of initial therapy; splenectomy in the PE era is reserved for refractory patients, with variable response rates; for antiplatelets agents a potential role in preventing relapse has been reported; vincristine, as in our two patients, can have favourable responses; anecdotal reports of others immunosuppressive therapies such as rituximab, cyclophosphamide and cyclosporine need more prolonged and controlled studies.

P059

NOT PUBLISHED

P060

DETERMINATION OF ADAMTS-13 ACTIVITY BASED ON VON WILLEBRAND FACTOR RISTOCETIN COFACTOR MEASUREMENTS

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Background and objective. Thrombotic thrombocytopenic purpura (TTP), a severe clinical disorder presenting with thrombocytopenia, microangiopathic haemolytic anaemia and organ dysfunction is often observed in cases of metastatic cancers at terminal stage. Most patients with idiopathic TTP present with severe deficiency of von Willebrand factor-cleaving protease (ADAMTS-13) activity, whereas patients with cancer-related TTP show only moderate reduction in ADAMTS-13 activity. Moreover, mild ADAMTS-13 deficiency is found in various cancer types without signs of thrombotic microangiopathy, which might suggest a pathological role of ADAMTS-13 in cancer. Quantification and characterization of ADAMTS-13 can be used to differentiate between idiopathic and cancer-related TTP, supporting appropriate therapy decisions. In addition, ADAMTS-13 assays might also be a valuable tool for investigating the role of this enzyme in cancer. This paper describes an ADAMTS-13 activity assay which can be performed on automated coagulation analyzers such as BCT™ and BCS®.

Materials and methods. Von Willebrand Factor substrate is digested at 37°C by the functional ADAMTS-13 of the plasma sample. Subsequently, the residual von Willebrand factor ristocetin cofactor is determined and used to calculate the ADAMTS-13 activity in the test sample. The procedure of the assay allows high throughput and does not require special laboratory equipment and expertise.

Results and Conclusions. The von Willebrand factor ristocetin cofactor based assay displayed an excellent concordance between expected and observed values with good inter-assay reproducibility on the BCT™ test system.

The method can also be used to detect ADAMTS-13 inhibitors and thus allows discrimination between congenital and acquired TTP.

P061

STUDY OF PREVALENCE OF POST TRANSFUSION INFECTION IN THALASSEMIC CHILDERN DASTGHIB HOSPITAL

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Introduction. This cross-sectional descriptive analytical study was carried out in order to evaluate the post transfusion (hepatitis B, C and Aids) in children suffering from major thalasemia as a consequence of transfusion.

Methods. The total number of 53 children of under 15 years old were detected to be suffering from major thalasemic at Dastghib hospital. A check list was completed for each case. ALT, HBsAg, anti–HCV and anti-HIV tests were carried out on all of the 53 children.

Results. The research revealed that 41.5% equivalent to the number of 22 of these children have been infected with ALT. Eighteen of patients (34%) have been confirmed to have positive anti-HCV. In all 18 patients with positive anti-HCV, ALT was also detected. All of the patients were found to be negative for anti-HBsAg and anti-HIV.

Conclusions. Determination of ALT level of the donated blood is useful for detection of chronic hepatitis. This test can be used as an easy screening tool.

P062

THE EFFECT OF LONG TERM ANTICOAGULATION ON THE QUALITY OF LIFE OF ADVANCED CANCER PATIENTS WITH VENOUS THROMBOEMBOLISM: THEMATIC ANALYSIS FROM PATIENT INTERVIEWS

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Background. The incidence of venous thromboembolism (VTE) in cancer increases with disease progression. The risks of anticoagulation with oral coumarins for palliative care patients are well documented and research supports low molecular weight heparin (LMWH) in the long term treatment of VTE. However, the impact of a daily injection on these patients' quality of life (QoL) has not been evaluated and there is a concern that the benefits of efficacy are overshadowed by the burden of treatment.

Aim. To assess the impact of anticoagulation on QoL in advanced cancer patients with confirmed VTE.

Patients and methods. Patients with advanced metastatic malignancy, receiving long-term LMWH for treatment of VTE were recruited. Semi-structured taped interviews exploring quality of life domains were carried out and transcribed. Transcripts were analysed for recurring themes and validated using the constant comparison method. Interview were continued until theoretical saturation was reached (i.e. no new themes emerged from interview).

Results. Forty patients were interviewed. Major emerging themes included acceptability and simplicity of LMWH leading to improved QoL and feelings of optimism and hope. Most importantly patients reported freedom to carry out their daily activities free from blood tests and anticoagulation clinic attendances. The use warfarin emerged as having a negative impact on patients' QoL. This was mainly due to the necessity of repeated INR checks and stress of uncertainty that the drug was working effectively.

Conclusions. Advanced cancer patients find LMWH preferable to warfarin in the long-term treatment of VTE. Warfarin may reduce overall QoL, unlike LMWH, which leads to overall improvement. LMWH should be considered in the first line treatment of VTE not only based on clinical efficacy but also for its positive psychological impact at a time that preservation of personal freedom and improvement in QoL is paramount.

P063

HOW DOES THROMBOPROPHYLAXIS IMPACT ON THE QUALITY OF LIFE OF INPATIENTS WITH ADVANCED MALIGNANCY? A QUALITATIVE STUDY

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Background. Less than 7% of British Palliative Care Inpatient Units have thromboprophylaxis guidelines and there appears a reluctance to use low molecular weight heparins (LMWH) for this purpose. There is a perception that daily LMWH may have a negative impact on patients' quality of life (QoL), outweighing the potential benefits of preventing venous thromboembolism (VTE).

Aim. To explore the views of palliative care inpatients with advanced malignancy regarding the effect that thromboprophylaxis has on overall quality of life.

Patients and methods. Palliative Care inpatients with advanced metastatic malignancy, receiving LMWH thromboprophylaxis were recruited. Semi structured taped interviews exploring their understanding and views about thromboprophylaxis were conducted. Transcripts were analysed for recurring themes and validated using the constant comparison method. Interviews were continued until theoretical saturation was reached (i.e. no new themes emerged from interview)

Results. Theoretical saturation was reached after 26 interviews. Major emerging themes suggested that patients were knowledgeable about the risks of VTE and the purpose of LMWH. Media coverage had raised awareness about VTE and many had previous experience of thromboprophylaxis. All found LMWH an acceptable intervention and many suggested that receiving thromboprophylaxis improved their QoL by giving them a feeling of safety and security. TED stockings were considered uncomfortable and had a negative impact on QoL. Patients expressed concerns that by nature of having advanced disease, they might be considered inappropriate for thromboprophylaxis.

Conclusions. LMWH is acceptable to Palliative Care inpatients with advanced malignancy and has a positive impact on overall QoL. TED stockings are an unacceptable intervention in this patient group. Thromboprophylaxis guidelines are urgently needed for Palliative Care Inpatient Units and Hospices.

P064

THROMBOPROPHYLAXIS IN BREAST SURGERY PATIENTS

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Background. Meta-analyses of randomised trials comparing low molecular weight heparin with placebo or no treatment, report >70% reduction in venous thromboembolism (VTE) in patients undergoing general surgery. Such data in breast cancer surgery is minimal. Risks of cancer-induced hypercoagulability are balanced against perceived risk of wound haematoma.

Aim. To investigate current prophylaxis practice policy amongst breast surgeons in the UK.

Methods. A questionnaire was sent to all breast surgeons (n=412) in the UK. Closed-ended questions were used to establish current practice in different clinical scenarios for VTE prophylaxis, and influences of current practice.

Results. Two hundred and seventy eight of 412 (68%) replies were received, of which 240 (86%) were suitable for inclusion.

	Clinical scenario		
Prophylaxis	*Healthy 35 year old, excision biopsy for fibroadenoma on triple assessment*	*Healthy 55 year old, wide local excision for TII, NI cancer on triple assessment*	*70 year old, ischaemic heart disease, mastectomy following partial response to neoadjuvant chemotherapy*
None	107(45%)	14(6%)	10(4%)
Compression	109(45%)	55(23%)	28(12%)
Anticoagulation	7(3%)	13(5%)	10 4%)
Compression and anticoagulation	14(6%)	158 (66%)	191 (80%)

Twenty-seven surgeons (11%) recognised cancer as a thromboembolic risk factor that would alter prophylaxis practice, but 141 (59%) would not stop hormone therapy for surgery. Risk of thromboembolism was estimated at less than one patient per year by 104 (45%) of surgeons. Eleven surgeons (5%) used no prophylaxis. Only three surgeons (1%) cited a hospital policy.

Conclusions. A lack of consensus for thromboprophylaxis exists amongst breast surgeons. Thromboprophylaxis use is currently influenced by subjective perceptions of VTE risk and anticoagulant side-effects. A prospective study is required to establish VTE risk in breast surgery. A national VTE database would allow identification of risk factors, stratification of patients and development of prophylaxis policies.

P065

THROMBOTIC COMPLICATIONS RELATED TO CENTRAL VENOUS CATHETERS

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Background. Central venous catheters (CVC) constitutes an integral part for cancer treatment. The incidence of CVC-related thrombosis and infections complications is highly variable among different studies. Most of these studies included several types of CVC and/or underlying diseases with different risk of catheter-related complications (CRC). *Aim.* We conducted a retrospective, observational, medical file-based study from patients derived from a register of PICC line and Broviac type catheters inserted over a 90-days period in order to characterize CRC.

Materials. 118 subjects were included into the study and divided in three groups: *group 1* : Broviac-catheter inserted for peripheral stem-cell marrow transplantation (PSCMT) for hematologic diseases (N=33), *group 2*: PICC line inserted for intravenous chemotherapy in solid tumour patients (N=49), *group 3* : PICC line inserted in predominantly non-cancer patients for indications other than chemotherapy (N=36).

Results. Demographics characteristics were 75 men, 43 women, median age 55 years old, mean catheter lifetime (days $\pm$ SD) in group 1,2,3 were respectively 42.6$\pm$15.3, 55.8$\pm$16.2, 35.3$\pm$12.7. Incidence of tip-occlusion in group 1,2,3 respectively were 0%, 6.1%, 11% where catheter-related central venous thrombosis (CR-CVT) in group 1,2,3 respectively were 0%, 8.1%, 5.5%. Global incidence of CR-CVT was 7% (6/85) translating into 1.15 per 1000 devices-days. Positive hemoculture rates were higher in Broviac 12.1% (4/33) representing 2.84 per 1000 devices-days compared to 0.78 per 1000 devices-days in PICC line. Tip-catheter colonization were higher in Broviac, 4.27 per 1000 devices-days compared to 0.78 per 1000 devices-days in PICC line.

Conclusions. Our study demonstrated these observations: (1) higher rate of tip-occlusion and CR-CVT in PICC line than Broviac with an overall symptomatic thrombotic of 8.2% tip-occlusion and 7.0% CR-CVT (2) a trend towards higher rate of CR-CVT in PICC line with an underlying active cancer (3) higher rate of tip-catheter colonization and positive hemoculture rates in Broviac inserted for PSCMT. *Ongoing study.* Due to the high management costs of CR-CVT in cancer, we wish to improve prophylactic methods. We postulate that low molecular weight heparin (LMWH) Tinzaparine will provide better prophylaxis. We are currently conducting such a study using Tinzaparine as a catheter PICC line instillation anticoagulant flush 300 UI/mL (3 mL in each lumen) compared to standard UFH 100 UI/mL (3 mL in each lumen) and monitoring thrombotic rate complications in cancer patients undergoing chemotherapy. We will discuss a preliminary report of a prospective, randomized, double-blind study.

P066

THERAPY OF VENOUS THROMBOEMBOLISM IN CANCER PATIENTS

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Background. The association between cancer and venous thromboembolism was first discussed as early as the 1820, and by the 1950 the theory gained more credence with the initiation of the first prognostic study. VTE has been classified into two categories, concomitant VTE and cancer (both symptomatic), and occult cancer in VTE (cancer symptomatic after VTE diagnosis). The prevalence of cancer with VTE differs according to age (being higher in older patients) and the depth of routine examination by physicians. Fibrin deposition around blood-borne malignant cells also plays a role in tumor development, acting as a scaffold for new vessel formation and assisting in protecting the cancer cells from the body's immune system. Inhibition of fibrin formation has, therefore, been considered as means to combat malignant disease progression.

Patients and methods. In non-surgical cancer patients, the majority of the clinical data involve breast cancer. Data have shown that in healthy women taking tamoxifen to prevent cancer development: 0.1–2% who had no cancer progression developed VTE; 0.2-0.9% who were node negative developed VTE; 1.6–9.6% who became node positive had VTE; and 17.6% who developed advanced cancer also had VTE. The increasing severity of cancer is associated with the incidence of VTE. Clinical studies investigating the effects of antithrombotic drugs have focused mainly on heparins and vitamin K antagonists, with heparins studied more extensively. Heparin has been shown to reduce primary tumor growth, prevent metastatic spread, and inhibit the adhesive properties of tumor cells and their migration through the vascular endothelium.

Results. Low dose unfractionated heparin (LDUH) has been shown to reduce the incidence of deep vein thrombosis and pulmonary embolism in cancer patients after surgery. Furthermore, low molecular weight heparin (LMWH) is more effective than LDUH in both non-surgically and surgically treated cancer patients. Prolonged prophylaxis after surgery beyond the hospital stay decreases the incidence of VTE even further.

Conclusions. We recommended primary prophylaxis with LDUH or LMWH in surgically treated cancer patients to reduce the risk of VTE development and added that to further reduce this risk, secondary prophylaxis with a LMWH after surgery should be implemented.

P067

EFFECTIVE HEMOSTASIS WITH RFVIIA IN PATIENTS WITH MALIGNANCY AND SEVERE BLEEDING-ASE REPORTS

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We report two patients with malignancy and severe bleeding disorder that were successfuly managed with recombinant activated factor FVII (rFVIIa).

Case 1. 49-year-old woman with glioblastoma of left frontal region, grade IV. The neurosurgery was carried out uneventfully and the tumour was removed. However, massive bleeding appeared several hours after operation from the site of tumour extirpation that was refractory to conservative treatment. Surgical cause of bleeding was excluded. Coagulation screeening showed normal values of prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), antithrombin (AT), fibrinogen and platelet count. rFVIIa was administered with the 1st dose of 120 ug/kg i.v. and the 2nd rFVIIa bolus of 96 ug/kg i.v. was given after two hours. The effect of rFVIIa administration was excellent, bleeding ceased and PT increased at 300% (INR:0,4) after the 2nd dose of rFVIIa.

Case 2. 51-year-old man admitted at Dept. of Surgery due to the gastrointestinal bleeding and jaundice with severe thrombocytopenia. After cessation of bleeding by conventional hemostyptic drugs the endoscopy discovered tumour in antral and pyloric part of stomach as an origin of bleeding. Computer tomography (CT) scan showed that the tumour infiltrated pancreas and caused the stenose of ductus choledochus close to duodenum and metastasized in the liver and lung. Bleeding did not continue but the jaundice progressively increased (bilirubin 725 µmol/L). The endoscopic retrograde cholecystopancreatography (ERCP) with papilotomy and stenting of stenose was necessary. Because of severe thrombocytopenia (15×10^6/L) the rFVIIa in a single dose of 1.2 mg was administered simultaneously with platelet transfusions before ERCP. The intervention with ERCP stenting was successful without bleeding. *Conclusions.* No adverse effects from the treatment with rFVIIa were observed. Our reports conclude that rFVIIa is safe and effective therapy in patients with malignancy and severe bleeding disorder.

P068

ADJUNCTIVE USE OF ANTICOAGULANTS WITH A TAFIA INHIBITOR DOES NOT FURTHER ENHANCE T-PA-INDUCED THROMBOLYSIS IN A RABBIT ARTERIAL THROMBOSIS MODEL AND COMPROMISES SAFETY

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Background. Previously, we reported that potato carboxypeptidase inhibitor (PCI), which inhibits the activated form of thrombin activatable fibrinolysis inhibitor (TAFIa), enhanced tissue plasminogen-activator (tPA)-induced thrombolysis in a rabbit model.

Aim. Using the same model, we examined whether anticoagulant addition to PCI would further enhance tPA–induced clot lysis.

Materials and methods. After generating a radiolabeled thrombus in the damaged and stenosed distal aorta, rabbits were treated with tPA (0.5 mg/kg) with or without PCI (60 Ìg/kg) in conjunction with hirudin (HIR; 0.4 mg/kg), unfractionated heparin (UH; 100 U/kg), fondaparinux (FOND; 1 mg/kg), or saline. Clot lysis was determined by subtracting the radioactivity of the residual thrombus at 90 min from that of the initial thrombus and expressing this value as a percentage of initial radioactivity. Cumulative blood loss from standardized ear incisions was used to assess safety.
Results. PCI enhanced tPA-induced clot lysis, but the addition of anticoagulants had minimal effects on clot lysis and increased blood loss (Table).

Conclusions. Addition of anticoagulants to PCI provides minimal enhancement of t-PA-induced clot lysis and compromises safety.

Table.

Treatment	Saline	*tPA*	tPA+PCI+Saline	*tPA+PCI+UH*	tPA+PCI+FOND	*tPA+PCI+HIR*
Clot lysis(%)	15.8±3.0	*67.9±10.5*	84.0±6.9	*91.7±3.8*	87.6±4.6	*93.9±3.8*
Blood loss(μL)	15.8±3.4	*109.6±31.8*	128.4±42.9	*167.1±43.9*	196.9±28.8	*485.9±302.9*

Values represent mean±SEM; n=6 rabbits per treatment group

P069

MANAGEMENT OF HAEMORRHAGE USING RECOMBINANT FACTOR VIIA (RFVIIA) IN PATIENTS TREATED WITH HAEMATOPOIETIC STEM-CELL TRANSPLANTATION FOR HAEMATOLOGICAL MALIGNANCIES

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Background. Haematopoietic stem-cell transplantation (HSCT) for haematological malignancy frequently causes thrombocytopenia, bleeding (e.g. diffuse alveolar haemorrhage, haemorrhagic cystitis, intracranial haemorrhage, gastrointestinal haemorrhage) and thrombosis (e.g. hepatic veno-occlusive disease). HSCT-associated graft-versus-host disease (GvHD) increases severe haemorrhage risk, and immunosuppressant medication increases the risk of sepsis leading to disseminated intravascular coagulation.

Aim. Presentation of case data showing the efficacy of rFVIIa in controlling HSCT-related haemorrhage in patients with haematological malignancies.

Patients and methods. Twelve cases, involving haemorrhage associated with HSCT for haematological malignancies in 11 patients, were identified from an international case registry of the investigational use of rFVIIa (haemostasis.com).

Results. Patients underwent HSCT for acute lymphocytic leukaemia (*n*=3), chronic myelogenous leukaemia (*n*=3), multiple myeloma (*n*=1), T-cell malignant lymphoma (*n*=1), non-Hodgkin's lymphoma (*n*=1) and unspecified haematological malignancy (*n*=2). HSCT-related bleeding occurred at nasal (*n*=1), pulmonary (*n*=2), gastrointestinal (*n*=5), urological (*n*=3), and unspecified (*n*=1) sites. rFVIIa was given as rescue therapy in all but one case (involving a single prophylactic dose covering dialysis-line insertion; conventional therapy was given for other bleeding in this case). Median total rFVIIa dose was 97.5 μg/kg (range 30–768 μg/kg). Six cases received ? 2 doses (one received 8 doses of 96 lg/kg). Bleeding was stopped (*n*=6), markedly decreased (*n*=1), decreased (n=1), unchanged (*n*=3) and not recorded (*n*=1) after rFVIIa administration. Cases of unchanged bleeding involved prophylactic rFVIIa administration, pulmonary sepsis, and GvHD plus pulmonary sepsis, respectively. Ischaemic stroke (*n*=1) at 2 days post-rFVIIa was *possibly* rFVIIa-related but other adverse events in the 12 cases were not rFVIIa-related. Deaths (*n*=7) among the 11 patients were neither bleeding- nor rFVIIa-related.

Conclusions. rFVIIa helped to stop or reduce HSCT-related bleeding in 8/12 cases after significant blood loss. These data supplement recent clinical trial data on rFVIIa in HSCT patients with bleeding complications[a] but rFVIIa benefits in this population need further evaluation in clinical trials.

[a]Pihusch M *et al.,* J Thromb Haemost 2005;3:1935-44.

PROCOAGULANT FACTORS OF TUMOR CELLS AND ENDOTHELIUM

P070

MICROPARTICLE-ASSOCIATED TISSUE FACTOR ACTIVITY: A LINK BETWEEN CANCER AND THROMBOSIS?

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Background. Cancer, in particular mucinous adenocarcinomas, is associated with an increased risk of venous thromboembolism (VTE). Tissue factor (TF) plays a central role in the paradigm that clotting and tumor growth form a vicious circle, in which hypercoagulability facilitates the aggressive biology of cancer and vice versa.

Aim. To investigate whether microparticles (MP) derived from malignant epithelial cells play a role in the initiation of blood coagulation via TF expressed on these MP.

Patients and methods. MP were isolated from peripheral blood samples obtained from healthy subjects (n=37), and unselected cancer patients, i.e. patients with primary breast carcinoma (before and after surgery, n=10), metastatic breast (n=17) and pancreatic carcinoma (n=23). TF procoagulant activity associated with isolated MP was assessed by a factor VIIa dependent factor Xa generation assay. MP were examined by flowcytometry, electron and confocal immunofluorescence laser scanning microscopy.

Results. Compared to healthy subjects, significantly increased MP-associated TF activity was found in patients with metastatic cancer. Pancreatic (n=5) and breast cancer patients (n=2) who presented with VTE had a 18-fold increase in MP-associated TF activity as compared to healthy subjects or 6 subjects with idiopathic VTE (p<0.003). In all individuals most of the circulating MP expressed the platelet antigen CD61. MP expressing the epithelial antigen MUC1 -most likely derived from malignant cells- were found in the majority of patients with metastatic disease. Co-expression of CD61 and MUC1 on MPs was shown by flowcytometry and confocal immunofluorescence microscopy.

Conclusions. Highly elevated MP-associated TF activity correlated with development of VTE and the presence of circulating $MUC1^{+}$-MP, suggesting a decisive role in the pathogenesis of the prothrombotic state in patients with mucinous carcinomas. Patients with a low level of TF-activity on MP that also lacked expression of mucin had a higher survival rate at 3-9 months follow-up than those with a high TF-activity and mucin present: the estimated risk of dying was 0.42 (95% CI 0.19– 0.94) for patients with these 2 predictor variables present, adjusting for the other factors (age cohort, type of cancer, VTE) in a Cox proportional hazards model.

P071

RAT C6 GLIOMA CELLS EXPRESS TISSUE FACTOR AND STRONGLY ASSEMBLE BLOOD PROCOAGULANT COMPLEXES

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Background. That there is a correlation between cancer and procoagulant states is well known. C6 glioma cell line was originally induced in random-bred Wistar-Furth rats and is morphologically similar to glioblastoma multiforme, the most common aggressive glioma resistant to therapeutic interventions.

Aim. In this study we investigated the procoagulant properties of C6 glioma cells.

Materials and methods. The assembling of blood procoagulant complexes on C6 glioma cells was investigated by using purified proteins.

Results. Increasing cell concentrations produced a significant decrease in the recalcification time of rat plasma. This observation was consistent with the presence of tissue factor (TF). Here, TF was identified by flow cytometric and functional (FXa formation in the presence of cells and FVIIa) assays. Alternatively, conversion of FX into FXa was also observed in the presence of C6 cells, FIXa and FVIIIa. This effect was both cell- and FVIIIa-dependent and is consistent with formation of the intrinsic tenase complex. C6 glioma cells were also able to potentiate prothrombin activation by FXa. This ability was dependent on FXa binding to the cell membrane, since no potentiating effect was observed with GLA-domainless FXa, a FXa derivative that has no phospholipid-binding properties. C6 cells also promoted formation of the prothrombinase complex (FXa/FVa), which efficiently activates prothrombin into thrombin. It is known that formation of both intrinsic tenase and prothrombinase complexes are dependent on phosphatidylserine (PS) exposure. Therefore, annexin V, which blocks PS binding sites, inhibited FX and prothrombin conversion by their respective C6-assembled activating complexes.

Conclusions. C6 glioma cells display a highly procoagulant pattern as a result of both TF exposure and the presence of the anionic lipid PS at the outer leaflet of their membranes. Therefore, this animal cell line may be used as a new model for studying the role of blood coagulation proteins in tumor biology.

P072

THE EFFECT OF APOPTOSIS AND LIPID PEROXIDATION ON THE TF CLOTTING ACTIVITY OF THP-1, JURKAT AND MOLT-4 CELLS

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Background. It is well established that leucocytes, especially monocytes, can exhibit procoagulant activity (PCA), mainly due to tissue factor (TF). Previous studies from our laboratory have shown that malignant T-cells, although predominantly showing a phospholipid-like PCA, do possess some TF activity.

Aim. The aim of this study was to investigate the effect of apoptosis and Lipid peroxidation (LP) on the TF clotting activity of one monocytoid cell line (THP-1) and two T-lymphoblastoid cell lines (Jurkat and Molt-4).

Materials and methods. Cells were cultured under standard conditions washed in RPMI, and suspended in Tris buffer. All cells used were >90% viable as measured by trypan blue exclusion before use in experiments. Apoptosis was induced by staurosporine (1μM). LP was caused by treating the cells with H_2O_2 (4 μM) and $CuSO_4$ (40 μM). TF activity was measured using a modified prothrombin time and caspase activity was measured chromogenically. The binding of annexin $A5^{FITC}$ and an anti-phosphatidylserine antibody (3G4) was measured by flow cytometry.

Results. Both apoptosis and LP induced a significant

increase ($p<0.05$) in TF PCA for THP-1 and Jurkat cells. After 24 hours apoptosis caused peak TF PCA levels of 89.1±3.1 and 25.8±1.5 compared with basal levels of 53.7±8.7 and 15.5±2.4 TF U/mL, respectively, a 1.7-fold increase. Four hours of LP induced peak levels of 197.2±29.3 and 59.8±10.4 TF U/mL, a 3.7 and 3.9-fold increase, respectively. Molt-4 showed a 1.5 and a 2.0-fold increase following staurosporine or LP treatment, with TF PCA levels of 2.3±0.1 and 3.0±0.4 being observed compared with a basal level of 1.5±0.2 TFU/mL, respectively. Apoptosis induced increase in TF PCA for Jurkat and THP-1 cells correlated with caspase 3/7 activity (r^2=0.56), annexin $A5^{FITC}$ (r^2=0.77) and 3G4 binding (r^2=0.80). Following LP treatment TF PCA for THP-1 and Jurkat cells correlated with TBARS levels (r^2=0.58) and annexin $A5^{FITC}$ binding (r^2=0.93) but not 3G4 binding (r^2=0.37).

Conclusions. Both apoptosis and LP were able to increase TF PCA supported by the cell lines with the effect of LP being greater than apoptosis. The differences between annexin $A5^{FITC}$ and 3G4 binding suggest that the PCA due to apoptosis is caused by increased phosphatidylserine exposure in close proximity to TF, whilst LP might increase the catalytic efficiency of the TF/FVII complex, by altering the net charge of the cell membrane.

P073

EXPRESSION OF ALTERNATIVELY SPLICED HUMAN TISSUE FACTOR IN HUMAN PANCREATIC CANCER CELL LINES AND ITS EFFECT ON TUMOR GROWTH *IN VIVO*

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Background. Tissue factor (TF) has been linked to tumor growth, progression, and thrombotic complications. Recently, a 25 kDa alternatively spliced form of human tissue factor (asHTF) was identified (Bogdanov *et al.*, 2003). In asHTF, exon 5 is deleted, resulting in a frame shift, loss of the transmembrane domain, and an alternative COOH-terminal domain. The function of asHTF in normal cells and cancer cells is not known.

Aims. (1) To examine the expression of asHTF in human pancreatic cell lines; (2) to assess the procoagulant activity of asHTF; (3) to determine the effect of asHTF expression on tumor growth in mice.

Materials and methods. RNA was isolated from a panel of six human pancreatic cancer cell lines. RT-PCR for TF, followed by sequencing, detected both wild-type TF (TF_{wt}) and asHTF. Immunoblots were done with anti-TF antibody (10H10) or a specific antiserum for asHTF. MiaPaCa-2 cells, which express no detectable TF, were transfected to establish lines containing vector alone, TF_{wt}, asHTF. A modified plasma clotting assay was used to test serum-free conditioned media for procoagulant activity. Transfected MiaPaCa-2 cells were subcutaneously injected into the flanks of nude mice, and tumor growth was monitored. Mice were sacrificed and tumor tissue was harvested, fixed, sectioned and stained.

Results. asHTF and TF_{wt} RNA and protein were expressed by ASPC-1, Capan-1, Capan-2, Hs766T, and HPaf lines, but not in MiaPaCa-2. Transfection of MiaPaCa-2 cells indicated that the TF_{wt} conferred procoagulant activity to the conditioned media, but asHTF did not. Interestingly, transfection of asHTF cDNA enhanced tumor growth of MiaPaCa-2 cells in nude mice, but transfection of TFwt did not result in enhanced tumor growth.

Conclusions. We now show that asHTF is expressed in 5 of 6 human pancreatic cell lines. Further, the asHTF protein has no detectable procoagulant activity. Although TF expression has been widely linked to enhanced cancer growth in animals and humans, we saw no effect from TF_{wt} on MiaPaCa-2 growth in mice. However, asHTF expression did enhance tumor growth in mice. Investigation of the mechanism of asHTF effect(s) on tumor growth and potential functions, and protein-protein interactions is in progress.

P074

CHARACTERIZATION OF PROCOAGULANT ACTIVITY OF A HUMAN MELANOMA CELL LINE -WM-266-4

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Background. The correlation between cancer and hypercoagulant states has been described for more than a century. Melanoma is an aggressive type of cancer and highly metastatic and there is evidence that tissue factor and thrombin contribute to this aggressive pattern.

Aim. Characterize the procoagulant activity of a human melanoma cell line, WM-266-4.

Materials and methods. The cells were grown in a 96-well plate 24h before the experiments. Procoagulant activity was assessed by assays of tissue factor (TF) in plasma, and intrinsic tenase and prothrombinase were assessed using purified coagulation enzymes. The expression of TF antigen and phosphatidylserine on the cell surface was measured by flow cytometry, using specific antibodies. *Results.* The human melanoma cell line – WM-266-4 expressed tissue factor antigen on the surface with a mean fluorescence intensity (MFI) of 11.7±2.8 relative to the control and also there was a cell concentration dependent tissue factor functional activity as measured by prothrombin time. This functional activity was inhibited by anti-TF antibody but not by Aprotinin, showing the activity was mainly due to the tissue factor present on the surface of these cells and not due to non specific serine-protease activity. Phosphatidylserine (PS), a negatively charged phospholipid that is related to binding of the coagulation enzymes on the surface of the cells was also measured. The MFI was 0.76±0.16 relative to the control. The procoagulant activity of the PS on the surface of the cell line was able to promote activation of FX to FXa and prothrombin to thrombin when assembled in intrinsic tenase and prothrombinase complexes respectively.

Conclusions. WM-266-4 melanoma cells have a high procoagulant activity that is not due just to tissue factor but also to a negatively charged surface and together they may contribute to the aggressive pattern of melanoma. Therefore, this human melanoma cell line may be used as a model for studying the role of blood coagulation proteins in tumour biology of melanomas.

P075

IMMUNOHISTOCHEMICAL LOCALIZATION OF PROTEIN C SYSTEM COMPONENTS IN HUMAN COLON CANCER TISSUE

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Background. Thromboembolic episodes are common complications of colon cancer. It has been recognized that blood coagulation proteins play a role in malignant tumor progression and metastatic dissemination.

An important inhibitory mechanism is provided by protein C (PC) system. What is of interest, recently novel biological activities have been desribed for protein C system components that do not relate to their hemostatic functions.

Aim. The purpose of the study was to elucidate the solid phase interactions between colon cancer tissue and components of PC system that may contribute to tumor progression.

Material and Methods. The tissues from colon cancer were obtained at surgical resection during radical treatment of previously untreated 66 patients. Tumor fragments were processed acc. to AMeX method and then embedded in paraffin. Immunohistochemical studies were performed using polyclonal antibodies against PC, protein S (PS) and thrombomodulin (TM).

Results. Weak expression of PC was observed in cancer cells of approx. 2/3 of the examined specimens while in 4/66 cases there was no detectable staining for PC in cancer cell bodies. One third of colon cancer fragments revealed strong expression of PC. Presence of PS was demonstrated in 64 cases of colon cancer, but its expression was irregular: a weak staining was observed in 60 specimens and strong one – only in 4 cases. Two cases of colon cancer tissue did not revealed any staining at all for PS in cancer cells.

Weak expression of TM was observed in 2/3 of the examined specimens, while a strong staining was revealed in 1/3 of colon cancer tissues. However the expression of TM was inconsistent: not all cell bodies were TM-positive. The presence of PC and PS, but not TM was demonstrated in tumor associated macrophages. Protein S and TM antigens were localized in tumor stroma.

Conclusions. The observed weak and irregular expression (or its absence) of PC system components in most cases of colon cancer may not sufficiently counteract blood coagulation activation and consequently facilitate tumor progression. Heterogeneous expression of PC, PS and TM in cancer cells may suggest that the proteins play a role in colon cancer growth.

P076

EXPRESSION OF PROTEIN Z AND PROTEIN Z-DEPENDENT PROTEASE INHIBITOR *IN SITU* IN HUMAN MALIGNANT TISSUES

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Background. Blood coagulation proteins play a role in malignant tumor progression. Recently a new mechanism of direct inhibition of factor Xa, that involves protein Z (PZ)/protein Z-dependent protease inhibitor (ZPI) system has been described. ZPI also attenuates the activity of factor XIa. The data on the presence of ZP and ZPI in malignant tumors *in situ* are obscure.

Aim. The purpose of the study was to evaluate the localization of PZ and ZPI *in situ* in colon, breast, gastric, laryngeal, pancreatic, renal, endometrial cancer, non-small cell lung cancer (NSCLC), malignant melanoma and glial neoplasms.

Materials and methods. Studies were performed on tumor fragments obtained at surgical treatment of previously untreated patients. Immunohistochemical procedures employed antibodies against PZ and against ZPI.

Results. Protein Z was localized in cancer cells in all types of malignant tumors examined. Staining intensity was more pronounced in less differentiated cancer cells of anaplastic gliomas. In contrast, more differentiated cancer cells of gastric cancer revealed stronger staining than less malignant ones. Cancer cells of NSCLC and renal cancer were characterized by weak intensity of staining for PZ. In pancreatic and renal cancer, as well as malignant melanoma, the intensity of staining for PZ in cancer cells was irregular: both high and low PZ expression was observed independently of the degree of malignancy. Protein Z was also revealed in association with macrophages in colon cancer, NSCLC and malignant melanoma. Expression of ZPI was observed in cancer cells in all examined specimens. The staining intensity for ZPI was irregular: both strong and weak expression of ZPI was observed. Various percentages of ZPI-positive cancer cells were revealed in different specimens of the examined tissues. Tumor infiltrative macrophages also revealed expression of ZPI in colon cancer. ZPI was also demonstrated in the stroma of renal cancer.

Conclusions. The results suggest that ZP and ZPI, being inhibitors of coagulation, may additionally exert regulatory effects on tumor growth and metastatic dissemination.

P077

CHARACTERIZATION OF CANCER PROCOAGULANT ACTIVITY IN MDA-MB 231 CELL LINE AND IN HUMAN AMNION CHORION MEMBRANE

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Background. Cancer procoagulant (CP) is factor X activating protease that has been found in a variety of human and animals malignant tissues and in human amnion chorion membrane (hACM) but was not detected in normally differentiated tissues. CP could be associated with thrombothic

complications in cancer patients.

Aim. Characterization and purification of CP from human breast cell line MDA-MB 231 compared with the activity from hACM.

Materials and methods. DE-52 chromatography column and glycerol gradient were performed to purify CP activity. Metal divalent ions, cysteine and serine protease inhibitors and three different detergents were used to characterize CP activity extracted from MDA-MB 231 cell line and hACM. *Results.* Differences in elution concentrations from glycerol gradient (52.2% vs. 42.2%) and chromatography on DE-52 column (0.35-0.7M NaCl vs. 0.2M NaCl) were observed in CP activity extracted from MDA-MB 231 vs. the activity extracted from hACM. Furthermore, differences in CP activity from the two sources were observed in the presence of divalent metal ions i.e; mix of Mn^{2+}, Mg^{2+}, Cd^{2+} and Ca^{2+} increased CP activity extracted from hACM by 2 fold but decreased the activity extracted from MDA-MB 231 cell line by 1.5 fold compared with the activities in the presence of Ca^{2+} alone. CP activity extracted from MDA-MB 231 and hACM was equally inhibited by $HgCl_2$ (98% inhibition) and leupeptin (70-98% inhibition), but not by the cysteine protease inhibitor iodoacetamide.The possible association between CP activity and lipoprotein was demonstrated by the decreased activity in the presence of detergents such as: triton X-100, tergitol and CHAPS.

Conclusions. Although CP activity from MDA-MB 231 cell line and hACM extracts were similarly inhibited by protease inhibitors and by detergents, differences were observed in elution concentrations from DE-52 chromatography column and from glycerol gradient and in CP activity in the presence of metal divalent ions. These may suggest differences in biochemical characteristics such as steric structure, electric charge, molecular size and ionic strength between CP extracted from MDA-MB 231 and hACM.

P078

CANCER PROCOAGULANT FROM HUMAN AMNION-CHORION MEMBRANES DOES NOT CONTAIN GAMMA-CARBOXYGLUTAMIC ACID RESIDUES

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Background. Cancer procoagulant (CP) is an enzyme directly activating coagulation factor X *in vitro*. The expression of CP is characteristic for rapidly proliferating cells like malignant and fetal cells. The hypercoagulation state in cancer patients can be abolished with anticoagulant drugs especially with warfarin. This coumarin anticoagulant, acting as a vitamin K-antagonist, prevents post-translational formation of gamma-carboxyglutamic acid (Gla) in the vitamin K-dependent carboxylation process. Some blood coagulation factors – II, VII, IX, X, PC and PS contain Gla residues. This amino acid is responsible for Ca^{2+} ions binding, protein adsorption to phospholipid membranes and protein-protein interactions. There are indirect evidences suggesting the presence of Gla residues in CP moiety. CP activity in animal tumors was depressed by warfarin while CP antigen was still present in tumor extracts. These results suggest that cancer procoagulant is a new vitamin K-dependent protein.

Aim. The aim of this study was to demonstrate directly the presence or the lack of gamma-carboxyglutamic acid in samples of cancer procoagulant isolated from human amnion-chorion membranes.

Materials and methods. Cancer procoagulant was purified from human amnion-chorion membranes by low-pressure ion-exchange chromatographies. CP and prothrombin – the positive test control – were prepared in the same manner; all of them were delipidated using butanol:DIPE solution. These samples were loaded onto the 11% polyacrylamide gels. One gel was stained with silver nitrate solution and the second one - with 4-diazobenzenesulfonic acid solution (DBS). Gla-containing proteins stain red whereas proteins not containing Gla obtain a yellow colour or do not stain at all with the DBS-staining method.

Results. We repeated this study for three different samples of CP. The CP activity of the samples was between 30-40 U/mg and the total protein concentration in purified samples was about 500 μg/mL. Prothrombin – the protein which contains Gla residues – was stained red with DBS solution. Although the DBS gel-staining method is very sensitive and the presence of CP in purified preparations was high (according to their activity and protein concentration) we observed no red stained band in all analyzed CP samples. *Conclusions.* Our analysis demonstrates that cancer procoagulant does not belong to proteins containing Gla residues.

P079

COMPARISON OF PROCOAGULANTS FROM HUMAN AMNION-CHORION MEMBRANES, SWINE'S AMNION-CHORION MEMBRANES AND RAT'S PROSTATE CANCER

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Background. Rapid proliferation is common for malignant and fetal tissue as well as for amnion-chorion membranes. It is accepted that these tissues can produce the unique enzyme called cancer procoagulant (CP) directly activating coagulation factor X. It has been suggested that CP is well-conserved protein and there is no species-to-species differences between CP isolated from different organisms.

Aim. The aim of the study was the comparison of enzymatic and antigenic properties of procoagulants isolated from different species. The effects of 1 mM divalent metal ions (Cu^{2+} and Mn^{2+}) and 1 mM iodoacetamide (IAA) on the FX-activating activity of the procoagulants and the recognition of the antigens by polyclonal antibody was examined.

Materials and methods. CP was isolated by ion-exchange chromatographies from human and swine's amnion-chorion membranes and from prostate tumors of Lobund-Wistar rats. The factor X-activating activity was quantified using 3-stage chromogenic assay. All analyzed CP samples were diluted to 5-7 mU/mL then incubated with 1 mM Cu^{2+}, 1 mM Mn^{2+} or 1 mM IAA. Their activity was measured and compared to untreated controls. To compare immunologic similarity all procoagulant preparations were diluted to ~2 U/mL to keep the same antigen concentrations. Microplate wells were coated with diluted procoagulants then the amount of CP/ polyclonal rabbit anti-human CP antibody complexes was evaluated by ELISA using anti-rabbit IgG-Alkaline Phosphatase conjugates.

Results. The enzymatic activity was always detected in fractions that were eluted from chromatography columns at the same conditions. 1 mM Cu^{2+} significantly reduced CP activity from human and swine's amnion-chorion membranes but not from rat's prostate cancer. The presence of 1 mM Mn^{2+} in the reaction environment activated human and rat CP but surprisingly reduced activity of CP from swine. 1 mM IAA inhibited activity of all procoagulants in the similar ran-

ge. Procoagulants from human and swine's amnion-chorion membranes were recognized by anti-human CP antibody in the resembling manner. However, the antibody reacted a little more weakly with procoagulant isolated from rat's prostate cancer.

Conclusions. All analysed procoagulants activated directly factor X and the activity was inhibited by iodoacetamide. This is a typical characteristics of cancer procoagulant (CP). Nevertheless, according to different effects of divalent ions and different recognition by polyclonal antibody, there are minor differences between procoagulants from human and swine's amnion-chorion membranes and rat's prostate cancer. It seems that CP is not as conserved protein as it was suggested previously.

P080

PACLITAXEL DOWNREGULATES TISSUE FACTOR IN CANCER AND HOST'S TUMOR-ASSOCIATED CELLS

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Background. Paclitaxel, a microtubule-stabilizing compound with potent antitumor activity, has been clinically used in a wide variety of malignancies. Tissue factor (TF) is often expressed by tumor-associated endothelial and inflammatory cells, as well as by cancer cells themselves, and it is considered a hallmark of cancer progression; moreover, a role of the plasminogen/plasminogen activator system in the metastatic process has been proposed.

Aim. We investigated whether paclitaxel could modulate TF, urokinase plasminogen activator (u-PA), tissue plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1) in human mononuclear cells (MN), umbilical vein endothelial cells (HUVEC), and the metastatic breast carcinoma cell line MDA-MB-231.

Material and *Methods.* Cells were incubated with or without paclitaxel at 37°C. At the end of incubation, conditioned medium was collected and tested for u-PA, t-PA and PAI-1 antigen levels by ELISA, and cells were disrupted and tested for procoagulant activity by a one-stage clotting assay and TF antigen by ELISA.

Results. Both the strong TF activity and antigen constitutively expressed by MDA-MB-231 and the TF induced by LPS and IL-1beta in MN and HUVEC were significantly reduced by paclitaxel at doses in the lower range of those attainable in plasma. Paclitaxel did not modulate u-PA and PAI-1 release from MDA-MB-231, while it strongly downregulated PAI-1 levels in LPS- and IL-1beta-stimulated HUVEC. Since paclitaxel has been shown to induce expression of inflammatory genes in monocytes and tumor cells, we tested whether paclitaxel could influence IL-1beta and IL-6 release from our cells. Neither the constitutive expression of these cytokines by MDA-MB-231 nor the LPS-induced release from MN and HUVEC were affected.

Conclusions. Our data support the hypothesis that the anti-tumor effects of paclitaxel may, in part, be mediated by the capacity of this drug to modulate the procoagulant/fibrinolytic potential of cancer and host cells.

P081

DOWNREGULATION OF THE PROCOAGULANT POTENTIAL OF HUMAN BREAST CARCINOMA CELLS BY INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM

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Background. The renin-angiotensin system (RAS) promotes angiogenesis and growth of neoplastic cells and its inhibition may protect against cancer, thus suggesting new treatment strategies of malignancies.

Aim. Since tissue factor (TF) and the plasminogen/plasminogen activator system play a role in the metastatic process, we determined whether interference with RAS could modulate TF, urokinase-type-plasminogen activator (u-PA) and plasminogen activator inhibitor-1 (PAI-1) expression in the metastatic breast carcinoma cell line MDA-MB-231. *Background.* To test this hypothesis, MDA-MB-231 cells were cultured and incubated with or without different concentrations of ACE inhibitors (captopril and enalapril) at 37°C. At the end of incubation, conditioned medium was collected and tested for u-PA and PAI-1 antigen levels by ELISA, and cells were disrupted and tested for procoagulant activity by a one-stage clotting assay and for TF antigen by ELISA.

Results. The strong TF activity and antigen constitutively expressed by the cells were significantly reduced in a dose-dependent manner by the ACE inhibitors. Since flow cytometry assays clearly showed the presence of the angiotensin II (AngII) receptor AT1 on MDA-MB-231 membrane, we tested whether blockade of AT1 could affect the procoagulant/fibrinolytic potential of the cells. Losartan, a competitive inhibitor of AT1, reduced TF activity and antigen at a degree similar to that exerted by ACE inhibitors. Similar results were observed when an anti-AT1 or AngII antibodies were used instead of losartan. Although a trend in downregulation of u-PA and PAI-1 could be obtained in all istances, statistical significance was not reached.

Conclusions. These *results.* could, at least in part, contribute to explain the effects of ACE inhibitors and AT1 receptor antagonists in some types of malignancy, and further support their use for tumor control.

P082

DOWNREGULATION OF TUMOR CELL TISSUE FACTOR PROCOAGULANT ACTIVITY BY ALL TRANS RETINOIC ACID IS ASSOCIATED WITH A LOSS OF TUMOR ANGIOGENIC CAPACITY

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All-trans retinoic acid (ATRA) is an anti-tumor agent capable of controlling the hypercoagulable state associated with malignancy. Among hemostasis-regulating functions, ATRA modulates the procoagulant tumor cell Tissue Factor (TF), which is also involved in tumor growth. In this study we evaluated whether the ATRA effects on TF of human tumor cell lines were associated to effects on their proangiogenic properties. Two breast cancer cell lines, i.e MCF-7 and MDA.MB.231, and an APL cell line (i.e. NB4), were exposed for 24h to 1 μM ATRA or vehicle (control). Tumor cell conditioned media (TCM) were collected from ATRA-treated

and control cells and tested for proangiogenic activity on endothelial microvascular endothelial cells (HMEC-1) by the matrigel assay. Tumor cells were tested for TF expression (evaluated as activity, antigen and mRNA), differentiation and cell proliferation. The results show that ATRA significantly affected TF activity expression in MCF-7 and NB4 cells (63% and 61% inhibition, respectively), with a comparable inhibition of both TF antigen and mRNA. Differently, only 15% reduction (p=n.s.) of TF was observed in MDA.MB.231. Furthermore, ATRA significantly increased cellular differentiation (p<0.05) and 30% inhibited proliferation in NB4 cells, with no such effects in the two breast cancer cell lines. TCM collected from untreated control cells, both breast cancer and leukemia, significantly increased HMEC-1 capillary-like tube formation. Pre-incubation of control-TCM with an anti-VEGF antibody, 90% inhibited tube formation. Differently, TCM from ATRA-treated cells did not affect HMEC-1 angiogenesis and had a significantly lower VEGF content compared to control-TCM. These results. indicate that the angiogenic activities of both leukemic and breast cancer cells can be modulated by ATRA, and this may be due at least in part to the downregulation of tumor-derived VEGF. The parallel reduction of tumor cell TF induced by ATRA suggests an involvement of TF in this process.

P083

TRANSCRIPTIONAL PROGRAM INDUCED BY VIIA/TF, PAR1 AND PAR2 IN MDA-MB-231 CELLS

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Background. Intracellular signal transduction leading to gene transcription is induced by factor VIIa (VIIa) upon its binding to tissue factor (TF). The MDA-MB-231 carcinoma cell line expresses high levels of TF and the protease activated receptors (PARs) 1 and 2. The cell line is highly responsive to VIIa and agonists for PAR1 and PAR2.

Aim. We used MDA-MB-231cells to study gene regulation induced by VIIa signalling via PAR2.

Results. Gene array analysis showed differential regulation (>2-fold) of 39 genes out of 8,500. Regulation by VIIa via PAR2 was indicated by *results.* showing that all genes regulated by VIIa were similarly regulated by PAR2 agonist peptide. A fraction of genes regulated by VIIa/PAR2 could also be regulated by stimulation with thrombin or PAR1 agonist peptide demonstrating redundancy between PAR1 and PAR2 signal transduction. An *inflammatoid* response induced by VIIa via PAR2 was suggested by up-regulation of inflammatory mediators, including CXCL1, CXCL8 (IL-8), CSF-2 (GM-CSF) and PTX-3 already at 1 h. Later at 6 h, CSF-1 (M-CSF), VEGFc, IL6 and acute-phase genes for serum amyloid A1 and PTX-3 were also up-regulated. The inflammatory mediators CXCL1, CXCL8, CSF-2, CSF-1 and VEGFc have also been ascribed a role in angiogenesis and wound healing. Other gene products found to be up-regulated by VIIa at 1h (CCN1 and CCN2) and at 6h (uPA and PAI-1) represent proteins involved in remodelling of the matrix during angiogenesis and tissue repair. Furthermore, VIIa/PAR2 stimulation regulated a number of genes coding for proteins involved in signalling and cell cycle control (p21, p35 and p57), and in regulation of apoptosis (Birc2, Birc3 and Pdcd6). Using qPCR analyses, three other high TF expressing cell lines, HaCat, N-Hek166 and SK-BR-3, were examined for VIIa-regulation of selected genes. The various cell lines did not respond with a general gene regulation pattern. Compared to MDA-MB231 cells, VIIa-specific genes were expressed at lower levels and several were below the detection level.

Conclusions. The gene repertoire induced by VIIa stimulation of MDA-MB-231 cells (showing regulation of proteins involved in the cell cycle, apoptosis, cell migration, inflammation, matrix remodelling and angiogenesis) is consistent with a wound healing type of response. This may give clues to the role of TF in wound repair as well as its role in the pathogenesis of inflammatory diseases and malignancy.

P084

OVEREXPRESSION OF TISSUE FACTOR PATHWAY INHIBITOR IN CORONARY ARTERY ENDOTHELIAL CELLS: INHIBITS PROLIFERATION, INDUCES APOPTOSIS VIA UPREGULATION OF TNF-α AND IL-1, EXPRESSION

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Background. Tissue factor pathway inhibitor (TFPI) is well known as the primary inhibitor of TF-induced coagulation. It has been reported that gene transfer of TFPI decreased the degree of neointimal formation. This may be because of anti-inflammatory and anti-proliferative effects of TFPI in addition to, or rather than via anti-coagulation.

Aim. To study the effect of TFPI overexpression on the cellular proliferation and apoptosis processes in coronary artery endothelial cells (CoAECs). We also explored the effect TFPI had on the global gene expression with special interest in alterations in the gene expression of cytokines. *Materials and methods.* Primary CoAECs were transfected with pcDNA3.1/V5-His-TOPO containing TFPI c-DNA plasmid by nucleofection. The cells were harvested 9 and 24 hours after transfection. The overexpression was measured at the protein level with TFPI ELISA and total protein in the corresponding cell lysate, and at the mRNA level by rt-qPCR measurements of TFPI and 18sRNA as an internal control. Proliferation studies were conducted with the WST-1 assay for 24 to 72 hours. Apoptosis was measured in the cell lysates by cell death detection ELISA kit. The Taqman cytokine gene expression plate was used to test the mRNA expression of 12 different cytokines. Agilent oligo microarrays were used to test the global geneexpression in the TFPI transfected cells.

Results. We obtained a 100-fold increase in TFPI mRNA expression in CoAEC by nucleofector transfection. From time course experiments we found a top in the TFPI expression after 9 hours. The increase in TFPI production was 70–fold and the concentration of TFPI reached an average of 120 ng/mL after 24 hours. Proliferation experiments explored that TFPI inhibited the process dose dependently up to 50%. The cell lysates from 24 hours increased the apoptotic process a two-fold in comparison to control. From the cytokine gene expression experiments we found that only two of the cytokines tested were altered: an upregulation of TNF-α and IL-1β. Microarray studies and subsequent verification by rt-qPCR revealed that TFPI downregulated the expression of PAI-1, BAT 8, IFI27, and upregulated PMFBP1 expression.

Conclusions. An overexpression of TFPI in CoAEC resulted in inhibition of the cell proliferation and induced apoptosis. These and other cellular effects may be induced by the upregulation of the cytokines TNF-α, IL-1β, and PMFBP1 gene expression, and by the downregulation of PAI-1, BAT 8, IFI27 expression.

P085

PRIMARY AND BONE METASTATIC RENAL CARCINOMA LINES INDUCE ADHESION MOLECULE AND PROINFLAMMATORY CYTOKINE EXPRESSION IN ENDOTHELIAL CELLS

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Background. It has been shown that adhesion molecules for leukocytes, expressed by endothelial cells after activation by an inflammatory stimulus, may also play a role in metastases formation and in osteoclast activation.

Aim. The aim of this research was to evaluate the effect of renal carcinoma cell lines on the expression of adhesion molecules and proinflammatory cytokines regulation in human endothelial cells.

Materials and methods. The effect of a cell line isolated from an osteolytic bone metastasis of a renal cell carcinoma (CRBM) was compared with ACHN and Caki-1, two renal carcinoma continuous cell lines. Intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) expression by HUVEC incubated with the conditioned media from renal carcinoma lines was evaluated by enzyme immunoassay on the cells and by gene-specific mRNA expression. ICAM-1 expression by renal carcinoma cell lines was also determined. Interleukin-6 (IL-6), interleukin-8 (IL-8), GM-CSF and monocyte chemotactic protein-1 (MCP-1) specific mRNAs were determined both in renal carcinoma lines and in HUVEC stimulated with the conditioned media.

Results. ACHN did not express ICAM-1 and induced a modest increase in ICAM-1 and E-selectin expression on endothelials cells. Caki-1 expressed low levels of ICAM-1; induced a significant ICAM-1 expression in HUVEC cells and a non significant increase of E-selectin. CRBM expressed a high level of ICAM-1, similar to that observed in LPS-stimulated HUVEC, and induced a significant expression of ICAM-1 in HUVEC and a non significant increase of E-selectin expression. VCAM expression was not induced by renal cancer cell conditioned media. The three cell lines constitutively expressed IL-6, IL-8 and MCP-1. The conditioned media from renal carcinoma cells induced IL-8 expression by HUVEC. Caki-1 induced MCP-1 and IL-8 expression and ACHN and CRBM induced IL-8 and IL-6. GM-CSF was not expressed in the carcinoma lines or in the carcinoma-stimulated HUVEC.

Conclusions. CRBM and Caki-1 induced a proinflammatory phenotype in HUVEC, while ACHN had a lower effect. CRBM, isolated from an osteolytic metastasis, expressed the ICAM-1 highest levels.

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P086

ENDOTHELIAL CELLS EXPRESS A MATRIX PROTEIN WHICH BINDS ACTIVATED FACTOR XII IN A ZINC-INDEPENDENT MANNER

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Background. Recent studies have shown that peptides identified as surface binding regions of high molecular mass kininogen (HK) and factor XII (FXII) inhibit the Zn^{2+}-dependent binding of FXII to confluent layers of human umbilical vein endothelial cells (HUVEC). This indicates that negatively charged FXII binding surfaces, such as sulfatides and dextran sulfate, may interfere with the binding of FXII to confluent layer of HUVEC.

Aim. To investigate if the negatively charged FXII binding surfaces affect the binding of FXII to HUVEC. Materials and Methods. HUVEC were grown to confluence on microtiter plates and removed by incubation with EDTA. Wells without cells but treated identical to those with cells were used as controls. The binding of FXII to Endothelial Cell Matrix (ECM) was analyzed by ELISA.

Results. The investigation unexpectedly showed that sulfatides enhanced a specific binding of FXII not to HUVEC but to a matrix protein expressed during growth of the endothelial cells and that this binding was independent of the presence of Zn^{2+} The function of sulfatides was partly to minimize unspecific electrostatic binding and partly to induce and enhance autoactivation of FXII generating αFXIIa. The affinity for binding αFXIIa and FXII premixed with sulfatides was identical (dissociation constant 12.5 ± 0.7 nM, n=6). FXII did not bind in the absence of sulfatides. The binding of αFXIIa to ECM was mapped to the heavy chain of αFXIIa as no binding was observed of the light chain, βFXIIa, containing the catalytic domain. HK, which previously has been shown to completely abolish the Zn^{2+}-dependent binding of FXII to confluent layers of HUVEC, did not affect the binding of αFXIIa to ECM but inhibited the sulfatide enhanced binding of FXII in accordance with the known interference of the autoactivation of FXII on negatively charged surfaces. Trypsin treatment of the matrix protein completely abolished the binding, and fibronectin but not laminin was found to be a suitable target for the binding.

Conclusions. The binding of activated FXII to the ECM suggests that αFXIIa in consort with fibronectin in the ECM controls cellular functions involved in matrix modulating processes including growth, migration, differentiation and survival of the endothelial cells. Furthermore, the results. addresses a fundamental observation of importance for understanding a possible functional role of FXII as a modulator of the vascular system with coagulant as well as anticoagulant, profibrinolytic, antiadhesive and proinflammatory activity.

P087

EFFECT OF CHEMOTHERAPY ON ENDOTHELIAL INJURY: CHARACTERIZATION OF APOPTOSIS, PROLIFERATION AND CELLULAR PROCOAGULANT PROPERTIES

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Background. Cancer patients are at increased risk for thrombosis. Among the predisposing factors for hemostatic imbalance in these patients, drugs have a potential role. Endothelial injury induced by various chemotherapy drugs was reported to be associated with occlusive phenomena like VOD, MI or stroke. Endothelial injury may initiate coagulation through different mechanisms, i.e: platelets adhesion and activation, local accumulation of tissue factor and exposure of procoagulant phospholipid membrane through cellular apoptosis.

Aim. To evaluate the association of injury and possible activation of coagulation following endothelial exposure to chemotherapy agents, i.e.: vincristine, doxorubicin, busulfan and methotrexate.

Methods. Effect of drugs on human umbilical vein endothelial cells (HUVEC) survival was evaluated by the XTT assay following cell exposure to pharmacological concentrations of the drugs for long periods (48hrs) . The cellular apoptotic course of drug effect was documented by annexin V and propidium iodide fluorescence assay. Procoagulant activity of the cells was measured by prothrombin assay using pooled normal plasma, cell extracts and $CaCl_2$. The assay was calibrated with thromboplastin (Innovin) diluted 1:10 which was referred as arbitrary 1000 units. Factor Xa generation was determined by chromogenic assay.

Results. Doxorubicin, vincristine and busulfan induced 50% cell death at concentrations of 0.7 μM, 150 ng/mL and 300 μM respectively. Methotrexate at concentration of 50 μM caused increased endothelial cell number by 50% but decrease of the malignant choriocarcinoma cell line JAR cells by 50% pointing to possible difference in effective mechanisms. Procoagulant activity of EC in response to vincristine and doxorubicin was elevated to 40 U/mL as compared to methotrexate busulfan and controls (10 U/mL) and was FVII dependent indicating TF activation. The drugs effect on both TF and factor Xa generation was not synergistic with LPS induced endothelial TF (up to 100 U/mL), however, cyclosporine – an immunologic modulator, induced LPS increase in TF.

Conclusions. The chemotherapy agents: vincristine, doxorubicine and busulfan caused increased apoptotic response in endothelial cells, which was partially associated with increased procoagulant TF activity. Whether interference of chemotherapy drugs with coagulation pathways may affect clinical outcome, should be further addressed.

ANTITHROMBOTICS AND CANCER

P088
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P089
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P090
SUPPRESSION OF P-SELECTIN FUNCTION AS DISTINCTIVE FEATURE OF ANTITHROMBOTIC AGENTS TO INDICATE THEIR INHIBITORY CAPACITY ON EXPERIMENTAL BLOOD-BORNE METASTASIS

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Background. Ample evidence suggests that many of the *in vivo* anti-metastatic effects by heparins reflect their actions on P-selectin-mediated binding. We hypothesized that the ability of widely used heparins and derivatives to interfere with P-selectin-dependent tumor cell interactions under flow *in vitro* could be used as distinctive feature to identify anticoagulants with advanced inhibitory functions on experimental blood-borne metastasis *in vivo*.

Aim, materials and methods. Therefore, we determined the impact of UFH, the LMWHs nadroparin and enoxaparin, and the synthetic fondaparinux with regard to their capacity to interfere with P-selectin-dependent tumor cell binding under flow conditions and correlated it with their capacity to block experimental melanoma metastasis in the B16.F10 model.

Results. Our data revealed that commonly used anticoagulants widely differ in their potential to interfere with P-selectin-mediated cell binding. The superior inhibitory capacity on P-selectin function of unfractionated heparin and low-molecular-weight heparin (LMWH) nadroparin as opposed to LMWH enoxaparin and synthetic heparin pentasaccharide fondaparinux (observed rolling interactions at 0.dyn/cm^2 were 46±4, 16±2, 19±4, 36±7 and 40±2 cells/mm^{2}min^{-1} for buffer, UFH, nadroparin, enoxaparin and fondaparinux each at 10 U/mL, respectively) strongly correlated with the individual 50% inhibitory drug concentrations (IC50) in experimental lung metastasis assay *in vivo* (IC50 expressed as units per mouse for UFH: 5.2, nadroparin: 6.4, enoxaparin: 35.4 and fondaparinux 297).

Conclusions. Hence, P-selectin inhibition may constitute a valuable feature to identify anticoagulants particularly suitable for anticancer therapy.

P091
ANTI-ANGIOGENESIS EFFECT OF LOW MOLECULAR WEIGHT HEPARIN IS PRIMARILY MEDIATED BY TISSUE FACTOR PATHWAY INHIBITOR

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Heparin and low molecular weight heparins (LMWHs) have both antithrombotic and anti-angiogenesis properties. The anti-angiogenesis activity of LMWH may be associated with the release of endothelial tissue factor pathway inhibitor (TFPI), an important endogenous inhibitor of tissue factor/Factor VIIa (TF/fVIIa). To evaluate the effects of LMWH and TFPI in a model of angiogenesis-mediated processes, we compared the effects of tinzaparin, and recombinant TFPI in inhibiting either basic fibroblast growth factor-2 (FGF-2)- or TF/fVIIa-induced endothelial cell tube formation in human umbilical vein endothelial cells (HUVEC). Our *results.* show that tinzaparin and recombinant TFPI both blocked endothelial tube formation induced by either FGF-2 or TF/fVIIa, in a concentration-dependent manner. Endothelial tube formation was only marginally inhibited by a potent and a specific anti-Factor Xa, recombinant tick anticoagulant protein (rTAP). A monoclonal anti-TFPI antibody reversed the inhibitory effects of either tinzaparin or recombinant-TFPI on HUVEC tube formation. Tinzaparin fractions in the range of 6,000-12,000 Da were the most effective in stimulating the release of TFPI from HUVEC and the most potent anti-angiogenesis fractions. Additionally, not all LMWHs are the same in term of endothelial TFPI release and their anti-angiogenesis efficacy. Increasing average molecular weight distributions of the LMWH as well as higher distributions of the sulfate/carboxylate functional groups resulted in a greater release of endothelial TFPI and hence greater anti-angiogenesis efficacy. These *results.* suggest that the greater inhibitory effects of the LMWH tinzaparin on endothelial tube formation and angiogenesis are primarily associated with stimulation of the release of endothelial TFPI, but not to due to its anti-Factor Xa activity.

P092
EFFECT OF MCM09, AN ACTIVE SITE DIRECTED INHIBITOR OF FACTOR XA, ON B16-BL6 MELANOMA EXPERIMENTAL LUNG COLONIES

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Background. Several anticoagulant and antithrombotic drugs showed potent antitumor activity, although the relationship with clotting inhibition was not clear. Selective inhibitors of activated Factor X (FXa) may inhibit not only clotting factors, but also FXa mediated signal transduction. *Aim.* The aim of our study was to evaluate the potential antitumor activity of MCM09, a newly developed, active site directed, small molecule inhibitor of Factor Xa (FXa) [WO0216312], and to relate the findings to anticlotting potency.

Materials and methods. MCM09 (0.1-10 mg kg^{-1}) or heparin, H (10 mg kg^{-1}), were injected intravenously (i.v.), with 5×10^4 B16-BL6 melanoma cells, in C57BL6/J mice. Mice were sacrificed after 18 days, to count lung colonies. *Ex vivo* anticoagulant activity was measured by activated partial thromboplastin time (aPTT) on mouse plasma.

Results. MCM09, a selective inhibitor of FXa (IC50 = 2.4 nM against human FXa), dose-dependently inhibited B16-BL6 melanoma lung colonies, given with tumor cells. Mean lung metastasis number was 20.9±4.8 in control mice (n=10), 1.2±0.4 in mice treated with H, 10 mg kg-1 i.v. (p<0.01), 0.9±0.3, 9.2±2.2 and 15.5±2.6 in mice treated with MCM09, at 10 (p<0.01), 1 (p<0.05) and 0.1 mg kg^{-1} i.v. (ns), respectively. MCM09 (10 mg kg^{-1}) i.v. significantly prolonged aPTT (57.1±10.2 sec) 30 min after injection, as compared to controls (25.3±1.6 sec) (p<0.05); clotting times were normal thereafter. Lung colonies were 74.2-72.6% reduced by MCM09 (10 mg kg^{-1}) given i.v. 60 or 120 min

before cells, but only 21.1% lower than controls by MCM09 given 60 min after tumor cells.

Conclusions. This is the first observation that a selective inhibitor of FXa may be active on a tumor model. MCM09 was effective when given simultaneously or before tumor cells, but not following them, suggesting a direct tumor cell interaction and/or inhibition of the release of pro-inflammatory cytokines by vascular and circulating cells. The persistence of antitumor activity, with a weak anticoagulant potency, is quite promising in view of the possible use of the compound, without undesired side effects.

P093

POTENT TUMOR GROWTH INHIBITION OF ORTHOTOPIC BREAST CANCER XENOGRAFTS BY AN ANTI-TISSUE FACTOR ANTIBODY IS MEDIATE BY FUNCTIONAL INHIBITION AND FC INTERACTION

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Background. Tissue factor (TF), a membrane receptor for factor VII (FVII) and the principle initiator of the extrinsic coagulation pathway, is constitutively expressed in many tumor cells and is associated with hypercoagulability in cancer. Tumor TF is associated with enhanced metastatic potential, peritumoral fibrin deposition, endothelial cell proliferation and migration, expression of angiogenic factors, and intracellular signaling through MAPK, Akt and PARs. CNTO 859 and CNTO 860 are humanized anti-TF antibodies that inhibit TF function by blocking FX binding.

Aim. CNTO 859 was previously shown to inhibit metastasis in an experimental model. Here we present additional evidence that CNTO 859 and CNTO 860 can also potently inhibit tumor growth of breast carcinoma in an orthotopic xenograft model.

Materials and methods. Four anti-TF antibodies were generated: PHD126, a murine IgG2a specific for murine TF; CNTO 859 and CNTO 860, humanized IgG4 and IgG1 antibodies, respectively, specific for human TF; and CNTO 859 Ala/Ala contains point mutations which abolish Fc binding and consequently, only possesses function blocking activity. All four antibodies bind to TF and TF:FVIIa and inhibit FX activation. For *in vivo* studies, MDA-MB-231 human breast carcinoma cells were implanted into the inguinal mammary fat pad of SCID Beige mice. Animals received weekly intravenous antibody therapy starting on day 3.

Results. CNTO 859 potently inhibited tumor growth in a dose dependent manner relative to control antibody treated animals. Isotype switching of the IgG4 to and IgG1, CNTO 860, conferred greatly enhanced ADCC activity in a chromium-release assay. In the same *in vivo* system, CNTO 860 therapy (0.1 mg/kg) inhibited tumor growth more effectively than its predecessor, reducing final tumor volumes by 95% ($p<0.0001$) vs. 75% ($p<0.0478$), respectively. Targeting host and tumor TF with combination therapy using CNTO 860 and PHD126 further reduced final tumor volumes and decreasing tumor incidence.

Conclusions. Antibody targeting of TF on both tumor and host stroma potently inhibits tumor growth of MDA-MB-231.

P094

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P095

GREATLY INCREASED DISEASE CONTROL RATE IN METASTATIC COLON CANCER PATIENTS UNDERGOING FIRST LINE FOLFOX 4 BASED CHEMOTHERAPY REGIMEN WITH CONCOMITANT HEPARINIZATION: A RETROSPECTIVE ANALAYSIS

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Background. Several cancer patients necessitate anticoagulation for a number of reasons. As is the case of thromboembolic complication, secondary prevention of venous thrombosis, bedridden status, central venous catheter insertion, thrombocytosis, etc. Administration of the thrombotic complication prophylaxis is commonly exercised through the use of oral anticoagulants and/or low-molecular weight heparins. Recent literature reports support the conviction that low-molecular weight heparins may improve the tumor response to chemotherapy based treatments and survival in cancer pts.

Aim. To investigate, retrospectively, tumor response and disease control rate in metastatic colon cancer patients undergoing first line FOLFOX based chemotherapy regimen with concomitant heparinization.

Patients and methods. The subjects were 28 heparinized Mcc pts who had undergone first line FOLFOX based chemotherapy regimen. Response rate was evaluated according to RECIST criteria. Moreover the authors collected data regarding heparins characteristics, anticoagulatory treatment duration and its safety. *Results.* Twenty-two pts were treated with calcium nadroparin, 4 pts with enoxaparin and 2 pts with reviparin. Patients completed a median of nine chemotherapy courses. Three pts achieved complete response (nadroparin group) (10%), 14 (50%) had partial response, 5 (18%) showed stable disease (for an overall response rate of 60% and disease control rate of 78 %) and 6 (22%) progressed. Median time to progression (21 evaluable pts to date): six month (range 2-12). Median duration of heparin treatment: 41 weeks (range 7-173). Major bleeding 1 pt (3%); minor bleeding 2 pts (7%).As regards heparin dose level, concerning nadroparin group (22 pts), a median dose level of 88 anti-Xa IU (range 68-90)/Kg/daily subcutaneously was administered.

Conclusions. Our results indicate that low molecular weight heparin treatment in combination with FOLFOX 4 chemotherapy regimen could produce a favourable additive effect against human colon cancer.These data point to a possible cooperative antitumor effect beetwen low molecular weight heparin based anticoagulant therapy and chemotherapy in Mcc pts.

P096

ANTICOAGULANTS INHIBIT TUMOR METASTASIS INDEPENDENT OF FIBRIN FORMATION

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Background. The association between thrombosis and cancer is well understood. Although a critical role for individual coagulation factors in cancer biology has been suspected for years, the molecular mechanisms underlying

the effects of the coagulation system on cancers and vice versa remains largely unknown.

Aim. To determine the antimetastatic effect of anticoagulants targeting at different levels of the coagulation cascade.

Materials and methods. Wildtype mice were injected with 3x10e5 B16F0 melanoma cells in the tail vein and lung metastases were scored 3 weeks later. Single doses of the following anticoagulant compounds were administered 30 minutes before injection of the melanoma cells: 1) Low molecular weight heparin (Fraxiparin, a combined FXa/thrombin inhibitor; 600 UaXa/kg); 2) Tick Anti-Coagulant protein (TAP, FXa inhibitor; 25 μL 200 mM); 3) Active-site inactivated FVIIa (2.5 mg/kg); 4) PEG-hirudin (specific thrombin inhibitor, 10 mg/kg) and 5) Ancrod (depletes fibrinogen; 1,5 U/mouse). In addition a group of mice was treated with PEG-hirudin 3.5 days after injection of melanoma cells. Finally, either tumor cells or mice were pretreated for 30 minutes with a PAR-1 blocking antibody (H-111, 20 μg/mL).

Results. All inhibitors diminished the number of metastases compared to the untreated control group. Pretreatment with PEG-hirudin showed the most striking effect by decreasing the number of metastases from 300±48 to 7±9 (p=0.003). The corresponding figures for the other compounds were 150±60 for Fraxiparin, 120±90 for TAP and 100±75 for FVIIai. Delayed treatment with PEG-hirudin diminished the number of metastases (150±60, p=0.05), however clearly less efficient as pretreatment, indicating that thrombin plays its role in metastasis at an early stage. Depletion of fibrinogen had no effect on the number of metastases (260±150, p=0.5), proving a coagulation independent effect of thrombin in tumor metastasis. Finally, blocking of PAR-1 on the tumor cells showed a significant reduction (to140±80) in metastases, whereas PAR-1 blocking of the endothelium did not affect metastasis (280±30).

Conclusions. These data indicate that anticoagulant drugs interfere with tumor cell metastasis by early stage thrombin-inhibition (probably inhibiting adhesion or migration of tumor cells) in a PAR-1 dependent but coagulation independent manner.

P097

COAGULATION FACTOR XA DRIVES TUMOR CELLS INTO APOPTOSIS THROUGH BH3-ONLY PROTEIN BIM UPREGULATION

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Background. Metastasis of primary tumor cells into distant organs is a major determinant in the malignancy of cancers. The ability of primary tumour cells to form a distant metastasis is, in part, dependent on activation of the blood coagulation cascade. The underlying mechanism is still elusive but one explanation would be that individual activated coagulation factors (factor (F)VIIa, FXa and thrombin) determine the fate of circulating cancer cells through the activation of specific receptors on the tumor cells thereby altering its metastatic properties.

Aim. To determine the effect of FXa (and the underlying molecular mechanism) on the growth and survival of different human cancer cell lines.

Materials and methods. Different cell types (either tumorigenic or not) were treated with FXa (0.75 U/mL) and cell survival was assessed using MTT-assays. The induction of apoptosis was analyzed by nuclear condensation and caspase-3 cleavage. After the identification of the underlying signal transduction elements (using Western blot analysis), siRNA transfected cells were used in the MTT-assays to confirm their involvement.

Results. FXa drives tumor cells of epithelial origin, but not endothelial cells or monocytes into apoptosis, whereas it even enhances fibroblast survival. FXa signals through the protease activated receptor (PAR)-1 to activate extracellular-signal regulated kinase (ERK) 1/2 and p38. This activation is associated with phosphorylation of the transcription factor CREB, and in tumor cells to up-regulation of the BH3-only pro-apoptotic protein Bim, leading to caspase-3 cleavage, the main hallmark of apoptosis. Transfection of tumor cells with dominant negative forms of CREB or siRNA for either PAR-1, Bim, ERK1 and/or p38 inhibited the pro-apoptotic effect of FXa. In fibroblasts, FXa-induced PAR-1 activation leads to down-regulation of Bim and pretreatment with PAR-1 or Bim siRNA abolishes FXa-induced proliferation.

Conclusions. We provide evidence that beyond its role in blood coagulation, FXa plays a direct role in cellular processes in which Bim is the central player in determining FXa-induced cell fate.

P098

THE LOW MOLECULAR WEIGHT HEPARIN DALTEPARIN IS MORE EFFECTIVE THAN UNFRACTIONATED HEPARIN IN CONTROLLING THE PROCOAGULANT PROFILE OF MICROVASCULAR ENDOTHELIAL CELLS

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Background. In clinical studies of thrombosis in cancer patients, heparins have demonstrated potential anticancer effects, with some advantages for LMWH over UFH in terms of disease outcome.

Aim. To understand the underlying mechanisms, we studied the impact of the LMWH dalteparin (DLT), versus UFH, on the coagulant features of the microvasculature (human microvascular endothelial cells [HMEC-1]), and the macrovasculature (HUVEC). Microvascular EC differ from those of the macrovasculature, and fibrin formation in the microcirculation favors tumor cell interaction and arrest.

Materials and methods. The two types of endothelia were incubated with either DLT or UFH (0.01 to10 IU/mL for both) ±10 μg/mL lipopolysaccharide (LPS), as pro-inflammatory stimulus. The expression of tissue factor (TF) procoagulant activity, TF antigen levels, and TF mRNA levels were measured, as well as TF pathway inhibitor (TFPI) and thrombomodulin (TM) antigen levels.

Results. The expression of TF procoagulant activity, antigen, and mRNA levels induced by LPS was significantly (p<0.05) inhibited by both DLT and UFH in HMEC-1 and HUVEC. In HMEC-1, DLT was more effective than UFH in inhibiting TF expression. For TF procoagulant activity, an inhibition of 80 % and 55% was observed with 10 IU/mL DLT and UFH, respectively. In contrast, in HUVEC the two heparins had a lesser effect, with no significant differences between them (37% and 40% inhibition of TF procoagulant activity with 10 IU/mL DLT and UFH, respectively). In addition, in HMEC-1, DLT significantly increased TM antigen levels (control vs 10 IU/ml DLT: 23±1.7 vs 30.2±1.8 ng/105 cells) and reversed the LPS-induced reduction of TM (LPS vs LPS/DLT: 11.8±1.1 vs 16.4±1.3 ng/105 cells), while UFH had no effect. In HUVEC, TM antigen levels were increased with both heparins, and the LPS-stimulated reduction in TM levels was prevented by pre-incubation with both heparins. In HMEC-1, TFPI expression (control: 14.8±1.2 ng/mL) was

significantly increased by incubation with DLT (32.4±2.8 ng/mL) or UFH (36.4±4.8 ng/mL), also in the presence of LPS. Similar findings were observed with HUVEC.

Conclusions. Suppression of inflammatory-mediated TF expression and stimulation of the anticoagulant proteins, TM and TFPI, are consistent with an endothelial anticoagulant phenotype. In this regard, our results. demonstrate that the LMWH dalteparin has significantly greater anticoagulant activity than UFH, particularly in the microvasculature.

P099

BIOCHEMICAL AND PHARMACOLOGICAL EQUIVALENCE OF GENERIC VERSIONS OF LOW MOLECULAR WEIGHT HEPARINS. IMPLICATIONS IN THE MANAGEMENT OF CANCER ASSOCIATED THROMBOSIS

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Generic versions of enoxaparin (ENOX) and dalteparin (DALT) have become available in some Asian and South American countries. Additionally, several drug manufacturers have filed for approval of generically equivalent versions of ENOX and DALT with North American and European regulatory agencies. Generic versions of these products are cl*aim*.ed to have similar anti-Xa potency and molecular component distribution. At this time, there are no published guidelines regarding acceptance of generic LMWHs with the exception of specifications in product patents and pharmacopoeial descriptions. This study compares 6 commercially available generic versions of ENOX and 2 generic versions of DALT with 2 batches of each innovator product. Molecular profiling by GPC and structural profiling by NMR were carried out. Oligosaccharide composition was investigated before and after heparinase I digestion. Potency evaluation was performed using the USP assay and an amidolytic anti-Xa assay in relation to the 1st and 2nd LMWH standards. Anticoagulant effects were compared using global clotting assays. Protamine neutralization profiles were determined at gravimetric and anti-Xa adjusted concentrations. The molecular profiles of generic ENOX and DALT were comparable and the anti-Xa potency was within the expected range. However, differences were noted in the oligosaccharide components, heparinase digestion profile and protamine titration studies for some of the generic ENOX. In the NMR analysis, specific signals for the 1,6-anhydrosugar groups at the reducing terminus and the presence of a double bond at the non-reducing end also varied. Marked differences were observed with some of the generic products in the clot-based assays. Differences in molecular profile and biologic activity were minimal with the generic versions of dalteparin. Both the DALT and ENOX have been used in clinical trials for the management of cancer associated thrombosis. *Ex vivo* analysis of plasma samples collected from the treatment trials indicate that down regulation of inflammatory mediators, release of TFPI, modulation of fibrinolysis are product specific. Thus, the generic versions of these products are required to exhibit similar pharmacodynamic characteristics. Such data is only obtainable in comparable clinical studies. As both DALT and ENOX have been used in multiple indications a direct comparison of the data obtained from the generic versions of these drugs is required to project the sameness of the generic versions of these drugs. These studies suggest that the current regulatory requirements in terms of anti-Xa potency specifications and molecular parameters may be inadequate as acceptance criteria for the generic LMWHs and underscore the importance of pharmacodynamic equivalence in specific pathologic settings.

P100

ENOXAPARIN AND WARFARIN DIFFERENTIALLY REGULATE TISSUE FACTOR, TISSUE FACTOR PATHWAY INHIBITOR AND THROMBIN ACTIVATABLE FIBRINOLYTIC INHIBITOR IN CANCER PATIENTS WITH THROMBOSIS

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Background. Increased TF levels in malignancy produce consumption of endogenous TFPI, activate platelets and upregulate fibrinolytic deficit. Anticoagulants such as E and W have been used in the management of cancer associated thrombosis.

Methods. To test the hypothesis that E and W differentially regulate TF mediated hypercoagulation, plasma samples were retrospectively analyzed from an open label, multidose, active comparator parallel design study in which all patients (*n*=110) were initially treated with E at 1-1.5 mg/kg s.c. for 5 days. These patients were further subdivided into two groups. The first group continued to receive E whereas the second group received W (INR target 2-3), for up to 12 weeks. Baseline blood samples (BL), 5 day post E (IPE) and 4-6 weeks sample from the E and W were analyzed for TF antigen, functional TFPI and antigen, functional TAFI and antigen and platelet marker CD 40L.

Results.

Group	BL	IPE	4-6weeks E	4-6weeks W
TF *(pg/mL)*	340±180	290±30	180±30	240±30
TFPI:F *(U/mL)*	1.7±0.75	2.1±0.7	2.5±0.9	2.4±0.6
TFPI:Ag *(ng/mL)*	74±110	151±34	168±27	82±15
TAFI:F *(%)*	98±30	76±14	68±12	108±12
TAFI:Ag *(µg/mL)*	12.5±1.4	9.6±1.3	9.1±1.2	12.8±2.5
CD 40L *(pg/mL)*	1280±180	590±125	560±87	312±65

The initial treatment of all of patients with E at 1.0-1.5 mg/kg SC for 5 days resulted in a decrease of the circulating TF, CD-40L and functional TAFI levels. An increase in both the functional and antigen TFPI levels was noted, whereas the TAFI antigen levels were decreased. Patients continued on E sustained these trends. However, patients treated with W exhibited a rebound increase in the TF antigen, CD 40L and functional TAFI levels, whereas TFPI levels were decreased. The W group at 4-6 weeks, exhibited a decrease in TFPI levels.

Conclusions. These results indicate that TF levels are upregulated in cancer patients with thrombosis. E is capable of down regulating TF and TF mediated upregulation of thrombogenic mediators, however W treatment fails to sustain this effect.

P101

HYPERHOMOCYSTEINEMIA IN CANCER PATIENTS WITH THROMBOSIS IS NOT ASSOCIATED WITH METHYLENE TETRAHYDROFOLATE REDUCTASE GENE MUTATIONS AND CAN BE DOWN REGULATED BY LOW MOLECULAR WEIGHT HEPARIN TREATMENT

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Background. Plasma homocysteine levels are considered an important surrogate marker of vasculopathy in thrombotic disorders. Methylene tetrahydrofolate reductase (MTHFR) gene mutations, nutritional factors (folic acid/ B_{12}) and anticancer drugs such as methotrexate and purine derivatives are primarily responsible for the observed hyperhomocysteinemia and homocysteine in cancer patients. In addition, it is known that cancer patients exhibit increased prevalence of thrombotic events, possibly due to the effects of homocysteine on endothelial function.

Aim. to demonstrate that an upregulation of homocysteine is multifactorial in cancer associated thrombosis. Initial screening of cancer patients showed that the malignancy related thrombotic state was independent of molecular defects in Factor V Leiden, Prothrombin 20210 and MTHFR (677 and 1298) variants (Hoppensteadt *et al.,* Proceedings ASCO 2003. 22:346;861).

Methods. In three groups of 210 patients who were diagnosed with cancer and thrombosis and in a group of medical patients (cancer free) with thrombosis enrolled in a treatment study with low molecular weight heparin (LMWH), (Reviparin, *n*=90), blood levels of homocysteine were measured using a highly specific ELISA method (Diazyme Laboratories, San Diego, CA). Molecular methods. utilizing PCR were used to measure MTHFR (677 and 1298) variants.

Results. On a cumulative basis the cancer patients with thrombosis exhibited a relatively higher level of homocysteine (12.8±4.9; range 2.2-39.4 μM/L) in comparison to normal (n=140, 4.8±3.9; range 2.8=6.9 μM/L) and medical patients with thrombosis (5.3±3.1; range 2.1-9.8 μM/L). Of the 210 cancer patients profiled for the molecular analysis 42/210 (20%) were positive for the 677 variant and 24/210 (11%) were positive for the 1298 variant. Similar results. were obtained in the medical patients and normal volunteers. One group of cancer patients (*n*=72) was treated with a LMWH for 4-6 weeks. Blood samples from these patients showed a down-regulation of homocysteine levels (4.8±2.5 μM/L).

Conclusions. These results suggest that cancer patients with thrombosis exhibited a relatively higher level of circulating homocysteine levels which are unrelated to a molecular defect in MTHFR. Cancer free patients with thrombosis do not exhibit this increase in circulating homocysteine levels. Thus, other factors such as the use of chemotherapeutic agents may contribute to this apparently acquired upregulation of homocysteine. These observations also suggest that LMWHs are capable of reducing the homocysteine levels.

P102

VEGF-A-MEDIATED ANGIOGENESIS *IN VIVO*: DALTEPARIN STIMULATES AND EPIRUBICIN INSIGNIFICANTLY AFFECTS ANGIOGENESIS FOLLOWING METRONOMIC TREATMENT, WHILE THE COMBINATION OF THE TWO SIGNIFICANTLY INHIBITS IT

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Background. Tumor growth is angiogenesis dependent and vascular endothelial growth factor (VEGF)-A-mediated angiogenesis is pivotal in most human and experimental tumors.

Aim. The aim of the present study was to elucidate whether continuous treatment (the extreme of metronomic scheduling) with the low-molecular-weight heparin (LMWH) dalteparin, the cytotoxic epirubicin or the two in combination modulates VEGF-A-mediated angiogenesis *in vivo*.

Materials and methods. Using the truly quantitative rat mesentery angiogenesis assay, dalteparin sodium (Fragmin®, mean molecular weight, MW, 6-kDa) and epirubicin (Farmorubicin®) were administered separately or combined by continuous subcutaneous infusion at a constant rate for 14 consecutive days. Dalteparin was given at 27, 80 and 240 IU/kg/day, a range used in the clinic, while epirubicin was given in a well-tolerated dose (3 mg/kg/w).

Results. Dalteparin alone stimulated angiogenesis inversely dose-dependently. Dalteparin thus exerted the opposite effect compared with that exerted by an MW 5.0-kDa fraction prepared from the commercial LMWH tinzaparin sodium (Innohep®/Logiparin®, MW 6.5-kDa), which inhibits VEGF-A-mediated angiogenesis in the same experimental setting, as reported previously. Epirubicin alone did not significantly affect angiogenesis. Combined treatment with dalteparin and epirubicin did, however, significantly inhibit angiogenesis in statistical terms.

Conclusions. Different LMWHs, most of which are distinct pharmaceutical entities, are able to significantly stimulate (dalteparin) or suppress (a 5.0-kDa tinzaparin fraction) VEGF-A-mediated angiogenesis in the rat model used. The combined treatment with dalteparin and epirubicin significantly inhibited angiogenesis, which is noteworthy as the treatment of cancer patients receiving chemotherapy with various LMWHs, including dalteparin, improves survival in clinical trials.