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THE IMPACT OF THE G58A POLYMORPHISM ON FIBRINOGEN A-CHAIN GENE ON FACTORS V, X AND THROMBIN IN PATIENTS WITH ADVANCED ATHEROSCLEROSIS

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Background: The G58A polymorphism on fibrinogen α-chain gene has been associated with increased fibrinogen levels in healthy individuals, but its effect on patients with coronary artery disease (CAD) regarding to the effects on thrombosis/coagulation is still unknown. In the present study we examined the impact of this polymorphism on factor V (FV), factor X (FX) and thrombin time in patients with CAD.

Methods: The study population consisted of 395 subjects, 246 of which angiographically documented for CAD. The G58A polymorphism was detected by polymerase chain reaction (PCR) and appropriate restriction enzymes. Factor X, (FV) and thrombin time were measured by standard coagulometry techniques.

Results: The genotype distribution was GG: 37.8%, GA: 39.4% and AA: 22.8% for patients with CAD, while GG: 33.5%, GA: 44.3% and AA: 22.2% for controls. There was a significant difference in thrombin time (sec) for CAD patients vs controls (19.7±4.8 vs 18.9±2.1, $p<0.05$), while this difference did not persist for 455G carriers vs 455AA homozygotes in CAD (19.3±2.1 vs 19.5±2.4, p=NS) and controls (18.9±2.0 vs 18.8±2.5). In addition, FV (%) was significantly higher in CAD patients than controls (121.7±28.4 vs 108.01±23.7, p=0.0011), while no difference was observed for 58G carriers vs 58AA homozygotes both in CAD patients (125.3±27.7 vs 126.5±30.3, p=NS) and controls (106.2±22.7 vs 119.2±30.8, p=NS). Finally, no significant difference was observed in FX (%) for CAD vs controls (94.0±35.4 vs 91.9±14.2, p=NS), as well as 58G carriers vs 58AA homozygotes in CAD (94.6±22.1 vs 96.1±21.5, p=NS) and controls respectively (92.1±13.5 vs 98.2±6.7, p=NS).

Conclusions: Our findings suggest that the G58A polymorphism on fibrinogen α-chain gene does not affect significantly coagulation markers such as factors V, X and thrombin time.

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PATENCY OF FEMOROPOPLITEAL BYPASSES AND THE TYPE OF THERAPY GIVEN TO PATIENTS: ANTICOAGULANT OR ANTIPLATELET

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Keywords: femoropopliteal bypasses, therapy

Aims: To establish the patency length of femoropopliteal bypasses on the basis of the therapy given to patients – anticoagulant or antiplatelet therapy.

Patients and methods: Documentation study and comparative method have been used. The subjects were patients operated in General Hospital of Pula, in the period from January, 1st, 1991, to December, 31st, 2001. 131 patients have been operated, and 147 femoropopliteal bypasses have been made.

Results: The patients have been divided into two groups – those whose bypasses are patent and those whose bypasses are not patent, and according to the type of therapy given to them. During the postoperative course 48 bypasses got occluded, 26 of which made with the saphenous vein and 22 with prosthetic graft. Bypasses in patients made with prosthetic graft got occluded in 17 cases when they were given pelentan and in 5 cases when they were given Marivarin. Bypasses in patients made with saphenous vein got occluded in 23 cases when they were given pelentan, 2 Andol, 1 aspirin. Bypass patency and the type of therapy given to patients have been analyzed with the chi-square test. The obtained hi-square is not significant (chi-square=5.945; ss=6; p=0.429). Consequently, the state of bypasses is not connected with the type of therapy prescribed after the operation. Another chi-square test has been done in which a comparison has been made between the subjects who were given Pelentan after the operation and those who were given any other therapy. The latter chi-square is also insignificant (chi-square=0.758; ss=1; p=0.384). There is no significant difference between the therapy with pelentan and other therapies as far as patency of bypasses is concerned.

Conclusions: The results have been analyzed with the chi-square test, and we have found out that the patency of bypasses is not connected with the type of therapy prescribed after the operation.

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PREGNANCY, DELIVERY AND POSTPARTUM PERIOD IN WOMEN WITH HISTORY OF STROKE

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Keywords: antenatal fetal loss, hyperhomocysteinemia, thrombophilia

Aims: Our objective was to evaluate the role of thrombophilia in pathogenesis of stroke, the effectiveness of our prophylactic strategy during pregnancy and outcomes for mother and fetus.

Materials and methods: We studied 37 women with history of stroke (32±5.5 years). In 4 pts pregnancy was interrupted in I-II trimester. 18 pts were followed prospectively (group I) in preconception period and during pregnancy (low-molecular-weight heparin (LMWH) guided by D-dimer, aspirin, antioxidants, vitamins of B group, folic acid). In 17 pts (group II) therapy was started in II-III trimester.

Results: Thrombophilia was detected in 100%: defects of homocysteine metabolism (70%), fibrinolysis defects (fibrinogen -455G/A, PAI-1 4G/5G, t-PA I/D) (60%), FV Leiden (16%), APA (50%). Stroke was associated with severe medical conditions (hypertension, rheumatic diseases, SLE, prosthetic valves, thrombosis) and obstetric complications. In group I mild-to-moderate obstetrics complications were detected in 7 pts (38.9%). All pts were delivered at term and all babies were alive. In group II 64.7% of pts had moderate to severe obstetrics complications, which required preterm delivery.

Conclusions: Thrombophilia might be the main pathogenic mechanism of stroke and obstetrics complications. Hypergomocysteinemia and APA were detected in most cases. Preconception treatment with LMWH allows preventing pregnancy complications, adverse fetal outcomes and recurrent thrombosis. The mode of delivery in pts with history of stroke is ultimately cesarean section.

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RELATIONSHIP BETWEEN DURATION OF ARTERIAL HYPERTENSION AND MICROCIRCULATION IN HYPERTENSIVE PATIENTS

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Keywords: hypertension, blood rheology, microcirculation

Aims: Associations between duration of arterial hypertension (AH) and blood rheology disturbances were studied in order to evaluate the potential influence of hypertension duration on microcirculation.

Methods: 57 patients with AH (mean age±SD, 51.26±1.94; 30 men and 27 women) were included in the study. All the subjects underwent to the reviewing their medical histories, investigation of blood rheology and clinical blood pressure measurements. According to the mediana of AH duration, all the patients were divided into two groups: < 7years and ≥ 7years. Patients with cardiovascular, peripheral and cerebrovascular diseases, diabetes mellitus, smokers, pregnant, obese as well as those with secondary forms of hypertension were excluded from the study.

Results: Patients with longer duration of AH (≥ 7years) had statistically significant higher value of platelet and erythrocyte aggregative activity (100.69±3.26 vs. 94.57±3.4; P=0.008 and 31.34±5.29 vs. 22.91±5.02; P=0.015; respectively), fibrinogen concentration (3.89±0.2 vs. 3.52±0.23; P=0.021) and plasma viscosity (1.81±0.1 vs. 1.68±0.1; P=0.031). Duration of AH positively correlated with platelet count (r=0.289, P=0.029), platelet aggregative and adhesive activity (r=0.426, P=0.001 and r=0.411, P=0.001, respectively), fibrinogen concentration (r=0.368, P=0.005), erythrocyte aggregability and deformability (r=0.410, P=0.002 and r=-0.272, P=0.041, respectively).

Conclusions: Based on the study data, we can conclude that patients with duration of AH more than 7 years have increased risk of vascular complications; furthermore, we can suggest that as long is duration of AH, as high is the possibility of the microcirculatory disturbances and consequently, potential risk of the hypertensive patient. Therefore, patients with longer duration of hypertension have to be paid with more attention by the health care professionals.

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BIOCHEMICAL PROFILING IN PATIENTS WITH CAROTID STENOSIS

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Keywords: biomarkers, carotid stenosis

Background/Aims: Atherothrombosis related diseases are a leading cause of death and impairment in developed countries. New biochemical markers are needed to improve risk stratification and prevent atherothrombosis progression. Aim of the study was to determine levels of biomarkers associated with inflammation (hs CRP, IL 6), fibrinolysis (fibrinogen, PAI 1), neurohormonal activation (BNP), endothelial dysfunction (homocysteine) and antioxidant defense (SOD, GPx, TAS, GLU-RED) in patients with carotid stenosis.
Materials and methods: The study enrolled 90 patients with carotid stenosis (48 men and 42 women). History of previous atherothrombotic events (CVI, TIA) was positive in 30 participants. Standard laboratory methods were employed, while statistical analysis included Student's t and Chi square test.
Results: Mean hs CRP, IL 6 and fibrinogen concentrations in our participants were 5.78 mg/L, 4.84 pg/mL and 5.43 g/L. Hs CRP, IL 6 and fibrinogen levels were increased in 88.5%, 16.1% and and 71.3 % respectively. Patients had mean PAI 1 activity 3.99 U/mL, while in 62.1% of them the activity was increased. The average BNP level in our group was 95.4 pg/L, and the increase was observed in 21.9% of cases. Homocysteinemia averaged at 14.3 µmol/L and the incidence of hyperhomocysteinemia was 65.2%. Mean activities of SOD, GPx, TAS and GLU-RED were 1327 U/g Hgb, 42.5 U/g Hgb, 1.43 mmol/L and 54.2 U/L and no decreased activity was observed. Gender related and differences concerning history of previous atherothrombotic diseases had no significant influence on above mentioned biomarkers.
Conclusion: Our preliminary results suggest the possible role of the above mentioned biomarkers both in etiopathogenesis and in clinical management of patients with carotid stenosis. Nevertheless, further clinical studies are mandatory to additionally evaluate the possible use of these markers.

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TISSUE-TYPE PLASMINOGEN ACTIVATOR –7351C/T ENHANCER POLYMORPHISM IN ISCHEMIC CEREBRAL STROKE

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Keywords: tissue-type plasminogen activator, enhancer polymorphism, cerebral stroke

Background/Aims: The majority of ischemic strokes occur because of thrombotic or thromboembolic occlusions. This forms the rationale for use of thrombolytic drugs. Out of several identified single nucleotide polymorphism (SNPs) at the Tissue-type Plasminogen Activator (tPA) locus, the -7,351 C/T enhancer SNP showed closest association to tPA release rates. Functional studies have shown that this SNP affects the binding of transcription factors, and that the T allele expresses less tPA compared with the C allele as well as increased risk of myocardial infarction. A recent study also reported an increased risk of ischemic stroke for the TT genotype. In this study the possible association between ischemic stroke and the tPA -7351C>T polymorphism was investigated in Egyptians.
Materials and methods: forty-eight ischemic stroke patients and 48 matched control cases were recruited in a case control study. DNA was extracted from venous blood samples using QIAamp® 96DNA Blood Kit (QIAGEN). Genotyping was performed by allele discrimination analysis using the 5' nuclease assay. PCR was performed in Rotor-GeneTM 6000 (Corbett Research). The results represented by two curves as: Yellow channel represents C probe labeled by (VIC) dye; this is the wild type. Green channel represents T probe labeled by (FAM) dye; this is the mutant allele.
Results: We observed that distribution of genotypes was consistent with that predicted by the Hardy-Weinberg equilibrium in both cases and controls. The respective genotype distribution among cases was 31.2% for CC, 52.1% for CT, and 16.7% for TT. Among the 48 control subjects, 56.25% were homozygous for the C allele (CC), 43.75% were heterozygous (CT), and 0% were homozygous for the T allele (TT). The overall allele frequencies for controls and cases were respectively 78.13%, 57.25% for the C allele and 21.87%, 42.75% for the T allele. Subjects carrying the –7351 T allele had an increased risk of cerebral stroke as compared to subjects homozygous for the C allele with an OR of 2.66,95% CI : 1.42 -5.01, P-value two tailed = 0.003. This association was independent of established risk factors.
Conclusion: This study supports the tPA-7351 C/T enhancer polymorphism being a risk factor for ischemic stroke. Larger studies will have to confirm our observation with stratification for stroke subtypes. The possible association between this polymorphism and the respective plasma protein level remain to be elucidated. The interaction between tPA gene and other susceptibility genes in large scale association studies will provide useful information for better understanding of their impact in the development of ischemic stroke.

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ANALISYS OF PLATELET GLYCOPROTEINS AND ENOS POLYMORPHISMS IN ARTERIAL ISCHEMIA OF LOWER LIMB IN TYPE 2 DIABETES MELLITUS PATIENTS

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Keywords: ischemia, diabetes, polymorphisms

Background/Aims: Several polymorphisms in platelet glycoproteins and eNOS genes have been studied as possible risk factors for development of thrombotic events. The aim of this work is to verify the possible association between platelet glycoproteins and eNOS polymorphisms and the presence of arterial ischemia of lower limb in type 2 diabetes mellitus patients.
Materials and methods: Genetic analysis was performed in a sample of 153 control individuals and 121 patients with type 2 Diabetes mellitus. It was used PCR-RFLP to analyze PlA1/A2 in GPIIIa, HPA-2, KOZAK and one VNTR in GPIbα, C807T in GPIa, and T-786C and G894T in eNOS gene. Hardy-Weinberg equilibrium was tested.
Results: KOZAK and PlA1/A2 polymorphisms were in Hardy-Weinberg disequilibrium for type 2 diabetes mellitus patients with and without arterial ischemia of lower limbs, respectively. VNTR polymorphism in GPIbα showed statistically significant differences when compared control group and type 2 diabetes mellitus group.
Conclusions: Our results suggest an association between the presence of arterial ischemia of lower limb in type 2 diabetes mellitus patients and platelet genetic markers. Further studies to investigate functional consequences of these polymorphisms in mature proteins will be necessary.

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DEVELOPMENT OF VASCULAR WALL STIFFNESS PARAMETERS IN DIABETES MELLITUS TYPE II - EVALUATION OF THERAPEUTIC EFFECT OF SULODEXIDE VS. NAFTIDROFURYL

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Background: Diabetes mellitus type II is a serious civilization disease with epidemic occurrence in our population. The most common complications of diabetes are micro- and macrovascular. Even other complications such as retinopathy and nephropathy are partially caused by microangiopathy. Progression of diabetic angiopathy is also preconditioned by the loss of glycosaminoglycans (GAG).
Aim: The objective of monitoring was to determine whether administration of GAGs in oral form (sulodexide) affects the progression of pathological vascular wall stiffness in diabetic patients. Naftidrofuryl was selected as a reference medication – as a substance with proven effect in the early stages of peripheral arterial occlusive disease.
Patients and Methods: Type II diabetic patients without anamnesis of developed atherosclerosis (CHD, PAOD, cerebrovascular disease) or other developed late complications of diabetes treated by diet or oral antidiabetic drugs were selected. Patients were divided into groups on sulodexide therapy, naftidrofuryl therapy and the control arm with no new medication. Healthy control group of non-diabetics has been added. Patients were followed for 3-6 months. Vascular wall stiffness parameters were repeatedly measured (Fukuda Denshi VaSera VS-1000) by determining both the CAVI (Cardio-Ankle Vascular Index) and ABI (ankle-brachial index) indexes.
Results: Significant decrease in vascular wall stiffness parameters was observed in the sulodexide group. A similar but less significant decrease was observed in the naftidrofuryl group. A slight increase of observed values was observed in the control group of healthy subjects, that corresponds to the natural aging of the vascular wall. The control group of diabetics without sulodexide or naftidrofuryl therapy showed a significant increase in vascular wall stiffness parameters. ABI index value did not show significant change in any of the evaluated groups, while the changes in CAVI values reflected quite well the hypothesis on the evolution of the vascular wall stiffness in each group.
Conclusions: Sulodexide therapy appears to be very effective in preventing progression of vascular wall stiffness as both, risk factor and a marker of micro-and macroangiopathy and therefore the organ damage development in diabetes mellitus type II. Using CAVI index is sufficiently sensitive method for detection of vascular wall pathology and its dynamics, especially in diabetics in early stages. Further evaluation of the effect of long-term therapy on morbidity and mortality in diabetic patients remains a question of continued or controlled randomized clinical trial.

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FETAL AND MATERNAL THROMBOPHILIA IN IUGR WITH OR WITHOUT MATERNAL HYPERTENSIVE DISEASE

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Keywords: pregnancy disease, thrombophilia

Background/Aims: Intrauterine growth restriction (IUGR), defined as a reduction from the physiological growth rate, depends on the capacity of the placenta of transferring oxygen and nutrients from the maternal to the fetal circulation. Placental insufficiency could be caused by impairment in maternal or fetal circulation by a thrombotic event, possibly associated with thrombophilic disorders. The aim of our study was to 1) define the role of maternal/fetal factor V Leiden and prothrombin G20210A in the development of IUGR diagnosed in utero and 2) to evaluate whether maternal hypertensive diseases would modify this association.

Methods: Prospective case-control study. IUGR was defined as: abdominal circumference <10th percentile of the age-related reference values, decrease >40th percentiles from the reference ultrasound growth curve and neonatal weight <10th percentile. Pregnancy induced hypertension (PIH) was defined as: blood pressure >140/90mmHg after 20 weeks of gestation. Preeclampsia (PE) was defined as: blood pressure >140/90mmHg and proteinuria (>0,3 gr/24h). Normal pregnancies showed normal fetal growth confirmed at birth.

Results: 263 normal pregnancies and 77 pregnancies complicated by IUGR (28 with and 49 without PE or PIH) were enrolled. Factor V Leiden was present in 3 (3.9%) mothers with IUGR (1 with PE/PIH, 2 without PE/PIH) and 4 (5.2%) neonates (1 with PE/PIH, 3 without PE/PIH) compared with 7 (2.7%) mothers and 8 (3.1%) neonates in the control group. Prothrombin G20210A mutation was present in 5 (6.5%) mothers with IUGR (1 with PE/PIH, 4 without PE/PIH) and 8 (10.4%) neonates (1 with PE/PIH, 7 without PE/PIH) compared with 9 (3.5%) mothers and 13 (5%) neonates in the control group. Only IUGR without PE/PIH was associated with a thrombophilic mutation carried by the neonate (OR 2.91, 95% CI 1.27-6.63 for factor V Leiden or prothrombin G20210A).

Conclusions: Conflicting results in previous studies regarding the role of thrombophilic mutation in pregnancy disease can be due to: 1) heterogeneity of cases; 2) lack of evaluation of neonates. The present study found an association only between IUGR not complicated by PE/PIH and fetal thrombophilia.

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DISCONTINUING THERAPEUTIC DOSAGE OF LMWH 12 HOURS BEFORE DELIVERY IS NOT AS SAFE AS 24 HOURS BEFORE DELIVERY

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Keywords: pregnancy, anticoagulation

Background: Women who are using low-molecular-weight heparins (LMWH) may be at increased risk for postpartum haemorrhage (PPH). Guidelines recommend discontinuing LMWH at least 24 hours before labour, but labour is mostly not a planned event.

Aims: To assess the bleeding risk in relation to time between last therapeutic dose of LMWH and delivery.

Patients and methods: From 1999 to 2009, we prospectively followed all women with an indication for anticoagulation during pregnancy in our centre. We report on all first pregnancies with anticoagulation. Women had no standard planned induction with withholding of LMWH, but LMWH was changed to twice-daily from 36 weeks of gestation and stopped at start of labour. We restarted 4-8 hours after delivery. PPH was defined as >1000 ml blood loss. Women were divided into subgroups by the various intervals between last dose LMWH and delivery (<12hrs, 12-24hrs, >24hrs).

Results: We analyzed 88 patients with anticoagulation during pregnancy. Median age was 30 yrs (range 20-43 yrs), 66% was nulliparous. Median gestational age was 39 0/7 weeks (28 3/7-42 3/7). Modes of deliveries were vaginal in 81% and section caesarean (SC) in 19% (9% elective, 10% emergency). Median blood loss was 300 ml (50-2000ml), 7% had a primary PPH. Risk of PPH was 0%, 11% and 4% for intervals of <12, 12-24 and >24 hrs, respectively (p=0.32). One woman (1%) experienced a secondary PPH (>24hrs). Median blood loss in women who delivered before 12 hrs after last dose of LMWH was comparable to 12-24 hrs and after 24 hrs (275vs350vs300ml; p=0.26). The table below shows the median blood loss in relation to modes of delivery and time between last dose of LMWH and delivery.

Conclusions: Discontinuing therapeutic dosage of LMWH 12 vs 24 hours before delivery does not increase median blood loss, but the risk of PPH is increased.

Table: Median blood loss in relation to mode of delivery and time between last dose of LMWH and delivery

Blood loss, ml	<12 hrs	12-24 hrs	>24 hrs	p
Vaginal delivery	249	300	300	0.36
Elective SC	-	375	350	0.80
Emergency SC	-	400	450	0.87

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PROTEIN Z GENE VARIANTS IN WOMEN WITH PREGNANCY LOSS

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Background/Aims: Reduced protein Z (PZ) levels were described in women with pregnancy loss (PL). We previously showed in a small group of women with PL that PZ levels below the 2.5 percentile can be due to sporadic mutations within the exon 8. The aim of the current study was to screen this locus for additional mutations in a wide group of women with a history of PL.

Materials and methods: Between January 2007 and November 2009, 629 women with a history of pregnancy loss and 661 women with no history of obstetric complications and at least one uneventful pregnancy, were enrolled as cases and controls, respectively. Early recurrent pregnancy losses (ERFL) were defined as consecutive 3 or more fetal losses at ≤ 14 weeks, late pregnancy loss (LPL) were defined as fetal losses at ≥15<20 and fetal death (FD) as losses ≥ 20 weeks. Exon 8 within the PZ gene was investigated by DNA direct sequencing (3100 ABI PRISM, Applied Biosystems). Cases and controls did not significantly differ as far as the age, gravidity and parity are concerned.

Results: Polymorphisms and sporadic mutations are shown in the **Table**. The analysis of the exon 8 revealed the presence of a previously described polymorphism (Arg295His) and two missense mutations (Leu264Pro in one case with PZ levels below the 2.5 percentile and Thr316Met in 2 patients). The patient carrying Leu264Pro had previous 1 FD at 20 weeks; both patients carrying Thr316Met showed 1 FD each and one of them also ERFL. With the exception of the Arg295His variant, all the variants were not previously described.

Conclusions: Sporadic mutations within PZ exon 8 could have a role in the occurrence of PL, and particularly those ≥ 20 weeks. Further functional and clinical studies are needed to investigate the role of novel gene variants identified.

Table: Clinical data and mutations in both groups investigated

	Controls n=661	*Cases* n=629
Age, median (range)	30 (18-40)	33 (18-46)
Gravidity, median (range)	1 (1-6)	3 (1-22)
Parity, median (range)	1 (1-5)	0 (0-4)
Thr235Met	1	/
Thr336Ala	1	/
Leu292Phe	4	/
Ala257Val	1	/
Glu259Val	7	11
Arg295His	42	4
Leu264Pro	/	**1**
Thr316Met	/	**2**

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CIRCULATING ENDOTHELIAL MICROPARTICLES IN PREECLAMPSIA

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Background: Microparticles (MPs) are small (diameter <1um) membrane-bound vesicles shed from the surface of different type of cells, after membrane activation or apoptosis. Throughout various mechanisms, MPs participate in haemostasis and have procoagulant potential in several diseases characterized by endothelial dysfunction and systemic inflammation. Preeclampsia (PE) is an hypertensive disorder that occurs in 5 to 8% of pregnancies; the etiopathogenesis of this pregnancy syndrome has not yet been clarified but it is known that the procoagulant state and endothelial dysfunction, due to maternal systemic inflammation, give a relevant contribution. The complex network linking MP and PE is currently a subject of intense investigation.
Aims: To investigate the endothelial derived MPs (EMPs) plasma levels in a cohort of patients with PE and healthy pregnant control subjects.
Materials and methods: After informed consent, 15 healthy pregnant women and 16 patients with PE, comparable for gestational age, were enrolled. MPs determination was conducted in platelet-poor plasma. Using flow-cytometric techniques (EPICS XL-MCL, Beckman Coulter, USA) total MPs identified by either a dimensional gate (defined by 1um beads) and by Annexin V- fluorescein isothiocyanate (FITC), and EMPs identified by a double labelling with anti-CD146 conjugated with R-phycoerythrin-cyanine 5.1 and with FITC Annexin-V were measured.
Results: Total MPs and EMPs plasma levels (mean±SD) were higher in PE patients than in control women. The difference was not statistically significant in both cases (Student's t-Test p value 0.1 and 0.8, respectively) **(Table)**.
Conclusions: Our data show no statistically significance difference in EMPs plasma levels between the two groups. These findings may reflect a mechanism of higher consumption of EMPs due to increased placental cell turnover and the presence of a smaller active placental surface that is the source of membrane fragments. Future studies on wider populations should reveal the mechanism underlying the observed associations.

Table

	Healthy pregnant women	Preclampsia	p value
Total MPs (MPs/ µL)	8556 ± 3149	10524 ± 5899	0,1
EMPs (MPs/µL)	4414 ± 1517	4518 ± 1636	0,8

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CARBETOCIN INCREASE THROMBIN GENERATION AFTER CESAREAN SECTION

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Keywords: thrombin generation, pregnancy, uterotonic agents

Background/Aims: To prevent post partum haemorrhage uterotonic prophylactic drugs are commonly used after caesarean section. Aim of the present pilot study is to evaluate thrombin generation(TG) after caesarean section (CS) in women treated with carbetocin and oxitocin, two different uterotonic agents.
Materials and methods: We enrolled 28 women, undergoing cesaren section, 14 treated with oxytocin and 14 treated with carbetocin . Patients, without previous bleeding or thrombotic events, were matched for age, weight, parity and race. Blood samples for TG study and blood cell count were collected before delivery (T0), 1 hour (T1) and 24 hour (T2) after drug infusion. Blood samples were immediate centrifuged and poor platelet plasma (PPP) stored at -80°. Thrombin generation was performed with a commercial assay (Technothrombin TGA, Technoclone). TG measured lag time, peak, velocity, endogenous thrombin potential (ETP). The activation of coagulation was obtained with adding small amounts of tissue factor and phospholipids. ETP results were expressed as nM-thrombin/min.
Results: No differences were observed in TG between two groups at T0. A significant increase in ETP at T1 was observed in the carbetocin group (ETP mean ± DS= 3810.8 ± 661.95), than oxitocin treated patients (ETP mean ± DS= 3588.7 ± 711.36) with $p<0.05$. T2 showed the persistent ETP increase in carbetocin group even if it did not reach the statistical significance. Also other parameters like peak and velocity were significantly increased in the carbetocin compared with oxitocin group both at T1 and T2. No differences in bleeding were observed in the two groups.
Conclusions: Our pilot study shows a major ETP increase in patients treated with carbetocin, probably reflecting an important uterotonic action and its longer half-life in comparison with oxitocin. These properties, as recently reported, may support carbetocin use during delivery to prevent postpartum blood loss.

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HEALTH CARE PROGRAM FOR THROMBOEMBOLISM PREVENTION IN PREGNANCY

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Keywords: thrombin generation, pregnancy, uterotonic agents

Background/Aims: VTE is the major cause of mortality and morbidity in pregnant women in western countries. Even if the rates are low and knowledge has increased, mortality didn't decrease during the last decade.
Aim of the program was: 1) To inform all new pregnant women about VTE risk and give them practical recommendations on VTE and lifestyle;
2) To recognize all new pregnant women at risk for VTE and apply different prophylactic/therapeutic regimen, through a risk assessment stratification model.
Materials and methods: The program started in 2007. We performed courses on VTE and pregnancy for hospital's staff, obstetricians, specialists and general practitioners to uniform clinical approach and management. A risk assessment dynamic model, with four different prophylactic/therapeutic approaches, was elaborated. VTE risk was evaluated during the three trimesters, at delivery and during puerperium. All new pregnant woman received an information brochure, translated into 8 different languages.
Results: From January 1st 2009 until December 31st 2009, Cremona Hospital reported a total of 1290 deliveries, 327 of whom were cesaren sections (22 in emergency) and 963 vaginal delivery. 553 out of 1290 were included into the preventive program (group A) and followed up for VTE risk. 104 of them started a prophylactic treatment (70 mechanical, 24 pharmacological prophylaxis and 5 therapeutical treatment). Among the 737 pregnant women (group B), who were not managed in the program, we observed 4 thromboembolic events : 1 DVT, 1 EP and 2 superficial thrombophlebitis (complication rate=0.54%). In group A we observed 1 DVT (complication rate=0.18%).
Conclusions: Our program in 2009 reached the 42.8 % of the new pregnant woman and showed a significant reduction in major thromboembolic complication through a dynamic individual risk assessment. The integration among different specialists, through a common approach, is the base for a standardized pregnancy VTE risk assessment.

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WHOLE BLOOD COAGULATION THROMBOELASTOMETRY (ROTEM®) PROFILE IN HEALTHY TERM PREGNANT WOMEN

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Background: Whole blood (WB) ROtation ThromboElastoMetry analyser (ROTEM®; Pentapharm, Germany) is an attractive laboratory tool for studying the simultaneous and integrated effects of different components (i.e. plasmatic factors, platelets, leukocytes, and red blood cells) involved in the dynamic process of clot formation and lysis. We compare ROTEM® coagulation profile between a group of healthy term pregnant women and a group of healthy women.
Materials and methods: Blood samples were collected from 15 pregnant women. A group of 35 non-pregnant women acted as control. The INTEM and EXTEM represent assays in which the intrinsic or the extrinsic coagulation pathways are triggered, respectively. NATEM (Non-Activated TEM) was used to assess WB clot formation in the absence of activation of the clotting cascade other than recalcification. The FIBTEM assay was used for the assessment of the specific role of fibrinogen following inhibition of the platelets by Cytochalasin D. Parameters considered were: Clotting Time (CT), the time from the beginning of the coagulation analysis until increase in amplitude of 2 mm; Clotting Formation Time (CFT), the time between an increase in amplitude from 2 to 20 mm; Alfa angle the tangent to the clotting curve through the 2 mm point; Maximum Clot Firmness (MCF) the maximum amplitude, Area Under The Curve (AUC) defined as the area under the velocity curve.
Results: ROTEM® depicted an hypercoagulable profile in cases characterized by a shorter CT and CFT and a higher a-angle, MCF and AUC than controls. The differences were statistically significant in all fours tests considered (Student's T-test $p<.005$).
Conclusions: ROTEM® identified a hypercoagulable state in healthy pregnant women as comparable with controls. Rotational thrombelastometry could be useful to observe changes in the coagulation of WB during pregnancy. Further studies are needed to investigate the role of ROTEM® in the risk of thrombosis or haemorrhage in pregnancy.

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PREGNANCY COMPLICATIONS AND ANTIPHOSPHOLIPID ANTIBODIES

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Keywords: antiphospholpid-pregnancy

Background/Aims: Women with acquired and hereditary thrombophilia are at increased risk of developing venous thromboembolism and other associated gestational vascular complications like fetal loss, preeclampsia, intrauterine growth restriction, and placental abruption during pregnancy. These complications are a major cause of maternal and fetal morbidity and mortality. The primary objective of this study was to determine if elevated antiphospholipid titers or presence of lupus anticoagulant were correlated with the presence of vascular placental complications in women admitted for obstetric consultation at Tlemcen hospital, Algeria.
Methods: The medical records of pregnant women between January 2007 and 2010 were retrospectively collected. Maternal and perinatal histories including demographic data, medications, obstetric histories, and neonatal clinical manifestations and laboratory data were analyzed. We tested anticardiolipin antibodies (aCL) (IgG and IgM isotypes) and anti-β2 GPI by enzyme linked immuno-assay (ELISA), LA activity (LAC) using both Drvv screen/confirm and Staclot LA. The possibility that the relationship between elevated antiphospholipid antibody titers and the outcomes of pre-eclampsia/eclampsia, recurrent miscarriages, fetal death, help syndrome may have been modified by the presence of SLE was evaluated in a multiple logistic regression model by creating a composite interaction term.
Results: 51 patients with pregnancy complications were included. 45 of these patients had experienced frequent spontaneous abortions (88%), and five had unexplained fetal deaths (9%). 3 had help syndrome and eclampsia, None of them had vascular thrombosis. Specific autoimmune antibodies were detected, including anticardiolipin antibody (n=8), anti-β2 glycoprotein I (n=3), and lupus anticoagulant (n=6). Women who had elevated antiphospholipid antibody titers IgG had an increased adjusted odds ratio for recurrent miscarriages ($p<0.01$).
Conclusions: There are several possible mechanisms by which antiphospholipid antibodies (aPL) may have adverse effects on placental functions. A strong correlation between elevated IgG antiphospholipid antibodies titer status and recurrent miscarriages was also found, however, whether or not there is an association between high titers of AP antibodies and pre-eclampsia in the absence of APS is unclear. Further investigations are needed to better understand how aPL induce obstetric complications and to better clarify the functional role of heparin in the human placenta, leading to more successful therapeutic options.

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RISK FACTORS FOR PRE-ECLAMPSIA, STUDY IN IRAN

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Keywords: pre-eclampsia, pregnancy induced hypertension (PIH), risk factor, blood group, urinary tract infection (UTI), parity, hemoglobin

Aims: Evaluation of some of the risk factors for pre-eclampsia.
Methods: This study was conducted as a retrospective case control study. 318 pre-eclamptic women in the case group and another 318 women who were normotensive at the time of delivery were considered as control group. The evaluated factors were maternal age, gestational age, nulliparity, educational status of mother, maternal BMI (Body Mass Index), maternal hemoglobin and blood Rh, familial history of pre-eclampsia, history of pre-eclampsia in a previous pregnancy, marital relations, urinary infection(UTI) during the present pregnancy, season, and the method of contraception were compared between the two groups.
Results: UTI, (P= 0.04, OR= 3.7, %95 CI 1.1-13.6), history of pre-eclampsia during previous pregnancy, (P= 0.003, OR= 9.4, %95 CI 2.1-41.2), and Winter (p=0.001, OR=2.1 %95 CI 1.3- 3.4), were risk factors for pre eclampsia. Maternal age of more than 20 years old (p=0.02, OR=0.6 %95 CI 0.6-0.9), high educational status of mother (p=0.004, OR=0.4 %95 CI 0.2-0.7),parity more than one (p=0.009, OR=0.5 %95 CI 0.3-0.8), and oral contraceptive pill (p=0.04, OR=0.4 %95 CI 0.2-0.9), were protective for pre-eclampsia. The rate of cesarean delivery was more common than the control group [245 cases (77%) vs 85 cases (26.7%), P=0.000]. The minute l Apgar score of neonates less than 8 was more common in the case group. (28.6 % v.s 47.4%, P= 0.000). Gestational age at the time of delivery was lower in the case group (36.48 ± 3.4 weeks v.s 37.12 ± 3.3 weeks, P= 0.000).
Conclusion: Awareness of risk factors of pre-eclampsia can help monitor patients and make diagnosis earlier and predict which patients are more likely to develop pre-eclampsia.

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LOW-MOLECULAR-WEIGHT HEPARIN FOR PREVENTION OF OBSTETRIC COMPLICATIONS IN CARRIERS OF FACTOR V LEIDEN OR PT–G20210A MUTATION

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Keywords: obstetric complications, FV Leiden, PT-G20210A, prophylaxis

Background: Whether the administration of low-molecular-weight heparin (LMWH) during pregnancy is effective for prevention of obstetric complications (early or late fetal loss, pre-eclampsia, fetal growth restriction) in women who are carriers of factor V Leiden (FVL) and/or prothrombin variant G20210A (PTM) is controversial.
Methods and results: During a 7-year period, 508 pregnancies occurring in 422 women (age, 18 to 45) who were carriers of either mutation were monitored up to delivery at five Italian centres. 183 women had experienced one or more previous obstetric complications. The decision as to administer LMWH with either enoxaparin or nadroparin in prophylactic doses during the whole period of pregnancy was left to discretion of attending physicians. 278 women received the LMWH treatment, while the remaining 230 did not. 46 obstetric complications occurred in the former group and 59 in the latter, leading to a relative risk (RR) of 0.6 (95% CI, 0.5 to 0.9). When the analysis was confined to the only women with previous adverse obstetric events, the reduction in the RR became even stronger: 0.4 (95% CI 0.2 to 0.8) in women with one event, and 0.5 (95% CI, 0.3 to 0.9) in those with more than one. No side effects were reported in women who had received the LMWH prophylaxis.
Conclusions: LMWH prophylaxis safely reduces the risk of obstetric complications in carriers of FVL and/or PT mutation. The benefit is greater in those with previous obstetric events.

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CHARACTERIZATION OF BLOOD PLATELET HETEROGENEITY IN PRE-ECLAMPTIC, NORMOTENSIVE PREGNANT AND NON-PREGNANT WOMEN

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Keywords: pregnancy, pre-eclampsia, platelet

Background/Aims: One of the occasions of severe obstetric complications is the development of hypercoagulation. Aim of the study was to evaluate the dynamics of vascular-thrombocytal parameters in normal and complicated pregnancy.
Patients and methods: 20 non-pregnant women, 25 pregnant women with pre-eclampsia and 20 with a physiological current of pregnancy are surveyed. We assessed the changes of platelet amount, platelet aggregation and living blood platelet heterogeneity by method of vital computer morphometry using computer phase-interference microscope (CPM) "Cytoscan".
Results: We identified 4 living platelet forms that have different morphological features according to various activation levels: I - resting platelets, II - platelets with low activation level, III - platelets with high activation level and IV - degenerated functionally incomplete platelets. The proportion of different morphological cell types of non-pregnant women were 52.3%; 36.6%; 10.3%; 0.8%, respectively. The analysis of dynamics of vascular-thrombocytal parameters in I, II and III pregnancy trimesters allowed to define the consistent patterns of adaptation and dysadaptation hemostasis mechanisms in normal and complicated pregnancy.
At III pregnancy trimesters we found a decrease in the platelet amount by 15-30% and an increase in platelet aggregation by 1.7 time in patients with pre-eclampsia in comparison with normotensive pregnancy. Platelets in patients with pre-eclampsia on average in population diameter, perimeter, area and volume exceeded the controls by 5-18%, phase cell thickness decreased by 15-2%, percentage of cells with low and high level of activation increased, functionally incomplete platelets degenerated.
Conclusions: The circulating platelet multiplicity reflects cell distinctions in size, density, metabolic, functional, biochemical features and the level of megakaryocyte polymorphism. The considerable alteration of dimensional living platelet parameters (10-15 % from gestational norms) and increasing of platelet activating status (1.5-1.7 times) are indicated to hemostasis dysadaptation at pregnancy, the failure of adaptable mechanisms and the threat of trombogenicity complications.

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FACTOR V LEIDEN, PT-G20210A, MTHFR C677T MUTATIONS AND THE RISK OF IMPLANTATION FAILURE IN WOMEN UNDERGOING ASSISTED REPRODUCTIVE PROCEDURES (IVF OR ICSI)

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Keywords: thrombophilia, IVF ICSI

Background: Thrombophilia is a well known factor for female infertility. Several studies in the literature since 1980s underline the relationship between inherited and acquired thrombophilia and obstetric complications. This condition can cause indeed recurrent early pregnancy losses, fetal losses, pre-eclampsia, abruptio placentae, intrauterine growth retardation (IUGR). Yet, recent studies underline a possible relationship between thrombophilia and repeated *in vitro* fertilization failure. It has been supposed that an impairment uteroplacental circulation due to hypercoagulability in the mother is a possible causes of implantation failure after IVF or ICSI.
Aims: The aim of the study was to evaluate the association between inherited thrombophilia and an increased risk of implantation failure in women undergoing assisted reproductive procedures.
Materials and methods: A prospective cohort study was carried out in women undergoing IVF or ICSI in our PMA Center of Padova University in the period between May 2007 and May 2009. 201 women between 23 and 45 years were screened for inherited thrombophilia. In these women 83 were negative for thrombophilia mutations. Sixteen were positive for factor V Leiden (12 heterozygous ad 4 homozygous), six were heterozygous carries for PT-G20210A mutation, 70 were positive for methylenetetrahydrofolate reductase gene mutation in heterozygosis, 26 in homozygosis. The median age was similar in the two group of women as well as the number of previous pregnancy losses and pregnancy. The patients were classified into different causes of infertility: male sterility, tubal sterility, poor responder, endometriosis, mixed causes and unexplained sterility.
Results: According to multivariate analysis of the thrombophilic carrier status in relation with implantation, considering the different infertility causes for each women and their age, it result that the presence of FV Leiden, PT-G20210A or MTHFR C677 is not a risk factor for implantation failure after IVF or ICSI: odds ratio 0.8 (95% CI 0.5 to 1.4) [p=0.6].
Conclusions: In conclusion, this study provides no evidence that thrombophilia due to FV Leiden, PT-G20210A and MTHFR gene mutations in heterozygosis and in homozygosis may influence implantation failure in women undergoing assisted reproductive procedures (IVF or ICSI). To confirm our data we need a larger cases study.

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THROMBOTIC AND OBSTETRICAL RISK FACTORS IN ANTIPHOSPHOLIPID ANTIBODIES SYNDROME

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Keywords: thrombosis, antiphospholipid

Background: Antiphospholipid antibodies syndrome (APS) could present thrombotic and obstetrical recurrent manifestations despite regular treatment. Previous studies have evaluated thrombotic risk factors in primary and secondary APS, but most of these studies have been performed before the new diagnostic criteria characterisation in APS.
Aims: To identify risk factors for new clinical events (thrombotic and/or obstetrical) in a APS cohort characterised with new diagnostic criteria.
Patients and methods: 58 patients (3 among them failed to complete the whole study), mean age 29+/- 10 years (ratio between females and males 4:1), have been enrolled in a five year prospective study (2005-2009). Patients have been included in the study on the basis of new diagnostic criteria of the last Consensus Conference for APS ((Miyakis S et al., 2006). All new clinical manifestations reported during the follow-up period, after the APS diagnosis and under treatment, were considered such as new clinical events.
Results: 19 new clinical thrombotic and/or obstetrical manifestations have been reported in 15/ 55 patients (27%). One case of lethal arterial thrombosis (stroke) has been reported during the follow-up period (1.8%). 3 cases of stroke, 3 myocardial infarctions, 7 deep venous thromboses, two foetal death during the sixth month of pregnancy, one pregnancy precocious loss. IgG >40 UI GPL anticardiolipin antibodies have been correlated with arterial and/or venous thrombotic events, and anti β2GPI antibodies and lupus anticoagulant with obstetrical manifestations. Multi-varied regression analysis have confirmed that IgG >40 UI GPL anticardiolipin antibodies represent an independent risk factor for thrombosis (odds ratio: 8.9; 95% CI, 1.75 - 45.4).
Conclusions: IgG >40 UI GPL anticardiolipin antibodies represent an independent risk factor for arterial and/or venous thrombosis and anti β2GPI antibodies and lupus anticoagulant for obstetrical manifestations in our APS cohort study. However, longer longitudinal studies in larger cohort patients are requestedfor a formal conclusion.

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LMWH CAN IMPROVE THE OUTCOME OF ART PROCEDURES

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Keywords: infertility, LMWH, pregnancy

Background: Implantation failure in patients submitted to ART assisted reproduction techniques is since many years a matter of debate. Low molecular weight heparin (LMWH) role is emerging as a possible factor improving the early phases of implantation.
Study design and population: Retrospective observational analysis of patients with at least two implantation ART failures, screened for the presence of thrombophilic factors and submitted to successive ART cycles with or without administration of LMWH.
Aims: Main aim of the study was to evaluate the pregnancy rate in patients with or without heparin administration.
Materials and methods: 265 patients fulfilled the enrolling criteria. 149 (56%) were primary infertile and 116 (4%) were secondary infertile. Their mean age was 36.3 ± 3.6 years and the basal FSH level was 7.73 ± 3.03. 81/265 (?) were positive for at least 1 thrombophilic mutation (G1691A FV, G20210A FII, homozygous C677T- MTHFR); 25/265 (9.4%) if we consider only G20210A FII e G1691AFV. They were submitted to 569 new ART cycles 512 (90%) non supported and 57 (10%) supported by heparin administration.
Results: 105 clinical pregnancies were observed in 569 cycles (18.80%). Stratified by age group, in term of previous pregnancies, previous ART cycles, basal FSH, number of retrieved oocytes, embryo transferred, BMI and smoking were not significantly different between the group with or without heparin administration; 17% (88/512) was the pregnancy rate in patients not treated with heparin and 33 % (19/57) in the heparin treated group (p=0.006).
Conclusions: A significant higher pregnancy rate in ART implantation failures was observed in patients supported by heparin administration. These interesting findings from our observational study should be confirmed by further randomized controlled trials before routine application of LMWH for ART cycles.

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ENDOTHELIAL ACTIVATION MARKERS DURING PHYSIOLOGICAL PREGNANCY

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Keywords: endothelium, pregnancy, activation, preeclampsia

Aims: To establish endothelial activation markers which could uncover endothelial damage during physiological pregnancy.
Type of study: Prospective study.
Patients and methods: We examined 403 pregnant women with a physiological pregnancy. Venous blood samples were collected from the women at the beginning of the pregnancy, a second sample was collected in the interval 24-28 weeks gestation. Parameters were examined using methods: t-PA – ELISA, PAI-1 – ELISA, VWF Ag – EIA ePCR – ELISA, MMP-2,9 – ELISA with fluorogenic detection, TIMP -2 – ELISA, endothelial microparticles- flow cytometry.
Results: The level of VWF antigen increased during the entire course of pregnancy (in the I trimester the average level was 152.32 %, in the II and III trimester 173.34% and 216.20% respectively). At the same time, VWF activity also increased (I trimester average level 130.20%, II and III trimester 150.09% and 181.91% respectively).
The level of thrombomodulin significantly increased during pregnancy (I trimester average level 19.05 ng/ml, II and III trimester 28.47 ng/ml and 39.86 ng/ml respectively). The level of soluble form of EPCR increased during pregnancy (I trimester average level 201.76 ng/ml, II and III. trimester 274.68 ng/ml and 324.07 ng/ml respectively). The level of PAI – 1 increased during the entire course of pregnancy (I trimester average level 36.14 ng/ml, II and III trimester 50.07 ng/ml and 60.12 ng/ml respectively). The level of t- PA did not change significantly during the course of pregnancy The levels of MMP-2, MMP-9 (I trimester average level 8371.90, II and III trimester 8290.81 and 7470.50 respectively), TIMP-2 or endothelial microparticles did not change significantly throughout the individual trimesters.
Conclusions: We confirmed the hypothesis regarding the significant influence pregnancy has on changes in levels of these markers.
Acknowledgement: Supported by the Grant of the Czech Ministry of Health IGA NR 9282-3/2007 a NS10319-3/2009

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ITALIAN REGISTRY OF ADVERSE PREGNANCY OUTCOMES AND VENOUS THROMBOEMBOLIC DISEASE IN PREGNANCY

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Keywords: pregnancy, thrombosis, abortion

Background: Venous thromboembolic disease (VTE) in pregnancy is an important cause of maternal morbidity and mortality. In women, over half of all venous thromboembolic events occurring in the reproductive age are related to pregnancy and about 15% of maternal death are related to pulmonary embolism. Several conditions have been identified as important risk factors for the development of VTE during pregnancy and post-partum: a previous episode of VTE, thrombophilia, operative delivery, increasing maternal age, obesity and prolonged immobilisation.
Obstetric complications occur in at least 10% of pregnancies. Several studies in the last few years have found an association between the presence of thrombophilia and adverse pregnancy outcomes. However, the size of this increased risk and the mechanism which explain this association are not clarified yet.
Low-molecular-weight heparin (LMWH) is the anticoagulant most often used in pregnancy for treatment and prophylaxis. Disadvantages include the risk of uncontrolled bleeding, allergic reactions, heparin-induced osteoporosis and heparin-induced thrombocytopenia.
At present there are no clear guidelines about doses and duration of treatment in pregnant women who develop a VTE or need a thromboprophylaxis.
Moreover, whether anticoagulant therapy will improve the outcome in women with pregnancy complications is unknown.
Aim: To collect data about thromboembolic events and obstetric complications during pregnancy, with particular regards to the thromboprophylaxis/ anticoagulant therapy used.
Materials and methods: Design: Registry (observational cohort study); duration: approximately 48 months: 36 months recruitment, 9 months observational period for the last patients, 3 months of follow-up; number of centers to be involved: 30
Inclusion and exclusion criteria: women who develop a thrombotic (venous or arterial) event or an obstetric complication during pregnancy will be eligible for study entry.
Primary outcomes: to collect data on:
° treatment of VTE in pregnant women
° thromboprophylaxis
° obstetric complications ad anticoagulant prophylaxis
° correlation with thrombophilia
Secondary outcomes: side effects of anticoagulant treatment or prophylaxis
Sample size: based on at least 1500 pregnancy/year for participant Center, a cumulative incidence of thrombotic events of 5:1000 (first thrombotic events, recurrences of thrombosis, thrombotic events in thrombophilic women) and an observational period of 45 months, 30 Centers should allow to observe about 600 thrombotic events.
With regard to obstetric complication 90 events/year for participant Center should be observed (we presume that many early foetal losses will be lost); then 8000 adverse pregnancy outcomes should be collected during an observational period of 45 months

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THE FEATURES OF LIVING PLATELET MORPHOFUNCTIONAL STATUS AT THE PREGNANCY COMPLICATED BY THE TYPE I DIABETES

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Keywords: pregnancy, platelet, type I diabetes

Aims: The purpose of this study was to estimate the features of platelet hemostasis at the pregnancy complicated by the type I diabetes using the basic standard hemostasis analysis and real-time monitoring of the morphofunctional status of peripheral blood platelets.

Materials and methods: 45 pregnant women have been examined throughout their gestational process: 25 with pregnancy complicated by the type I diabetes of various seriousness, 20 with physiological pregnancy. We measured the following hemostasis parameters: prothrombin time (PT), partial thromboplastin time (PTT), D-dimer, fibrinogen, the soluble fibrin monomer complex (SFMC), plasminogen, von Willebrand factor antigen (vWFA) by "Sta compact" (Roche). Morphofunctional status of peripheral blood platelets we determined by method of vital computer morphometry using computer coherent phase-interference microscope (CPM) "Cytoscan".
The pregnant women with type I diabetes were afflicted by the prevalence of coagulative potential and formation of physiological hypercoagulation irrespective of severity level and a stage of indemnification of a diabetes. We have analyzed the optic-geometrical parameters of each isolated living platelet and the distribution of platelets by sizes to detect the heterogeneity of cell population. The average metric platelet parameters (diameter, perimeter, thickness, area and volume) in diabetic pregnancy were constituted 3.6±0.9mkm; 9.7±2.7mkm; 0.9±0.4mkm; 6.2±3.0mkm2; 2.9±2.0mkm3 (M±σ) contrary to 3.1±0.8mkm; 9.2±3.4mkm; 1.2±0.5mkm; 5.4±2.1 mkm2; 2.2±1.3mkm3 in physiological pregnancy ($p<0,05$). It is important that the phase thickness objectively reflects granule contents in platelets. In diabetic pregnancy patients had the high level of platelet activating state: we registered an increase in the activating and degenerating platelet forms. The standard hemostasis studies confirmed our morphometric data.
Conclusions: The computer morphometry of living platelets has guaranteed rapid and objective analysis of the platelet hemostasis, showing early appearances of platelet complications in patients with diabetic pregnancy. Platelet structural and functional impairments could be most marked in the phase of decompensation of type I diabetes during pregnancy.

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THROMBIN GENERATION TEST IN PREGNANCY

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Keywords: Pregnancy, thrombin generation

Background: Generation of thrombin is the pivotal step of haemostasis. Thrombin activity can be registered by continuous measurement of chromogenic or fluorescent substrate cleavage resulting in thrombin generation curve. From this curve various parameters as time until thrombin burst, peak amount of generated thrombin or total amount of thrombin generated (area under the thrombin generation curve) can be inferred . It was shown that thrombin generation is increased in thrombophilia patients. Recently, it was showed that thrombin generation based on the individual's blood composition is associated with the risk of the first venous thrombosis.
Methods: Thrombin generation test venous blood was collected into 0.1 volume of 3.6% trisodium citrate, centrifuged for 20 minutes at 2000g and stored at −80°C until time of analysis. Thrombin generation was determined using an assay kit (Technothrombin TGA). Three major parameters of thrombin generation curve were used for analysis – maximum of thrombin generation (thrombin peak), endogenous thrombin potential (ETP, area under curve) and lag phase. The sample for analysis was taken in 40 healthy pregnant women with no anamnesis of thrombotic complication.
Software-processed results of the examination of the fluorescence reader, the curve with the main parameters - Lag phase, maximum concentration of thrombin (Cmax) and endogenous thrombin potential (ETP) were also examined as well as standard thrombophilia markers.The reference group was a set of healthy blood donors.
Results: In groups of 40 pregnant patients Cmax, ETP and Lag Phase parameters were higher compared to the control group. There were also significant differences between all three trimesters.
Conclusion: TGT is the exciting new possibility for thrombophilia laboratory setting. Limitations for routine use is the lack of standardization and technical and economic performance test.
Acknowledgment: Supported by the Grant of the Czech Ministry of Health IGA NR 9282-3/2007 a NS10319-3/2009

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USE OF THE DELPHI METHOD TO FACILITATE ANTITHROMBOTICS PRESCRIPTION DURING PREGNANCY.

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Keywords: pregnancy, venous thromboembolism, risk score, guidelines, risk assessment

Background/Aims: Management of pregnant women at risk for venous thromboembolism (VTE) remains complex. Guidelines do not definitively fix optimal strategies due to limited trial data. Our objective was to build an easy-to-use tool allowing individualised, risk-adapted prophylaxis. A Delphi exercise was conducted to collect 19 French experts' opinions on pregnancy-related VTE.

Results: Experts with an active interest in clinical research and care of VTE and placental vascular complications were selected. The risk score was classified by an anonymous computer vote. A scoring system for VTE risk in pregnant women was developed, each score being associated with a specific treatment: graduated elastic compression stockings, aspirin, prophylactic low-molecular-weight-heparin (LMWH: variable durations), or adjusted-dose of LMWH through pregnancy and postpartum.

Conclusions: Our simple consensual scoring system offers an individual estimation of thrombosis risk during pregnancy together with its related therapeutic strategy, in accordance with most of the new international recommendations. The accuracy of our individual risk score-based therapeutic guidance is currently being prospectively evaluated in a multicenter trial (Clinical trials.gov registry no: NCT00745212).

Final risk score obtained at the end of the DELPHI Method development and application. The retained risk components are classified into 4 categories according to their main characteristics. The final proposed treatment, detailed at the bottom, depends on the total value obtained after addition of the various risk components of the score.

Figure: Final risk score obtained at the end of the DELPHI Method development and application. The retained risk components are classified into 4 categories according to their main characteristics. The final proposed treatment, detailed at the bottom, depends on the total value obtained after addition of the various risk components of the score.

Past history of venous thromboembolic events		Score
Multiple personal VTE events (including one proximal DVT or PE) or long-term anticoagulants		12
Unique personal VTE event (*)	- PE or proximal DVT	5
	- distal DVT	2
(*) with a triggering factor		- 2
(*) during pregnancy, post partum or OC intake		+1
1st degree relative, proximal VTE: idiopathic or multiple or severe		2
Family history: non-severe VTE (distal DVT or triggering factor or >60 years)		0
Past history of arterial thromboembolic events		
Stroke, vasculitis, arterial embolism, symptomatic atherothrombosis		0/A

Thrombophilia	Score
Antithrombin deficiency	10
Proteins C, protein S deficiency	4
Factor V Leiden, Factor II 20210 A polymorphism - heterozygosity	3
- homozygosity	5
- compound heterozygosity	4
High factor VIII concentrations,	0
hyperhomocysteinaemia	0/B
LA / aPL antibodies +/- placental vascular complications	9/A
LA / aPL + arterial and / or venous thrombosis	12

Placental vascular complications	Score
IUFD	+2/A
Recurrent miscarriages (≥ 3) or one loss ≥ 9 weeks	0/A
Preeclampsia or HELLP syndrome or Placental Abruption	+1/A
IUGR	+2/A

Other risk factors	Score
Multiparity > 3, Varicose veins, Age > 35 years, Obesity, Post-phlebitis sequels, Systemic lupus erythematosus without LA	0

1 to 3: Postpartum prophylactic LMWH, 6 weeks
4: Third trimester and postpartum prophylactic LMWH
5 to 11: Antepartum and postpartum prophylactic LMWH
≥ 12: adjusted-dose LMWH
A: low-dose aspirin (if no adjusted-dose LMWH)
B: folic acid

Legenda: VTE: venous thromboembolism; PE: pulmonary embolism; DVT: deep vein thrombosis; OC: oestrogen-containing oral contraceptives; AT: antithrombin; LA: positive lupus-like anticoagulant; aPL: positive antiphospholipid antibodies, i.e. anticardiolipin and/or anti-β2 GP1 antibodies; IUFD: intrauterine foetal death; IUGR: intrauterine growth restriction; LMWH: low molecular weight heparin

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LOW MOLECULAR WEIGHT HEPARIN IN PROPHYLAXIS OF RECCURENT PREGNANCY COMPLICATIONS IN WOMEN WITH THROMBOPHILIA

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Keywords: thrombophilia, pregnancy complications, low-molecular-weight heparin

Aims: Our objective was to evaluate maternal and fetal outcomes in women with history of pregnancy complications receiving the preconception treatment.

Materials and methods: 400 patients with fetal loss syndrome, 160 patients with severe preeclampsia, 80 patients with venous thromboembolism (VTE), 100 patients with placental abruption, 60 patients with antenatal death and 500 healthy controls were tested to have genetic thrombophilia and antiphospholipid antibodies. Women with history of pregnancy complications received treatment in the preconception period and during pregnancy: low molecular weight heparin (LMWH) guided by D-dimer, natural progesteron up to 24 weeks, aspirin, antioxidants, vitamins of B group, folic acid (up to 4 mg in women with hyperhomocysteinemia).

Results: Thrombophilia was found in 75% patients with fetal loss syndrome and antenatal death, in 96% patients with recurrent preeclampsia, in 70% in patients with history of 1 episode of preeclampsia, in 80% patients with placental abruption and in 100% in patients with VTE. In the study group nobody had moderate or severe form of preeclampsia, mild preeclampsia was observed in 16%. All babies were alive. Preconception therapy allowed preventing recurrent fetal loss syndrome in 66%; 96% pts were delivered after 37 weeks. Patients had not recurrence of placental abruption or VTE.

Conclusions: Thrombophilia might be the main pathogenetic mechanism of recurrent pregnancy complications. Due to thrombophilia involvement in trophoblast invasion and placentation early treatment is essential.

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THROMBOPHILIA IN WOMEN WITH PREGNANCY RELATED VENOUS THROMBOEMBOLISM AND IT`S CONNECTION WITH LOCALIZATION AND THE PERIOD OF THROMBOSIS OCCURRENCE

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Keywords: thrombophilia, pregnancy, thrombosis

Background: Pregnancy and puerperium are independent risk factors for the development of venous thromboembolism and this risk is further increased by the presence of thrombophilia.

Aims: The determination of the prevalence of thrombophilia in women with pregnancy related venous thromboembolism (VTE) and investigation of the connection between the period of VTE occurrence (antepartal vs postpartal) as well as thrombosis localization in various types of thrombophilia

Materials and methods We have investigated 209 women with first episode of venous thromboembolism during pregnancy and puerperium and 128 healthy women with at least one uncomplicated pregnancy. The antithrombin, proten C and protein S activity, APC resistance, lupus anticoagulant, anticardiolipin antibodies, fasting homocystein level, as well as the presence of mutations FV G1691A. FII G20210A and MTHFR C677T have been determined.

Results: Inherited thrombophilia was diagnosed in 113 patients (54.1%) and 11 controls (8.59%). The prevalence of FV Leiden and FII G20210A heterozygous mutation, antithrombin, PC and PS deficiencies taken together, combined thrombophilia and APA in the investigated group was 20.1%, 9.8%, 6.7% 8.8% and 3.8% respectively, with OR 15.37 (CI 3.65-64.76) and 4.36, 95%CI 1.27-14.99) for FV Leiden and PGM. VTE have occurred more frequently in the iliofemoral segment in women with thrombophilia, compared to nonthrombophilic (72vs 37, p=0.000). PTE occurred more frequently in nonthrombophilic women comparing to thrombophilic ones (25 vs 7, P=0.000). Women with deficiencies of natural inhibitors and combined thrombophilia had significantly more frequent antepartal occurrence of VTE, p=0.011 and 0.032 respectively.

Conclusions: Connection of inhibitor deficiencies and combined thrombophilia with antepartal VTE occurrence is the most important finding of this study. Thrombophilia screening in women with pregnancy related VTE is of great importance, since both the duration of treatment and the management of future pregnancies are highly influenced by the presence of certain types of thrombophilia.

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THE EFFECTIVENESS OF D-DIMER AS A BIOMARKER IN SCREENING PROTEIN C DEFICIENCY AMONG THE LOW RISK PATIENTS DURING PREGNANCY

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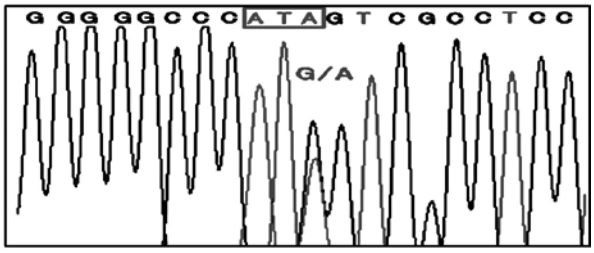
Keywords: pregnancy, D-dimer, proteinC

Background: The prevalence of protein C deficiency is 2 to 5 per 1000, and inheritance is autosomal dominant. The risk of thromboembolism in pregnant women is between 3 to 20 percent and most occur during the peurperium. However, establishment of strategy for screening thrombophilias during pregnancy is difficult as the interpretation of biomarkers is problematical.
The values of D-dimer at 28th weeks of gestation have measured for the purpose of screening thrombophilia among low risk pregnant women from January 2009 to December 2009. The value of D-dimer increases during pregnancy comparing with that of non-pregnant women, and normal range said to be less than 2.7μg/ml. There was 3 patients among 277 whose data exceeded upper limit, and one of them was the case of 16 years old young woman who has been completely healthy except for her data of D-dimer (8.0μg/ml) at 28th weeks. The genetic analysis was performed, and the result is that she has the "Protein C Osaka2 (Met 406Ile) mutation ". She treated using LMWH, and delivered her healthy infant at full term without any thromboembolism.
Results:D-dimer might be one of the useful biomarkers screening thrombophilias such as protein C deficiency during pregnancy among low risk patients.

Figure: Met 406 Ile mutation



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PATHOGENETIC PROPHYLAXIS OF RECURRENT THROMBOEMBOLISM DURING SUBSEQUENT PREGNANCY AND POSTPARTUM IN WOMEN WITH THROMBOPHILIA

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Keywords: antenatal fetal loss, hyperhomocysteinemia, thrombophilia

Background/Aims: Despite intensive research, thromboembolism still accounts for significant maternal. Our aim was to determine thrombophilia in patients with thromboembolism during pregnancy and to evaluate the efficiency of antithrombotic prophylaxis in patients with thrombophilia for prevention of recurrent thromboembolism.
Materials and methods: Group I: n=47 (28,7±4,2 years), subgroup I (n=25) women with history of thromboembolism associated with pregnancy, subgroup (n=22) women with thromboembolism during current pregnancy, group II (control) – healthy pregnant women (n=45) were screened for genetic thrombophilia and antiphospholipid antibodies (APA). Subgroup I received prophylaxis in preconception period, during pregnancy and at least 6 weeks postpartum: low molecular weight heparin (LMWH), omega-3-acides, vitamins of B group, folic acid (up to 4 mg/day), aspirin (80 mg/day).
Results: In the subgroup I 40% had familial history of venous thromboembolism, and 60% had personal history of pregnancy complications (fetal loss syndrome, preeclampsia, placental abruption) ($p<0,05$ vs. control). In the group I thrombophilia was detected in 100% (4-6 mutations concomitantly including homozygous forms): MTHFR C677T (40,5%), FV Leiden (27%), prothrombin G20210A (18,9%), multigenic fibrinolytic defects (84%); APA (56,7%). In the control group were detected APA (8,6%) and genetic thrombophilia without homozygous forms (20%) ($p<0,05$). In subgroup I anyone had recurrent thromboembolism, 12% had pregnancy complication (mild preeclampsia) ($p<0,05$ vs. subgroup II).
Conclusions: Thrombophilia might be the essential pathogenetic mechanism of thromboembolism associated with pregnancy. LMWH was effective in 100% cases for prevention of recurrent thromboembolism and obstetric complications.Women with personal or family history of thromboembolism or with history of obstetric complications should be screened for thrombophilia.

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THE ANTIOXIDANT EFFECT OF THE AMINOESTROGEN BUAME

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Keywords: antioxidant effect, aminoestrogen

Background: Estrogens have antioxidant actions, suppress lipid peroxidation, and decrease cell death caused by oxidative stress.
Aims: We aimed to test the antioxidant effect of a 17-beta butyl-amino derivative of estrogen.
Materials and methods: Male adult CD1 mice weighing 30-40 g were kept under controlled temperature (21-24 °C) and light-dark cycles of 12 h. Food and water were given ad libitum. Buame was dissolved in dimethylsulfoxide (DMSO). After 7 days of pre-treatment (0.75 mg/100 g body weight, subcutaneously) or DMSO (control group), a sample of blood was obtained by cardiac puncture under anesthesia. For each assay, blood was inmediatly anticoagulated with citrate and centrifuged at 4°C. The antioxidant effect was measured through determining the final concentration of the ferrous ions formed from ferric reduction employing potassium ferricianide as a chromogen.
The protocol of this study followed all the ethical and legal regulations outlined for animal experiments.
Results: Table shows that Buane has 2.5 more ferric reducing power than control
Conclusions: The aminoestrogen Buame increase 2.5 the antioxidant capacity of plasma and could efficiently reduce systemic oxidative damage.

Table: Buane has 2.5 more ferric reducing power than control

Treatment	FRP (μmol/L) Mean ± SE
Vehicle	441.8 ± 69.3
Buame (0.75mg/100g s.c.)	* 1137.6 ± 183.6

Mean ± SE. *P < 0.05 were considered as significant.
Statistical analyses were, t-student, performed by using Prisma 4.0.

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QUALITY OF HEMOSTASIS IN WOMEN WITH MISSED ABORTION AND MISCARRIAGES: THE ROLE OF MOLECULAR AND GENETIC THROMBOPHYLIA FACTORS

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Keywords: thrombophylia, miscarriages, missed abortion

One of the reasons of obstetric complications is development of hypercoagulation produced by different congenital and acquired thrombophilia factors. The changes in the coagulation and the fibrinolytic system which may be related to the early losses indicate an interference of the activation of both coagulation and fibrinolysis. During termination of missed abortion and missed labour disorders of the hemostasis may occur acutely. The aim of this study was to evaluate the role of hemostasis disruption in genesis of first- and second-trimester miscarriage.
75 pregnant women of reproductive age in dynamics were examined: 55 women with reproductive losses in the anamnesis and 20 healthy pregnants. We analyzed the following hemostasis parameters: prothrombin time (PT), partial thromboplastin time (PTT), D-dimer, fibrinogen, the soluble fibrin monomer complex (SFMC), plasminogen, von Willebrand factor antigen (vWFA) by "Sta compact" (Roche). Morphofunctional status of peripheral blood platelets we determined by real-time method of vital computer morphometry using computer coherent phase-interference microscope (CPM) "Cytoscan". Homocystein plasma levels were measured by polarization fluorescent immunoanalyzer. Gene mutation was studied by allele-specific polymerase chain reaction.
It was determined that the damages of platelet hemostasis in reccurent miscarriage are consisted cell morphology and functional activity. We detected the frank heterogeneity of circulative platelet population which was connected to increasing quantity of macro-platelets with different image density. The 78% of patients had the high level of platelet activating state with failure of compensation (43%- the resting platelets; 41% - activating forms; 16% - degenerating forms).We registered the tendency to increasing of platelet aggregation. No essentiel changes in the plasmatic system of coagulation and fibrinolysis were found.
The hemostasis disruptions induced the deficit of microcirculation, thrombosis of blood vessels, infarcts of placenta. Thus, we may conclude that practically more than 50% of early reproductive losses are connected to molecular and genetic thrombophilia factors.

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INHERITED THROMBOPHILIA IS ASSOCIATED WITH RECURENT FETAL LOSS IN TUNISIAN POPULATION

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Keywords: fetal loss, protein S deficiency, activated protein C resistance

Serious obstetric complications including recurrent fetal loss (RFL), fetal growth retardation, preeclampsia and placenta abruption is a significant clinical problem, occurring in 1% to 5% of reproductive females. Majority of Case–control and cross-sectional studies have demonstrated that thrombophilia is more prevalent in cohorts of women with pregnancy loss. The objective of our study was to determine the prevalence of thrombophilia in women with unexplained RFL in Tunisian population. We have studied blood samples from 140 non pregnant women with unexplained RFL or intrauterine fetal death antecedents and 100 healthy blood donors' controls. Antithrombin activity (AT), protein C activity (PC), free protein S (PS), activated protein C (APC) resistance (after dilution in factor V deficient plasma) were determined using STAGO reagents on STA-Compact automate. At least 1 inherited thrombophilic defect was found in 40 (28.5%) of 140 women with repeated foetal losses. The most common thrombophilic abnormality was PS deficiency in 20 patients (14%). APC resistance was found in 14 (10%) women with RFL and in 6% of 100 controls. We report 3 patients (2.1%) with protein C deficiency. RFL was associated with AT deficiency in 3 cases (2.1%). Our study shows an association between hereditary thrombophilia and RFL in Tunisian population. PS deficiency may be involved frequently in the pathogenesis of recurrent fetal loss.

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THE MEASUREMENT OF THROMBOGENIC POTENTIAL OF MICROPARTICLES IN PREGNANCY

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Keywords: microparticles, pregnancy

Background/Aims: Thrombin generation test (TGT) can be used for many aplications. We used this test for observation of thrombin generation in women during normal pregnancy. Aim of our study was to compare generation of thrombin with and without microparticles in plasma sample to determinate the influence of platelet and endothelial microparticles at normal pregnancy.

The pregnancy is associated with activation of blood coagulation. During pregnancy rise the levels of coagulation factors and drop to inhibitors of coagulation. The situation with levels and thrombogenic potential of microparticles wasn't being still clear.

Platelet (PMP) resp. endothelial (EMP) derived microparticles are fragments released from the plasma membrane of platelet or endothelial in course of their rise, activation and destruction. Through surfactant immunoglobulin PMP and EMP activate coagulation cascade and in this way they supply the function of platelets.

Meterial and methods: Thrombin generation was measured using a Ceveron Alpha (Technoclone Austria) with an excitation wavelenght of 360 nm and an emission wavelenght of 465 nm. The amount of PMP was measured by flow cytometry using monoclonal antibody CD 51. The amount of EMP was be detected by using monoclonal antibody CD 144. The microparticels were removed before TGT by using ultrafiltration unit Ceveron MFU 500 (Technoclone, Austria).

Results: We analyzed group of 35 pregnant women in comparison to 35 healthly blood donors. We determined generation of thrombin in both group and evaluated results in relation to the level of microparticles.

The results are compared and statistically analysed in detail. On this basis, we assume significant correlation between higher thrombogenic potential of microparticles and pregnancy.

Conclusions: Our data indicated that during pregnancy rice as the absolute amount of micropraticles as their thrombogenic potential.

Acknowledgements: Supported by the Grant IGA of Min. of Health Czech Republic IGA NS 10319-3/2009 86-14 and NR 9282-3/2007 86-12.

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ANTITHROMBIN CONCENTRATE AS A TREATMENT OF PREECLAMPSIA INDUCED THROMBOPENIA: THROMBOELASTOMETRIC AND BIOLOGICAL DATA

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Keywords: antithrombin, preeclampsia, thromboelastometry, thrombopenia

Preeclampsia (PE) is a major cause of maternal death. Thrombopenia is observed in 15% of the severe PE and may be explained by immunologic or microthrombotic or microangiopathic comsumptive mechanisms. Drastic decrease in platelet count is a criteria for pregnancy medical termination. In this case report, a severe PE with thrombopenia and acquired antithrombin (AT) deficiency was treated with AT concentrate (Aclotine® LFB France) and monitored by thromboelastometry (ROTEM® Pentapharm Munich).

Case: Mrs V.S . 5pare, 38SG, 28 years old, BMI 39 kg/m², was admitted in a tertiary care obstetric unit for severe preeclampsia: moderate hypertension, epigastric pain, platelet count: 37,109/mm3, elevated hepatic enzymes >10 N, hemolysis. Induction of labor was decided. Laboratory results and ROTEM® showed a hypercoagulable state and AT deficiency 40%. (CT and CFT were decreased, alpha angle and MCF in normal range and FIBTEM amplitude increased). AT concentrate (Aclotine ® LFB France) [1000 UI/30 minutes then 2000 UI/12h] was administered in order to obtain AT activity 72% and platelet count 99 109/mm3. Haemostasis remained in the normal range for the course of labor and caesarean section (**Table**). No postpartum haemorrhage HPP=950 ml was observed. Post-partum treatment was nicardipine® and enoxaparine® 2*6000 UI/j. Mother and child discharged at Day 5 from from the hospital without complication. Decrease in AT activity is known to predict the severity of preeclampsia. Terao and Paternoster have studied the benefit of AT administration on the course of maternal and fœtal disease (2, 3). However AT is used carefully because of a potential increase in hemorrhagic risk. In this case report, hypercoagulability, AT efficacy and safety are documented by thromboelastometry.

References

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Table: Clinical and biological parameters before and after treatment with AT

	8° month	Admission 38 SA	Labor induction prostaglandins	Labor induction oxytocin	After cesarean	Post-partum	D3
Clinical Symptoms	0	HELLP Epigastric pain		HELLP HTA	hemolysis	hemolysis	
Diuresis		hemoglobinuric	Oliguria hemoglobinuric	Oligurie hémoglobinurique	hemoglobinuric		
Vascular loading		SSI 1000	SSI 1000	SSI 1000	HES 500ml		
Aclotine		avt TTT	a reçu 1000 UI /30mn	a reçu 2000 UI/12H	0	enoxaparin 2* 6000 UI/j	enoxaparin 2* 6000 UI/j
Hb g/dl	13.9	9.1	10	11.0	8.2	9.5	8.6
Ht		26.9	29.7	32.2	24.5	28.0	26.4
Platelet 10^3/mm^3	204	37	63	99	115	128	320
Fibrinogen		6.5	7	7.1	6.5	6.7	6.4
AT		40	72	85	70	69	94

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GENETICALLY DETERMINED HYPERHOMOCYSTEINEMIA AND PATHOGENETIC PROPHYLAXIS IN PARENTS WITH HISTORY OF ANTENATAL FETAL DEATH AND FETAL BIRTH DEFECTS

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Keywords: antenatal fetal loss, hyperhomocysteinemia, thrombophilia

Aims: We have assessed the role of folates and homocysteine in the developmental abnormalities and intrauterine fetal death.

Materials and methods: We measured plasma homocysteine levels and genetic forms of thrombophilia (MTHFR C677T, MTRR, 2756 MTS, 1958 G/A MTDD, 1298 A/C MTAC) in 100 married couple with a history of antenatal fetal loss in term 28-39 weeks after 2-12 months after episode of fetal death, who received preventive therapy (folates and vitamins group B) before conception, and in 50 healthy controls.

Results: Homocysteine level was elevated in 86% of mothers with history antenatal fetal death and in 84% of fathers. In 96% homocysteine was elevated in both parents. In 70% of mild hyperhomocysteinemia there were diagnosed fetal abnormalities such as malformations, Down syndrome, neural tube defects and cardiovascular system defects ($p<0.05$ for all comparisions).

Conclusions: Genetic hyperhomocysteinemia is associated with the involvement of folate metabolism and homocysteine in developmental abnormalities and in pregnancy complications.

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MUTANT MATERNAL AND FETAL THROMBOPHILIC GENOTYPES AS A RISK FACTOR FOR PREECLAMPSIA

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Keywords: genetic thrombophilias, maternal gene, fetal gene, preeclampsia

Aims: 1. to determine the mutant thrombophilic genotypes (factor V Leiden mutation, 20210G→A mutation in the prothrombin gene, 677C→T and 1298A→C mutations in the metylenetetrahydropholate reductase gene) in the 26 pairs – preeclamptic mother / newborn of preeclamptic mother and 33 pairs – mother with normal pregnancy / newborn of mother with normal pregnancy; 2. to analyse the fetal thrombophilic genes in association with maternal genes as risk factors for preeclampsia.
Materials and methods: Mutant and normal genotypes for the four thrombophilic mutations were determined using the PCR-RFLP methods.
Results: The risk of preeclampsia was 4.36 (p= 0.2), 5.55 (p= 0.003) and 1.25 (p=0.8) in association with factor V Leiden, 677C→T and 1298A→C, respectively mutation. None of the subjects included in the study was identified as positive for the 20210G→A mutation. The risk of preeclampsia was significantly increased in the case of presence of multiple thrombophilias (OR 4.4, p= 0.019). The 677C→T mutation represents higher risk factor for mild and severe preeclampsia (OR 12.5, p= 0.03 and OR 19.6, p= 0.005, respectively). Multiple thrombophilias confers a risk of 7 (p= 0.02) for the severe preeclampsia. The frequency of 677C→T mutation and multiple thrombophilias in newborns of preeclamptic mothers was significantly more increased compared to the frequency in the group of newborns from normal pregnant mothers (73.07% vs. 39.39% and 38.46% vs. 12.12%, respectively). The risk of preeclampsia is significantly increased when the 677C→T mutant genotypes or multiple thrombophilias are present both in the mother and the child (OR 6.53, p=0.002 and OR 4.65, p=0.06, respectively).
Conclusions: Our results emphasize the role of genetic thrombophilias in the development of different forms of preeclampsia. The presence of multiple thrombophilias in both the mother and the child is a risk factor for the development of preeclampsia.

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Mechanisms of thrombosis

Thursday 8th July, 2010

☺ **P81**

EFFECT OF MECHANICO-STIMULATION ON ENDOTHELIAL CELL (EC) EXPRESSION OF TISSUE FACTOR (TF)

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Background/Aims: The expression of TF mRNA in normal vascular EC *in vivo* is increased in a variety of pathological conditions, including atherosclerotic plaques. Since EC are perpetually exposed to continually changing physical forces induced by the pulsatile circulation, but have different patterns depending on the location in the vascular tree, we hypothesized that these mechanical forces can influence EC response differently.
Materials and methods: Human umbilical vein EC were exposed to five types of mechanical forces, which were steady laminar flow, pulsatile forward flow, To&Fro flow, orbital shear and cyclic strain for 2hrs, 4hrs and 6hrs. TF expression was determined using real time quantitative PCR. Fold changes were calculated by Pfaffl method and expressed as mean±SD n= 4.
Results: TF RNA expression in EC exposed to laminar, pulsatile and To&Fro flow for 2hrs showed 10.44 ±3.5, 4.28 ±1.2 and 6.63 ±0.8 fold increases compared to static control, respectively (p<0.05, ANOVA). TF expression in EC exposed to orbital shear and cyclic strain for 2hrs showed 1.41±0.5, 1.17±0.3 fold increases, respectively, which were not significantly different from static control. While EC exposed to laminar shear for 6hrs showed 5.02±2.0 fold increased TF expression, which was significantly lower than that at 2hrs, TF RNA expression in HUVEC exposed to To&Fro shear for 6hrs was 5.41±2.2 fold, which was not significantly different from that at 2hrs.
Conclusions: Steady laminar flow, pulsatile forward flow and To&Fro flow induced TF RNA expression in EC, while orbital shear and cyclic strain did not. TF RNA expression in EC induced by To&Fro shear tended to sustain longer than that by laminar shear, suggesting that To&Fro shear causes prolonged activation of coagulation cascade. This provides a physiologically relevant model by which to study mechanical and chemical signal cascades interactions that ultimately control EC phenotype.

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☺ **P185**

☺ = **bestposter**

THROMBIN GENERATION ASSOCIATED WITH MICROPARTICLES: REGULATION BY PHOSPHATIDYL SERINE AND TISSUE FACTOR PATHWAY

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Keywords: microparticles, thrombin generation, phosphatidyl serine, tissue factor pathway

Background: Circulating microparticles (MP) are vesicles phosphatidyl serine (PS) positive, resulting mostly from blood and vascular cells. Membrane MP facilitates the thrombin generation (TG) because they provide tissue factor (TF) and a procoagulant PS surface. Various methods of detection for MP include flow cytometry and functional assays. However, assays that measure the procoagulant functions of MP and how these tests are influenced by MP levels has not been extensively investigated.
Aims: To analyze, in normal subjects, the level of MP and their procoagulant activity dependent upon the PS and the TF pathway.
Methods: The study included 39 healthy subjects. Platelet-free plasma (PFP) and MP-free plasma (MPFP) obtained from citrated blood were assessed. The TG was measured by the calibrated automated thrombogram method (CAT, Thrombinoscope) in presence or absence of Annexin-V, Anti-TF and tissue factor pathway inhibitor (TFPI). Peak (nM) from the thrombin curve was calculated (Thrombinoscope-software). The total MP-PS positive (0.5-1µm) was analyzed through flow cytometry (EPICS-XL, Beckman-Coulter) by labeling with FITC-Annexin-V. A microbeads standard was used (0.5–3.0 µm, Megamix, BioCytex) to set the MP gate. Circulating TF was measured by ELISA (Imubind,AD).
Results: In PFP, TG reached 178±84 nM and decreased to 8.4±13.5 nM in MPFP. The levels of MP-PS positive were 1277±551/µl in PFP and decreased a 95% in MPFP. A correlation between TG and MP levels was observed (r=0.46, p<0.01). No thrombin was generated when Annexin-V (6 ng/ml) or TFPI (70 ng/ml) was added to PFP. However, only a partial TG inhibition was reached (36%) when anti-TF (0-5 µg/ml) was added. Circulating TF slightly decreased in MPFP (190±63 pg/ml) compared with PFP (211±75 pg/ml; p<0.05). Circulating TF correlated with MP levels (r=0.46, p<0.05) but not with TG. Conclusion: In healthy subjects, plasma TG is associated with MP levels, and is regulated by PS and TF pathway.
FIS-PI-080124

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☺ **P219**

PLATELET CGMP: A POTENTIAL IN VIVO BIOMARKER

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Keywords: platelet cGMP, nitric oxide, biomarker, NO-donors

Background: Nitric oxide (NO) is produced endogenously and plays a wide range of fundamental physiological actions in different tissues. Cyclooxygenase-Inhibiting Nitric Oxide Donators (CINODs) are a new class of anti-inflammatory agents, which combine a mechanism of cyclooxygenase (COX) inhibition along with nitric oxide (NO) donation. Despite important insights into the understanding of endogenous NO pathway, lack of appropriate in vivo biomarkers of NO deficiency and/or restoration, is hampering further studies with novel NO donors.
Aims: To explore whether would be possible to use *ex vivo* platelet cGMP as an indicator of *in vivo* NO availability. Platelet cGMP levels were measured in mice following endogenous NO depletion with L-NAME, and then treated with either a representative CINOD (NCX 429) or a reference NO donor (molsidomine).
Methods: Male C57Bl6/J mice received either 100 mg/kg L-NAME or 0.1 mL saline intraperitoneally two hours before sampling. Platelet extraction was performed using a citrate dextrose buffer. A commercially available kit was used to quantify cGMP levels. Vehicle, molsidomine (5 mg/kg), or a cyclooxygenase-inhibiting nitric oxide donator (CINOD) NCX 429 (30 and 100 mg/kg) were given orally 15 min after L-NAME.
Results: Following *in vivo* NO depletion with L-NAME, platelet cGMP levels were lower (1.1 ± 0.06 pmol/mg of protein) than those in vehicle treated animals (1.9 ± 0.2 pmol/mg of protein; p<0.01). Interestingly, platelets cGMP levels can be restored to normal using the two different NO donors, molsidomine (1.8 ± 0.4 pmol/mg of protein) and NCX 429 (30 and 100 mg/kg) (1.55±0.14 and 1.65±0.14 pmol/mg of protein, respectively; p<0.01).
Conclusions: Current data encourage further characterization studies to validate the use of platelet cGMP as a biomarker both of endogenous NO dysfunction and NO donor treatments.

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☺ **P187**

ROSIGLITAZONE INDUCES SHEDDING OF PROCOAGULANT MICROPARTICLES FROM HUMAN MONOCYTES

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Keywords: microparticles, ischemic heart disease

Introduction: Rosiglitazone (Rz) is an exogenous peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonist; it belongs to the thiazolidinedione group of insulin sensitizers. Recently it has been associated with a significantly increased risk of myocardial infarction and an increased risk of death from cardiovascular causes, but the mechanisms remain uncertain.
Microparticles (MP) are membrane fragments shed by virtually all eukaryotic cells upon activation or during apoptosis. It has been proven that platelet-derived MP bind factor VIII so they act like an ideal catalytic surface promoting the assembly of the characteristic enzyme complexes of the coagulation cascade, called tenase and prothrombinase, able to activate factor X. This observation demonstrates that MP are able to exert important biological activities.
Aims: We evaluate the hypothesis that a mechanism of increased cardiovascular risk by Rz is linked to the generation of monocytes/macrophages derived procoagulant MP.
Materials and methods: The generation of MP by monocytes/macrophages induced by Rz was investigated through two methods: a one-stage clotting assay, that investigates the procoagulant activity mediated by tissue factor (TF), and the prothrombinase assay. This test measures the concentration of negative charges on the cell membrane, expressed as phosphatidylserine (PS) concentration. We also confirmed the data with the naturally occurring agonist, 15-deoxy-delta12,14–prostaglandin-J2 (15d-PGJ2).
Results: Data show that both PPAR-gamma agonists are able to significantly increase the generation of procoagulant MP by monocytes/macrophages. GW9662, a specific inhibitor of PPAR-gamma, and PD098059, a specific inhibitor of ERK, both inhibited the agonist effect (**Fig. 1A, B**). Coagulation tests suggest that MP induced by PPAR-gamma agonists contain the procoagulant molecule, TF, on their membrane (**Fig. 1C**).
Conclusions: Association of Rz with a significant increase in the risk of myocardial infarction could be due to an increased shedding of procoagulant MP by monocytes/macrophages.

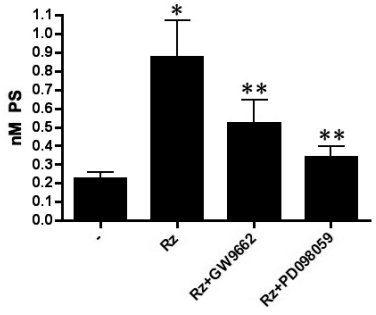

Figure 1A

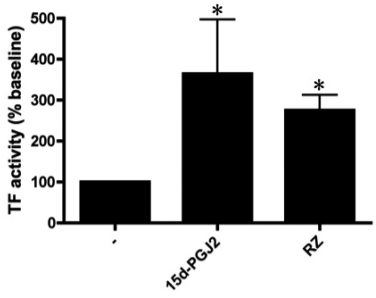

Figure 1B

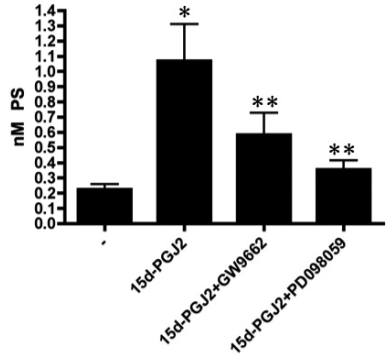

Figure 1C

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☺ **P307**

☺ = **bestposter**

CONJUGATED LINOLEIC ACID ISOMERS DOWNREGULATE TISSUE FACTOR EXPRESSION IN HUMAN MONONUCLEAR CELLS AND MACROPHAGES

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Background/Aims: Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid. CLA is a mixture of dietary fatty acids that exerts various beneficial effects including decrease in proliferation, atherogenesis, diabetes and inflammation in animal models.
Atherosclerotic plaques contain significant amounts of tissue factor (TF), the trigger of the clotting cascade. TF is mainly expressed by infiltrating inflammatory cells, in response to cytokines and other inflammatory mediators, and is considered one of the main contributors to the thrombogenicity associated with atheroma.
In this study we investigated the effect of the main isomer of CLA, cis-9, trans-11 (c9,t11), and of the blend of isomers: 80% (c9, t11) and 20% (t10, c12) on TF expression in mononuclear cells (MNs) and macrophages (MØ).
Materials and methods: MNs from peripheral blood of healthy donors and MØ, obtained by spontaneous differentiation of blood monocytes in culture, were incubated with (c9, t11) or blend with or without lipopolysaccharide (LPS) for 6 hours at 37°C. At the end of incubation, supernatants were drawn and cells were then disrupted by freezing and thawing and procoagulant activity was assessed by a one-stage clotting time. TF mRNA levels were assessed by real time RT-PCR.
Results: CLA (c9, t11) and Blend inhibited TF activity of LPS-stimulated MNs in a dose dependent way. Down regulation of TF activity was accompanied by a decrease in TF mRNA levels. The decrease in TF activity was observed also in the presence of other agonists, namely IL1-β and TNF-α, used to stimulate MNs.
CLA and blend inhibited TF expression on MØ. Interestingly, TF inhibition was accompanied by a decrease in TNF-α release as assessed by ELISA.
Conclusions: Our results suggest that CLA or blend intake, by decreasing TF expression, could exert a beneficial effect on risk factors associated with the atherosclerotic process.

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☺ **P64**

METALLOPROTEASES AND INHIBITORS: NEW BIOMARTKERS OF SEVERITY AND MORTALITY IN SEPSIS

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Keywords: MMP,TIMP-1, sepsis

Background: Matrix metalloproteinases (MMPs) play a role in infectious diseases through extracellular matrix (ECM) degradation. The objective of this study was to determine the predictive value of MMP-9, MMP-10, and TIMP-1 on clinical severity and mortality in patients with severe sepsis.
Methods: Multicenter study carried out in 192 (125 surviving and 67 nonsurviving) patients with severe sepsis and 50 age- and sex-matched healthy controls. Serum levels of MMP-9, MMP-10, TIMP-1, tumor necrosis factor (TNF)-alpha, and interleukin (IL)-10 were measured in patients with severe sepsis at the time of diagnosis.
Results: Sepsis patients had higher levels of MMP-10 and TIMP-1, higher MMP-10/TIMP-1 ratios, and lower MMP-9/TIMP-1 ratios than did healthy controls ($P < .001$). An association was found between MMP-9, MMP-10, TIMP-1, and MMP-9/TIMP-1 ratios and parameters of sepsis severity, assessed by the SOFA and APACHE-II scores, lactic acid, and markers of coagulopathy. Nonsurviving sepsis patients had lower levels of MMP-9 ($P = 0.037$), higher levels of TIMP-1 ($P < 0.001$) and lower MMP-9/TIMP-1 ratio ($P = 0.003$) than did surviving patients. An association was found between MMP-9, MMP-10, and TIMP-1 levels and inflammatory markers (TNF-alpha and IL-10). The risk of death in sepsis patients with TIMP-1 values greater than 531 ng/ml was 80% higher than that in patients with lower values (RR=1.80; 95% CI = 1.13 to 2.87; $P=0.01$).
Conclusions: In patients with severe sepsis, reduced MMP-9/TIMP-1 ratios and increased MMP-10 levels may be of great pathophysiologic significance in terms of severity and mortality. TIMP-1 levels may represent a biomarker to predict the clinical outcome of patients with sepsis.

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THE INFLUENCE OF GENETIC VARIATIONS ON

CIRCULATING LEVELS OF MATRIX METALLOPROTEINASE 9 IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Keywords: MMP-9, polymorphisms, cardiovascular disease

Background/Aims: The matrix metalloproteinase-9 (MMP-9) plays an important role in atherosclerosis and plaque rupture. In patients with coronary heart disease (CHD) we have investigated the association between two functional polymorphisms in the MMP-9 gene, as related to disease entities and serum levels of the protein.
Materials and methods: The promoter -1562C/T - and the exon 6 R279Q G/A polymorphism were studied in 1001 patients with angiographically verified stable CHD (mean age 62 yrs, 22% women) and in 200 healthy controls (mean age 55 yrs, 28% women). The polymorphisms were determined by the Applied Biosystems 7900HT Fast Real-time PCR system. Serum levels of MMP-9 and its inhibitor TIMP-1 were measured in the CHD population only, by the use of commercial ELISA kits (R&D Systems, Europe).
Results: No differences in allele frequencies between CHD and controls were observed. In the CHD population, the T allele of the -1562C/T- and the A allele of the R279Q G/A polymorphism were associated with higher levels of MMP-9 ($p=0.007$ and $p=0.065$, respectively). None of the polymorphisms were associated with myocardial infarction (MI), diabetes mellitus type 2 (DMT2) or gender, and no differences in their respective phenotypes were observed. However, TIMP-1 levels were significantly higher in MI patients and in patients with DMT2 ($p=0.03$ and $p=0.002$, respectively).
Conclusions: Variations in the MMP-9 gene strongly influenced the circulating levels of MMP-9 in patients with CHD. However, genotypes were not related to clinical manifestations of MI and DMT2, which presented with elevated levels of TIMP-1.

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DETECTION OF THE IMBALANCE OF PRO- VS. ANTI-COAGULANT FACTORS IN CIRRHOSIS BY A SIMPLE LABORATORY METHOD

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Keywords: global coagulation test, hypercoagulability, cirrhosis

Cirrhotic patients possess a pro- vs anti-coagulant imbalance due to increased factor-VIII and decreased protein-C. This imbalance can be detected by thrombin-generation assays performed in the presence/absence of thrombomodulin (predicate-assay) that are not readily available in clinical laboratories. We sought to assess this hypercoagulability with a simpler thrombin-generation assay performed in the presence/absence of Agkistrodon contortrix contortrix venom (ACCV), a snake venom that activates protein-C in a manner similar to thrombomodulin (new-assay). We analyzed blood from 105 cirrhotics and 105 healthy subjects (controls). Results for the predicate- or the new-assay were expressed as ratio (with-to-without-thrombomodulin) or as ACCV-induced-coagulation-inhibition (ACCV-ICI%).
By definition, high ratios or low ACCV-ICI % translate into hypercoagulability. The median (range) ACCV-ICI % was lower in patients [74%(31-97%)] than controls [93%(72-99%)] $p<0.001$ indicating that cirrhotics are resistant to the action of ACCV. This resistance resulted in greater plasma hypercoagulability in patients of Child C than A-B. The hypercoagulability of Child C [63%(31-92%)] was similar to that observed for factor-V-Leiden patients [69%(15-80%)]$p=0.59$. The ACCV-ICI % values were correlated with the levels of protein-C (rho=0.728, $p<0.001$) or factor-VIII (rho=-0.517, $p<0.001$). Finally the ACCV-ICI % values were correlated with the predicate-assay (rho=-0.580, $p<0.001$). In conclusion, the hypercoagulability of plasma from cirrhotics can be detected with the new-assay that compares favorably with the other markers of hypercoagulability (i.e, high factor-VIII and low protein-C) and with the predicate-assay based on thrombin-generation with/without thrombomodulin. Advantages of the new- over the predicate-assay are easy performance and standardized results. Prospective trials are needed to ascertain whether it is useful to predict thrombosis in cirrhotics.

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ACTIVATION OF BLOOD COAGULATION: A POSSIBLE EXPLANATION FOR THE INCREASED THROMBOTIC RISK IN BULLOUS PEMPHIGOID

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Keywords: coagulation, thrombosis, tissue factor, bullous pemphigoid

Background: Bullous pemphigoid (BP) is an inflammatory blistering skin disease mainly affecting elderly subjects and associated with an elevated thrombotic risk. It is caused by autoantibodies to hemidesmosomal proteins, with eosinophils participating in blister formation.
Aims: Since eosinophils are a source of tissue factor (TF), the main initiator of blood coagulation, we evaluated the local and systemic activation of coagulation in BP.
Methods: We studied 60 patients with active BP (sixteen re-evaluated during remission) and 60 sex- and age-matched healthy controls. The coagulation markers prothrombin fragment F1+2 and D-dimer were measured in plasma of all subjects and in both plasma and blister fluid of patients with BP. TF was evaluated immunohistochemically in skin specimens from 40 patients and in 20 normal samples. In lesional skin specimens from 5 BP patients, we evaluated TF mRNA expression by in situ hybridization as well as the colocalization of TF and eosinophil cationic protein (a classic eosinophil marker) by confocal microscopy using immunofluorescence techniques.
Results: F1+2 and D-dimer levels were higher in plasma of patients with BP than in plasma of controls ($p=0.0001$ for both), and were very high in blister fluid ($p=0.0001$ vs plasma). Plasma and blister fluid F1+2 and D-dimer levels paralleled blood and tissue eosinophilia and disease severity. In the sixteen patients re-evaluated during remission, there was a marked reduction in F1+2 (from 600±377 to 193±151 pmol/L; $p=0.0001$) and D-dimer (from 3225±3010 to 561±476 ng/ml; $p=0.0001$). Immunohistochemistry revealed strong TF reactivity in BP skin ($p=0.0001$) confirmed by in situ hybridization. Colocalization studies by confocal microscopy identified eosinophils as a source of TF.
Conclusions: The coagulation cascade is activated in BP most likely via TF expressed by eosinophils. The hypercoagulability correlates with the severity of the disease and possibly contributes to inflammation, tissue damage, blister formation and thrombotic risk in BP.

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P616

PREOPERATIVE ACETYLSALICYLIC ACID CESSATION AND PERIOPERATIVE MYOCARDIAL INFARCTION IN PATIENTS UNDERGOING ELECTIVE BYPASS SURGERY.

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Keywords: ASA, perioperative myocardial infarction, oxidative stress

Background: Platelet activation and oxidative stress are affected in the pathogenesis of perioperative myocardial infarction in patients undergoing bypass surgery. ASA discontinuation augments platelet activation and for this reason, current recommendations state that ASA should be maintained to the day of operation, in almost all patients.
Aims: Evaluation of platelet activation and oxidative stress in relation to perioperative myocardial infarction in elective CABG patients who self eliminated ASA at least 7 days before the bypass surgery.
Materials and methods: We studied 108 elective CABG patients not taking ASA at least 7 days prior to surgery. Thromboxane B2 (TXB2), β-thromboglobulin (β-TG) and 8-isoprostaglandin F 2α (8-iso PGF 2α) were measured at baseline and 5-7 days after the procedure (after 4 doses of 150 mg of ASA).
Results: Baseline levels of TXB2, β-TG, and 8-iso PGF 2α were elevated and rose following surgery despite postoperative ASA administration.13 patients (12%) with perioperative myocardial infarction have had higher baseline levels of TXB2, 8-iso-PGF2-a and β-TG than the remainder ($p<0.05$ for all comparisons). On multiple regression analysis, including preoperative and perioperative variables baseline and postoperative TXB2 and 8-iso-PGF2 concentrations were the independent predictors for perioperative myocardial infarction. Postoperative β-TG and 8-iso-PGF2a levels correlated with postoperative TXB2 concentrations ($p< 0.0001$ for both markers).
Conclusions: Preoperative ASA discontinuation before CABG procedure significantly correlated with increased TXB2 concentrations in postoperative period, regardless of early ASA administration. Elevated TXB2 levels were a strong predictor for perioperative myocardial infarction and were associated with the level of oxidative stress.

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P514

RECOGNITION OF ACTIVATED PLATELETS INDUCES THE FORMATION NETS (NEUTROPHIL EXTRACELLULAR TRAPS) VIA AN HIGH MOBILITLY GROUP BOX -1 (HMGB-1) DEPENDENT MECHANISM.

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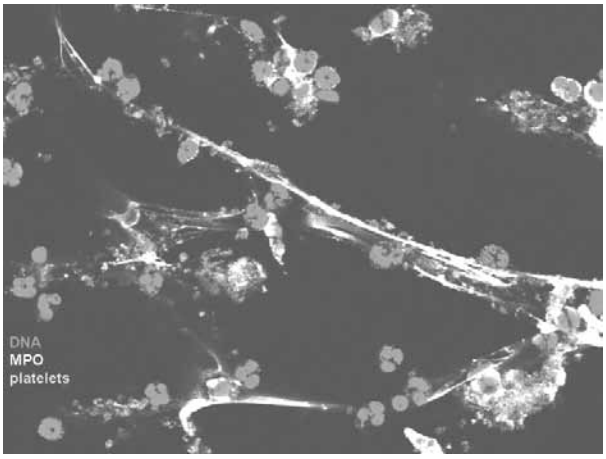
Keywords: neutrophils, platelets acute myocardial infarction

Background: Neutrophil activation and degranulation is a critical event in the acute myocardial infarction and is accompanied by a transient burst of interaction with circulating platelets. In addition to the more traditional mechanism, activation of neutrophils causes the release of web-like structures of DNA (neutrophil extracellular traps, NETs). NETs contain proteolytic activity that can damage tissues
Methods: We analyzed 25 coronary thrombi from patients with acute myocardial infarction and 10 thrombi from patients with peripheral artery disease by confocal microscopy. The in vitro NETs formation was induced using IL8 or challenge with resting or activated platelets and monitored by confocal microscopy and by assessment of released DNA. Cellular markers of activation were determined by flow cytometry.
Results: Ex vivo: The confocal analysis of thrombi indicates that degranulated neutrophils are the principal cellular component in coronary, but not in peripheral, arterial thrombi. We observed the presence of extracellular DNA structures, compatible with NETs, physically associated with activated platelets in coronary but not in peripheral arterial thrombi. The platelet associated to NETs were positive for P-selectin and HMGB1. *In vitro* assays demonstrated that, HMGB1 is present in cytoplasm of resting platelets. After activation with thrombin 36.7±5.3% of platelets expressed HMGB1. The co-incubation of neutrophils with activated platelets led to robust neutrophil activation, NETs formation with consequent DNA release. This event was time-dependent and abated in the presence of antibodies against the HMGB1, RAGE (the HMGB1 counter receptor on neutrophils) and by of the HMGB1 antagonist Box-A. Similar results were obtained when neutrophils were treated with human recombinant HMGB1.
Conclusions: our data indicate that NET formation is a possible outcome of the cross-talk between activated platelets and neutrophils. Moreover they reveal HMGB1 as a possible candidate involved in the interaction.

Figure: Profound NET formation is accompanied by the binding of activated platelets



Table

Treatment	Released DNA (ng/mL)
None	290±21
recombiant Interleukin 8	813±37
Resting platelets	321±26
Platelet secretome	420±18
Activated platelets for 10 min	435±43
Activated platelets for 20 min	653±68
Activated platelets for 40 min	850±19
Activated platelets + mAb anti HMGB1	381±37
Activated platelets + Box A	323±28
mAb anti RAGE + activated platelets	292±43
rHMGB1	625± 9
mAb anti RAGE + rHMGB1	301±25

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THROMBOGENIC POTENTIAL OF CIRCULATING BLOOD-PLATELET DERIVED MICROPARTICLES IN AN ELDERLY POPULATION

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Keywords: microparticles, elderly

Background: Microparticles (MP) play a role in hemostasis and thrombosis because of the possible presence of negatively charged phospholipids (PL) and tissue factor (TF) on their surface. Increasing age is associated with an elevated risk for thrombosis.
Aims: To investigate correlations between blood platelet derived MP (BP-MP) counts and their thrombogenic potential in an elderly population.
Methods: Our study included 27 elderly persons, recruited in a nursing home, with mean (±SD) age of 83.5 (±6.0) years, not taking vitamin K-antagonists. Platelet function was assessed by a Platelet Function Analyzer (PFA), and plasma was frozen for subsequent analysis of coagulation parameters (aPTT, PT, fibrinogen, FVIII, FXI) and MP. BP-MP numbers were determined by flow cytometry. Thrombinoscopy was used to measure thromobogenic potential of MP, both by PL-independent (4μM PL, no TF) and PL-dependent (differential measurement with and without filtering through a 0.1μm filter, no PL, 5pM TF) assays.
Results: Flow cytrometry demonstrated that 94% of total BP-MP (CD42a+) stained positively for annexinV. Total BP-MP numbers correlated with their PL-dependent (R2=0.25, p=0.01), but not their PL-independent thrombogenic potential. Subanalysis demonstrated that this correlation was present only for the annexinV+, and not the annexinV- BP-MP. Surprisingly, the mean annexinV fluorescence intensity of the former population was negatively correlated with the PL-dependent thrombogenic potential (R2=0.17, p=0.03). Nor BP-MP numbers, nor their thrombogenic potential correlated with blood platelet numbers, blood platelet function (PFA) or any coagulation parameter. Mean (±SD) FVIII levels were 211% (±42), demonstrating the increased thrombogenic risk in this population.
Conclusions: In elderly persons, blood platelet derived microparticle numbers correlate with their PL-dependent thrombogenic potential, independently of blood platelet numbers or blood platelet function. These findings indicate in a population at risk for thrombosis, that microparticles constitute an independedent thrombogenicity risk factor.

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TARGET ORGAN DAMAGE IN AN ASYMPTOMATIC POPULATION AT INTERMEDIATE CARDIOVASCULAR RISK AND ADJUNCTIVE MAJOR RISK FACTORS: THE CARDIOVASCULAR PREVENTION SACCO STUDY (CAPRESS)

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Keywords: cardiovascular risk, atherosclerosis, c-IMT, PAD

Background: Cardiovascular (CV) risk score has limited accuracy, in particular in intermediate risk. Assessment of Target Organ Damage (TOD), early atherosclerosis marker, could improve stratification in selected individuals.
Aims: To evaluate the prevalence of TOD at carotid, cardiac, renal and peripheral vascular level in subjects at intermediate risk and Adjunctive Major Risk Factors (AMRF).
Methods: From March 2007 to July 2009 in 979 subjects at intermediate risk assessed by "progetto CUORE" algorithm, sensitized by one or more AMRF, i.e. family history for CVD, overweight/obesity and relevant smoking (more than 10 cigarettes/day) we measured common carotid intima- media thickness (cc-IMT) and plaque at any level, left ventricular mass index (LVMI), urine albumin/creatinine ratio (UACR), ankle-brachial index (ABI).
Results: The prevalence of at least one TOD was 63% (n=617), increased value of cc-IMT was present in 48,2% (n= 472), abnormal UACR in 14,1% (n=138), increase in LVMI in 12,6% (n=117) and pathological ABI was observed in 9,1% (n= 89). In those with carotid damage a plaque was present in 423 subjects (43,2% of total population). At multivariate analysis including conventional and AMRF, the presence of age 50-69 years, systolic arterial pressure levels, relevant smoking and CV risk ≥15 were significantly associated to having at least one TOD and carotid damage. Among AMRF considered, peripheral arterial disease was associated to relevant smoking with a O.R. 3,00 (C.I. 1,80-4,97; P<0,0001), while both overweight and obesity showed a selective association to cardiac damage with a O.R. 2,75 (C.I. 1,2-6,3; P<0,01) and O.R. 3,89 (C.I. 1,61-9,73; P<0,01) respectively.
Conclusions: A substantial proportion of subjects at intermediate risk and almost one AMRF have at least one TOD considered a high risk marker of athero-thrombotic events. The evaluation of subclinical disease may improve the accuracy of CV risk prediction in this selected population and should be considered in common clinical practice.

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HOMOCYSTEINE IN VASCULAR BEHÇET'S DISEASE: A META-ANALYSIS

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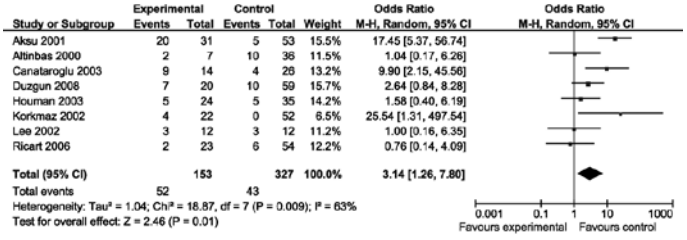
Keywords: Behçet's disease, hyperhomocysteinemia, thrombosis

Background: Behçet's disease (BD) is a vasculitis of unknown aetiology, characterized by the triad: oral and/or genital aphtous ulcerations and uveitis. Homocysteine (hcy) is an independent risk factor for venous and arterial thrombosis. The association between hyperhomocysteinemia and thrombosis has been investigated in some studies in BD patients. However, information on this association is based only on the results of small studies with conflicting results. Thus, no definitive conclusions have been reached yet.
Aims: To compare the plasma levels of hcy in BD patients with and without history of thrombosis.
Materials and Methods: Data Sources: MEDLINE, EMBASE databases (up to July 2009) Study Selection: Two reviewers performed study selection independently. Studies were included if hcy was measured in adult BD patients with and without thombosis Data Extraction: Two reviewers independently extracted data on study and population characteristics. The mean value of hcy in BD patients and the presence of hyperhomocysteinemia in patients with and without thrombosis were collected. Association between thrombosis and hyperhomocysteinemia, and the mean difference of hcy levels in BD patients with and without thrombosis were calculated.
Results: Sixteen studies for a total of 979 patients were included. Hyperhomocysteinemia was more prevalent in patients with thrombosis than in those without (Odds Ratio 3.14; 95% CI 1.26, 7.80) Mean levels of hcy were significantly higher in patients with thrombosis in comparison to patients without (mean difference 3.30 µmol/l; 95% CI 2.09, 4.51).
Conclusions: Our results suggest that hyperhomocysteinemia may be considered associated with thrombosis in BD patients.

Figure:



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P201

ENDOTHELIAL DYSFUNCTION AND INFLAMMATION IN PATIENTS WITH PSORIASIS

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Keywords: circulating endothelial cells, psoriasis, von Willebrand factor

Background/Aims: The moderate-severe psoriasis and the psoriatic arthritis is a chronic pathology that is characterized by an increase of the inflammation that can be associated with changes in the vascular endothelium. The aim of this study is to quantify the circulating endothelial cells (CECs), in patients with psoriasis, and to analyze its relationship with the established markers levels of vascular damage and inflammation.
Material and methods: We studied 20 patients and 20 healthy subjects. As markers of endothelial damage we quantified CECs a von Willebrand factor (vWF). Quantification of CECs was determined by an immunomagnetic technique.Antigenic levels of vWF were measured by an immunoturbidimetry assay. As markers of inflammation we quantified circulating levels of soluble E-selectin (sEsel) and interleukin 6 (IL-6) by ELISA.
Results: CECs quantifies in peripheral blood were significantly higher in psoriasis patients than in controls (78±47 vs 9±5 cel/ml, $p<0.001$). Levels of vWF, IL-6 and sEsel were significantly higher in patients than in controls (vWF, 150±48 vs 115±22 %, $p<0.05$; IL6, 1.8±2.2 vs 0.7±0.5 pg/ml, $p<0.05$; sEsel 31.7 ± 12.7 vs 21.2 ±8.5 ng/ml, $p<0.05$). A high number of patients (95%) present a value of CECs that exceeds the 99th percentile of the control group. In patient group, 47%, 35% and 33% of patients have a value of vWF, sEsel and IL-6, respectively, exceeding the 99th percentile of the control group. In the group of patients, the values of the CECs are correlated significantly with the values of vWF (Spearman, r=0.48; $p=0.01$).
Conclusions: The moderate-severe psoriasis and /or peripheral psoriatic arthritis are associated with a high number of CECs. The existence of correlation between vWF and CECs and the absence of correlation of CECs with sEsel and IL6, prove that in patients with psoriasis endothelial damage is not associated with the inflammatory process.

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ENDOTHELIAL NITRIC OXIDE IS CRITICAL FOR VASCULAR TONE REGULATION BUT NOT FOR PLATELET INHIBITION IN ENOS-/- MICE

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Keywords: nitric oxide, vascular tone, platelet functioning, eNOS-/- mice

Background: Prostacyclin mediated compensation in case of nitric oxide (NO) impairment is suggested to explain the increased cardiovascular risk observed when inhibiting cyclooxygenase in patients with endothelial dysfunction. In this regard Cyclooxygenase-Inhibiting Nitric Oxide Donators (CINOD) is a promising new class of drugs, which combine cyclooxygenase inhibition along with nitric oxide donation. Despite numerous studies illustrating the key role of endothelial NO on both blood pressure and platelets regulation, not many studies have addressed the occurrence of additional sources of NO and/or compensatory mechanism in case of endogenous NO impairment.
Aims: To assess whether the specific depletion of endothelial NO production in eNOS-/- mice affects blood pressure as compared to bleeding time and platelet cGMP, to the same extent as inhibition of total endogenous NO production by L-NAME.
Methods: Male C57Bl6/J wild type (WT) and eNOS-/- mice were treated with either L-NAME 100 mg/kg (i.p.) or vehicle. Basal and post-treatment measurements of systolic BP were carried out by tail-cuff. Additional animals were used for measurements of tail tip bleeding time and platelet cGMP content.
Results: Untreated WT, L-NAME treated WT and untreated eNOS-/- mice resulted in BP values of 96±2, 119±3 and 121±3 mmHg, respectively. In L-NAME treated eNOS-/- mice BP was 122±4 mmHg. As regarding bleeding at 120 seconds, untreated WT, L-NAME treated WT, untreated eNOS-/- and L-NAME treated eNOS-/- mice showed 40%, 0%, 60% and 40% of mice bleeding, respectively. Finally, platelets cGMP in L-NAME treated WT, untreated eNOS-/- and L-NAME treated eNOS-/- mice exhibited the following levels of platelet cGMP depletion: 46%, 26% and 55%, respectively.
Conclusions: Whereas eNOS is the only NO provider for vascular tone regulation, an alternative source of NO and cGMP contributes to platelet homeostasis in eNOS-/- mice.

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NITRIC OXIDE DONATION OVER COX INHIBITION DOES NOT CHANGE BLEEDING TIME IN THE MOUSE MODEL

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Keywords: nitric oxide, NSAIDs, CINOD, Bleeding Time

Background: Cyclooxygenase-Inhibiting Nitric Oxide Donators (CINODs) are a new class of anti-inflammatory agents. They combine a mechanism of cyclooxygenase (COX) inhibition along with nitric oxide (NO) donation. NO is aimed to counteract gastrointestinal and vascular side effects of NSAIDs. Given that COX inhibition and NO interact with different mechanisms underlying platelet aggregation we wanted to assess whether CINODs show additive bleeding effects compared to the respective NSAIDs.
Aims: To assess the bleeding time profile of CINODs as compared to NSAIDs. A representative CINOD (NCX 429), harboring both naproxen and a NO releasing moiety, was compared against the reference naproxen.
Male C57BL/6J mice were orally treated with vehicle, NCX 429 or naproxen at equimolar doses, and followed or not one hour later by the inhibitor of the three isoforms of NOS, L-NAME 100 mg/kg (i.p.). Finally, two hours after drug administration, bleeding time was assessed following the well established method of tail transection .
Results: In normal conditions, the two drugs did not produce significant changes in bleeding time as compared with vehicle-treated animals. On the contrary, L-NAME significantly shortened the bleeding time by about 50% (~250 sec) as compared to vehicle treated animals (~500 sec) demonstrating that indeed endogenous NO plays an important role in the platelet homeostasis. In those conditions of NO-deprivation by L-NAME, the two drugs were similarly effective in restoring bleeding time to normal.
Conclusions: The examined CINOD (NCX429) does not alter bleeding time in the mouse model when compared with its reference naproxen. Interestingly, lack of difference is between naproxen and NCX429, was registered either in normal conditions or in the presence of impairment of the endogenous NO pathway.

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TWO TYPES OF PARTICULATE MATTER TRIGGER

DIFFERENT INFLAMMATION BUT SIMILAR MILD COAGULATION ACTIVATION

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Keywords: particulate matter, coagulation

Background: Epidemiological findings suggest an association between chronic exposure to particulate matter (PM) in air pollution and VTE.
Aims: To investigate PM-induced dose- and time-dependent changes in inflammation and coagulation in mice.
Methods: Urban particulate matter (UPM, Mexico City) or diesel exhaust particles (DEP) were intratracheally instilled in C57Bl/6 mice in dose-response studies (sampling 24h after the instillation of 25, 100 or 200 µg/mouse) and time-response studies (sampling 4h, 10h or 24h after one instillation, and 24h after three consecutive daily instillations of 100 µg/mouse).
Results: A single instillation of PM dose-dependently increased total WBC count (mainly neutrophils, $p<0.001$) and levels of cytokines (IL-6, KC, MCP-1, RANTES, MIP1α, $p<0.05$) in broncho-alveolar lavage fluid, with peaks at 4h. Macrophage numbers were only mildly increased at the highest dose of UPM. Exposure to PM induced upregulation of pulmonary mRNA expression of VCAM1, ICAM1, tissue factor and thrombomodulin. In general, pulmonary inflammation was more pronounced after UPM than after DEP. Repeated instillation further enhanced the levels of macrophages (UPM and DEP) and MCP-1 (only DEP). Serum IL-6 increased 4h after instillation of UPM, but no other systemic inflammatory changes were measured. UPM and/or DEP significantly increased levels of FVII, FVIII and fibrinogen, but only at the highest dose (max. increase: 20-32%), without shortening the aPTT or PT, or increase in thrombin-antithrombin complex levels. DEP, but not UPM, upregulated hepatic FVII and protein C mRNA expression. Repeated instillation did not further alter coagulation parameters, except fibrinogen after DEP (+21±13% vs vehicle, $p<0.05$).
Conclusions: Despite extensive pulmonary inflammation, more pronounced for UPM than DEP, prothrombotic changes were mild and similar for both types of PM. Significant changes were only reached at the highest doses, exceeding comparable daily inhaled doses in humans. More chronic exposure models are needed to investigate the association between long-term PM exposure and DVT.

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P552

PLATELET FUNCTIONS IN NEWBORNS: IN RELATION WITH ENDOTHELIUM

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Keywords: platelet, endothelium, newborn

Background/Aims: The newborn period has different properties which are more significant morphologically and functionally than other periods of childhood and adulthood. One of these differences is that their platelets have deficient functions compared with the adult platelets. The purpose of the present study was to examine the platelet functions of newborns. We also planned to show the relationship between platelet and endothelial functions. Platelet aggregation and secretion was observed to invastigate the platelet function. Plasma nitric oxide (NO) level as a marker of endothelial function and cGMP as the signal molecule of NO in platelets were measured in comparison with adults.
Patients and methods: Twenty-nine healthy term newborns infants (aged 1-9 days) and 27 healthy volunteers (aged 20-30 years); those who were non-smokers and had not taken any medication within the last two weeks were enrolled in the study. ADP-induced platelet aggregation were evaluated by optical technique. Bioluminescence was used for measuring platelet ATP secretion. Platelet cGMP and plasma NO levels were measured by using EIA. Student's t test was used for statistical analyses.
Results: ADP-induced platelet aggregation and ATP secretion was lower in newborns than in the control group ($p<0.01$) . Platelet cGMP and plasma NO levels of newborns were higher than those of controls ($p<0.01$).
Conclusion: It was thought that lower platelet aggregation and secretion in newborns 1-9 days after the birth was due to higher NO levels and platelet cGMP levels induced by NO. High NO levels in newborns were suggested to be the result of circulation dynamics. Future studies on platelet and endothelial functions and interaction between these cells under spesific circulation conditions of newborns are needed.

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PROTHROMBOTIC PROFILE OF CIRCULATING PLATELETS AND LEUKOCYTES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Keywords: neutrophils, inflammation, autoimmunity

Background: Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular morbidity and death compared with age- and sex-matched community controls. Traditional cardiovascular risk factors may understimate the RA-associated risk. More specific predictive factors are required for these patients.
Aims: Main goal of this study was to dissect molecular pathways that link inflammation and thrombosis in patients with RA, and to evaluate whether they are affected by antirheumatic treatments.
Methods: We verified by flow cytometry and confocal microscopy and compared the tissue factor expression by circulating blood cells, the expression of platelet P-selectin and the fraction of circulating heterotypic aggregates in 53 RA patients (11 untreated, 15 treated with methotrexate alone, 19 with etanercept and 8 with adalimumab as well), as described in 50 sex- and age-matched healthy donors and in 10 patients with osteoarthritis.
Results: RA patients had a consensual increase in the expression of tissue factor by circulating platelets, neutrophils and monocytes compared with healthy controls and osteoarthritis patients. Moreover, platelets P-selectin expression and platelet-leukocyte heterotypic aggregates were as well significantly higher ($P<0.005$ vs both control groups). The leukocyte activation was significantly associated with the extent of platelet activation (P-selectin expression, heterotypic aggregates). Interestingly, the treatment with anti-TNF agents, which was clinically effective on disease severity, failed to influence the prothrombotic profile of circulating leukocytes, the fraction of platelet-leukocyte aggregates or the platelet P-selectin expression.
Conclusions: The results suggest that P-selectin dependent interactions between platelets and leukocytes occur in AR. These interactions may contribute to the risk of plaque instability and cardiovascular death observed in AR patients and suggest novel therapeutic strategies.

	Platelet P-selectin (%)	Platelet Tissue factor (MFI)	Neutrophil Tissue factor (MFI)	Monocyte Tissue factor (MFI)
Healthy	6.4±0.6	161.2±14.9	237.1±16.8	230.0±43.1
Osteoarthritis	7.0±3.22	159.0±11.6	244.8±36.7	182.6±11.3
RA untreated	18.9±3.1	225.1±24.7	423.0±15.7	334.0±42.1
RA methotrexate	13.3±3.4	238.5±10.4	437.3±13.7	358.3±31.2
RA anti-TNF	17.2±1.2	248.4±17.1	468.6±17.5	370.7±26.7

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IMPACT OF NEUTROPHIL APOPTOSIS ON HAEMOSTATIC ACTIVATION IN CHRONIC LIVER DISEASE PATIENTS

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Keywords: hypercoagulability, liver disease, neutropenia, neutrophil

Background: Recent studies suggest the impact of apoptosis on the mechanisms leading to hypercoagulability.
Aims: We aimed to clarify the potential role of neutrophil apoptosis in neutropenia and hypercoagulable state encountered in chronic liver disease patients.
Patients and methods: This study was conducted on fifteen normal controls and fourty five patients with chronic liver disease classified according to modified Child Pugh classification into, Child A, B and C groups (15 cases each). Haemostatic parameters studied include, prothrombin time (PT), partial thromboplastin time (PTT), tissue factor (TF), protein C antigen (PC), protein S antigen (PS), and markers of haemostatic activation (prothrombin fragment 1+2 {F1+2}, thrombus precursor protein {TpP} and D-dimer). Flowcytometric study was done for quantitative assay of neutrophil apoptotic subpopulations to detect the percentage of early and late apoptotic, and necrotic neutrophils using AnnexinV-FITC/Propidium iodide dye. Semiquantitative assay of apoptotic neutrophils showing DNA fragmentation was performed on neutrophil culture using terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end labeling (TUNEL) test. In addition to enzyme linked immunosorbent assay (ELISA) for soluble Fas (s Fas) (APO-1/CD95) in culture supernatant.
Results: The results revealed a rise in the neutrophil apoptotic and necrotic markers with progression of the disease, and they were inversely correlated with the absolute neutrophil count. The apoptotic neutrophil cells showed a significant positive correlation with several haemostatic parameters (TF, F1+2, TpP and D-dimer). Regression analysis proved that apoptotic parameters are independent determinant of prothrombotic markers, which further incriminate the apoptotic mechanisms in the hypercoagulable state encountered in this clinical setting.

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THE HORMONES OF THE HYPOTHALAMIC-PITUITARY-

THYROID AXIS ARE ESSENTIAL REGULATORS OF THE ACTIVITY OF PROTEIN C ANTICOAGULATION PATHWAY IN RATS

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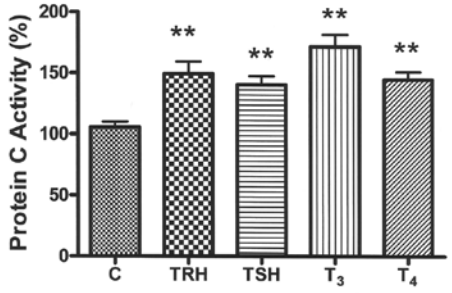
Keywords: hypothalamic-pituitary-thyroid axis, protein C, protein S

Background: Data about involvement of thyroid hormonal axis in hemostasis are relatively limited, controversial and are mainly based on clinical observations on patients with hyper- and hypothyroidism.
Aims: This study was aimed to investigate the effects of the hormones of the hypothalamic-pituitary-thyroid axis on some basic parameters of the activity of protein C anticoagulation pathway in rats.
Materials and methods: Five groups of rats were treated by Thyreotropin releasing hormone (TRH) (0.06 mg/kg b.m.), Thyreotropin (TSH) (1 MU/kg b.m.), Liothyroninum (T3) (0.08 mg/kg b.m.), Levothyroxinum (T4) (0.08 mg/kg b.m.) and saline s.c. for three consecutive days. Citrated plasma was checked for protein C antigen, activated protein C, protein C activity, soluble form of endothelial protein C receptor, total protein S, free protein S and protein S activity by ELISA kits.
Results: All the four hormones increased significantly ($p<0.001$) protein C activity, activated protein C, free protein S and protein S activity, and reduced the soluble endothelial protein C receptor. Protein C antigen and total protein S were significantly elevated only by TRH and TSH, but they were not affected by T3 and T4 treatment. Both prothrombin time and activated thromboplastin time were elongated after the application of all the hormones.
Discussion: The general hypocoagulability found by TRH, TSH, T3 and T4 harmonizes with the increased activities and of protein C and protein S and decreased soluble endothelial protein C receptor. The lack significant effects on protein C antigen and total protein S by T3 and T4 and the elevation of those parameters by TRH and TSH is an indication the latter not only regulate other hormones of thyroid axis, but they are directly involved in regulation of hemostasis.
Conclusions: The hypothalamic-pituitary-thyroid axis regulates protein C anticoagulation pathway in rats and as a whole imposes a general tendency of hypocoagulability.

Figure Effects of hormones of hypothalamic-pituitary-thyroid axis on protein protein C activity.



Abbreviations: TRH - thyrotropin releasing hormone; TSH - thyroid-stimulating hormone; T3 - 3-I-thyronin; T4 - thyroxin. Data are presented as MEAN±S.E.M. (** $p<0.01$).

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NITRIC OXIDE DONATION FROM THE CINOD NAPROXCINOD DOES NOT ALTER BLEEDING TIME OVER CYCLOOXYGENASE INHIBITION IN RATS

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Keywords: nitric oxide, CINOD, cyclooxygenase

Background/Aims: Naproxcinod is the first-in-class cyclooxygenase-inhibiting nitric oxide donator (CINOD) and differs from non steroidal anti-inflammatory drugs (NSAIDs) for its ability to release nitric oxide (NO), a key regulator of vascular tone and platelet function. Aspirin and traditional NSAIDs, by inhibiting platelet cyclooxygenase (COX)-derived thromboxane, impair platelet aggregation and can increase bleeding. Since NO donors have been shown to inhibit *in vitro* platelet aggregation, we tested whether NO donation from naproxcinod may contribute to prolongation of bleeding in addition to the effect derived from COX inhibition *in vivo.*
Male Sprague Dawley rats were orally treated with naproxcinod (5, 7.5, 15 or 30 micromol/kg), naproxen (5, 10, 20 or 30 micromol/kg) or vehicle. Thirty minutes afterwards, rats were anesthetized and 2 mm of the tip of the tail surgically removed, followed by tail immersion in heated saline. The time necessary to completely stop bleeding was measured. Plasma and serum were collected at the end of the experiment for determination of naproxen and thromboxane B2 (TxB2) concentration.
Results: Both naproxcinod and naproxen dose-dependently inhibited plasma TxB2 (complete suppression at 30 micromol/kg, both compounds), indicating effective platelet COX-1 inhibition. This was paralleled by a dose-dependent increase in bleeding time from 373±113 (control) to 736±292 and 732±241 sec. with 30 µmol/kg of naproxcinod and naproxen, respectively. Estimated median bleeding times depended linearly on naproxen plasma concentrations ($p<0.01$) and similarly linear correlation was seen for serum TxB2 concentrations. There was not statistical difference between the two compounds.
Conclusions: Oral administration of naproxcinod dose-dependently inhibited TxB2 formation, similarly to naproxen, indicating efficient COX-1 inhibition. Naproxcinod and naproxen did not differ in prolonging bleeding time following tail cut in anesthetized rats. In this model, NO donation from naproxcinod does not seem to contribute to prolongation of bleeding time, solely dependent on COX-1 inhibition.

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EFFECTS OF CIGARETTE SMOKING ON HUMAN PLATELET PROTEOME

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Keywords: platelet proteome, smoking, platelet function

Background/Aims: Cigarette smoking is one of the major risk factors of coronary artery disease, peripheral vascular disease and stroke. We tested platelet function of smoker or non-smoker healthy volunteers and compared their platelet proteomes.
PFA-100 collagen/ADP closure time and markers of platelet activation (P-selectin expression and PAC-1 binding) were measured in whole blood both in basal and stimulated conditions. Platelet proteomes of smokers or non-smokers were resolved by 2-DE DIGE, compared by Decyder software and identified by MALDI-TOF MS and LC-MS/MS. Regulated proteins were further analysed by Ingenuity Pathway Analysis which builds hypothetical networks from identified proteins and other proteins, on the basis of a regularly updated database.
Results: No significant difference between the two groups (n=14 each) was apparent in the PFA-100 closure time or in markers of platelet activation. In-gel analysis of platelet proteins from smokers or non-smokers by Decyder software revealed 5 significantly different protein spots ($p<0.05$): three proteins (factor XIII precursor, platelet glycoprotein IIb and β actin) were significantly higher in smokers, while WDR1 protein and chaperonine HSP60 were down-regulated. These five proteins were used for Ingenuity Pathway Analysis which identified one high ranking network (score 14). The cluster "Cellular Development, Lipid Metabolism, Small Molecule Biochemistry" consists of a network of 35 proteins, including the five identified by DIGE-based proteomics and 29 recognized to be related because of their reported interactions with the previous ones.
Discussion and conclusions: These data show for the first time an effect of cigarette smoking on platelet proteome. The proteomic approach may help clarify the disease mechanisms of smoke and identify biomarkers useful for risk prevention. The proteins identified are mainly involved in disease-associated inflammatory responses. Factor XIII might represent a candidate biomarker of disease resulting from tobacco exposure.

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NEW HUMAN FIBRINOGEN EFFICACY AND SAFETY IN A MODEL OF BLEEDING AND THROMBOSIS IN NORMOVOLEMIC AND NORMOTHERMIC RABBIT

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Keywords: arterial thrombosis, bleeding, fibrinogen, rabbit

Background: Human fibrinogen (Clottagen®-LFB) is increasingly used to secure haemostasis in hemorrhagic clinical settings such as trauma and surgical patients. A new manufacturing process including two steps of viral inactivation and a viral elimination step has been developed by LFB. In order to document the efficacy and safety of this new fibrinogen (FGTW), a rabbit model of bleeding and thrombosis has been used.
Materials and methods: Forty five rabbits were anaesthetized, ventilated and monitored for blood pressure and carotid flow. Folts model was used: a stenosis (75%) and an injury were carried out on the carotid artery, inducing thrombosis. Blood flow decreased as thrombus size increased until the pressure gradient was such that the thrombus was released and local arterial blood flow was suddenly restored. This is known as a cyclic flow reduction (CFR). After counting baseline CFRs during a 20 min-period, rabbits were randomized blindly to one of 3 groups: FGTW 300 mg/kg, Clottagen® 300 mg/kg and Control (NaCl). Then CFRs were recorded over a second period (P2). At the end of the experiment, a hepato-splenic section was performed and the amount of blood loss was recorded. After each period the following parameters was measured: ear immersion bleeding time, hematocrit, platelet account, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, a tromboelastrography measurement (ROTEM) and Thrombin Generation Assay (TGA).
Results: Doubling fibrinogen concentration had little influence on bleeding time, blood loss, PT, and aPTT, but increased the maximum firmness of clot measured through ROTEM analysis. It also augmented the endogenous thrombin potential and peak height in TGT measurement, possibly reflecting the antithrombin potential of fibrin (antithrombin-I): increase of fibrin would permit higher sequestration of thrombin.
Conclusions: Overall, FGTW was more effective than the corresponding dose of Clottagen®, without challenging safety. In vitro studies confirmed that FGTW was indeed more active, suggesting a higher clottable capability.

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EFFECT OF INCREASED BLOOD PRESSURE VARIABILITY ON PLATELET ADHESION AND AGGREGATION

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Keywords: blood pressure variability, sinoaortic denervation, platelet, adhesion, aggregation

Background: Blood pressure (BP) is not constant, but has a continuously spontaneous variation. This variation is defined as blood pressure variability (BPV). Chronic sinoaortic-denervated (SAD) rats exhibit an increased BPV with a normal BP level. It was found that SAD produced a remarkable damage in heartblood vessel, brain and kidney. Moreover, a previous study suggested that pulse blood flow in vitro may activate platelets.
Aims: The present study was designed to investigate the effect of increased blood pressure variability on platelet adhesion and aggregation.
Methods: Male Sprague-Dawley rats underwent SAD or sham operation at the age of 10 weeks. At 2 and 16 weeks after SAD or sham operation, homodynamic parameters in rats were continuously recorded in conscious unrestrained condition, and 24-hour SBP, DBP, SBPV and DBPV was calculated. Platelet adhesion to collagen type III was evaluated with a perfusion chamber at shear rate of 300s -1. Collagen- and ADP-induced platelet aggregation was detected with a particle counting method using light scattering. Expression of platelet surface P-selectin was analyzed by flow cytometry.
Results: Compared with time-matched sham-operated rats, in SAD rats 2 and 16 weeks after operation, SBP and DBP level remained unchanged, while SBPV and DBPV were significantly increased. Platelet adhesion and aggregation obviously increased in SAD rats. P-selectin positive platelets in SAD rats were significantly higher. In addition, platelet adhesion and aggregation were positively related to SBPV and DBPV, but were not related to SBP and DBP.
Conclusions: The blood pressure variability may enhance platelet adhesion and aggregation function.

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DISPOSAL OF ACTIVATED CIRCULATING PLATELETS IN GIANT-CELL ARTERITIS AND POLYMYALGIA RHEUMATICA

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Keywords: neutrophils, inflammation, autoimmunity

Background: Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are both associated to substantial inflammation and inflammatory mechanisms involved in the regulation of hemostasis and thrombosis. Blood cells of both GCA and PMR have an overt expression of leukocyte tissue factor. These features were not influenced by specific clinical features or by the extent of systemic inflammation and may fail to reveal the extent of ongoing smoldering vascular inflammation. We have recently observed that platelet P-selectin recognition culminates in the removal of activated platelets from the bloodstream, a physiological regulation of the haemostatic potential of activated platelets; conversely, a failure may contribute to persistent vascular inflammation and thrombosis.
Methods: We here investigated by flow cytometry and confocal microscopy the fraction of neutrophils and of monocytes that phagocytosed platelets in consecutive patients with CGA (n=8) and with PMR (n=8) and in sex- and age -matched donors (n=16).
Results: Only a small, even if consistent, level of platelet phagocytosis was detectable in healthy controls. In contrast, a striking increase in platelet phagocytosis was observed in PMR patients, which is only partially explained by platelet counts that did not differ between PMR patients and controls or platelet P selectin expression.
Discussion/Conclusions: Excess of activated platelets is apparently effectively removed by circulating leukocytes, quenching thrombocytosis and limiting P-selectin expression in PMR patients. Platelet phagocytosis is significantly less effective in GCA patients; this is not due to a lower stimulation, since CGA patients recruited in this study had a significantly higher number of platelets than PMR patients and an higher fraction of P-selectin expressing platelets.

Table

	Healthy	PMR	GCA
Platelet count (x10^3/µL)	225.7±6.4	238.7±49.6	318.9±27.1
P-selectin (%)	6.4±0.6	10.1±3.7	18.1±3.9
Neutrophils with intracellular platelets (%)	2.5±1.8	37.2±7.1	6.5±2.1
Monocytes with intracellular platelets (%)	0.6±0.5	15.4±2.9	5.8±1.5

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STICKY PLATELET SYNDROME AND FLOW CYTOMETRY

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Keywords: thrombophilia, sticky platelet syndrome, flow cytometry

Background/Aims: Sticky platelet syndrome (SPS) is a hereditary, autosomal dominant thrombophilia associated with an increased incidence of arterial and venous thrombosis. Light transmission aggregometry (LTA) reveals in SPS a platelet hyperaggregation induced by low concentration of platelet inducers – by adenosinediphosphate (ADP) and epinephrine (EPI). The cause of SPS still remains unknown but some studies suggest the abnormalities of platelet surface glycoprotein (GP) receptors that lead to their hyperfunction. Aim of the study was to detect any abnormalities in the expression of platelet membrane GP receptors in patients with SPS.
Patients and methods: Seventy-five patients with SPS were included in the study and examined by flow cytometry to assess the expression of platelet surface GP receptors (CD41, CD62P, CD61/63, CD36/63, CD29/49b and CD51).
Results: There were significant differences between the patient and control groups detected in the expression of CD62P, CD51 and in the co-expression of CD61/63. These GP receptors are neoantigens expressed on the platelet surface only after platelet degranulation (CD62P, CD51, CD63) or their expression is much higher (CD41/61) after platelet activation.
Conclusions: On the basis of our measurements we can say that platelets in SPS patients are activated compared to controls. We suggest that the expressions of CD62P, CD63 and CD51 may serve as predictors of thrombophilia in SPS patients.

Acknowledgement: Supported by grant VEGA 1/0067/08 and by the European Regional Development Fund (ERDF) Project "Center of Excellence for Perinatological Research".

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COMPOSITION OF AMBIENT PARTICLES INFLUENCES PULMONARY AND SYSTEMIC INFLAMMATORY RESPONSES IN RATS

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Keywords: air pollution, total suspended particles, metals, nitric oxide, oxidative stress, factor VIII

Background: Air pollution is associated with increased morbidity and mortality and these associations persist even at lower concentrations and short-term exposure.
Aims: Assessing the association between the components of three different sources of Total Suspended Particles (TSP), and local and systemic inflammatory responses in healthy rats.
Methods: TSP from automotive, industrial and biomass burning sources were collected on glass-fiber filters, extracted in distilled water by ultrasound and instilled into the trachea of 45 rats (15 in each group). Twenty micrograms of TSP were administered to each animal. The same amount of graphite particles in distilled water was used as control. Inflammatory markers of local and systemic responses were measured 24 hours after the exposure.
Results: Automotive-generated TSP presented high percentage of sulfur, iron and copper and produced local and systemic adverse effect evidenced by increases in exhaled nitric oxide (6.22 ppm ± 3.8), platelets (743.7 x 103/mm3 ± 73.4), fibrinogen (312.1 ± 42.7) and factor VIII (185.1 ± 37.2) levels, higher than those observed in the black carbon group. Biomass burning TSP, with high percentage of iron and copper, and moderated of sulfur produced the greatest pulmonary and cardiac oxidative stress when compared to black carbon and automotive groups. Industry-generated TSP, with relevant amounts of iron and copper and high percentages of calcium showed pulmonary and cardiac oxidative responses higher than those observed for the control group.
Conclusions: These findings showed that inflammatory reaction induced by ambient particles are influenced by their composition.

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MORNING PRO-THROMBOTIC MARKERS IN SUBJECTS WITH CHRONIC INSOMNIA

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Keywords: insomnia, sleep, PAI-1

Background/Aims: Insomnia, a highly common sleep complaint in contemporary society, has been associated to higher risk for cardiovascular morbidity and mortality. The direction and pathophysiological mechanisms involved in this relationship remain to be clarified. Poor sleep, chronic stress and hyper-arousal are significant key components of insomnia which theoretically have the potential to induce haemostatic changes know to be implicated in atherogenesis and its complication. The aim of this study was to assess morning plasma concentrations of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) in insomniacs compared to good sleepers.
Patients and methods: We studied 8 non obese unmedicated subjects (6 women, aged 44 ± 7 years) complaining of chronic insomnia (i.e. at least 3 times per week for at least 6 months) in the absence of any significant medical or psychiatric co-morbidity. Presence of sleep apnea and other sleep disorders was ruled out by full polysomnography. Subjects were compared to 6 good sleepers matched by age, sex and BMI. Blood samples (15 ml) were collected in the morning, after 30 minutes rest in supine position, in plastic tubes containing 3.2% sodium citrate. PAI-1 antigen and TF were determined by ELISA.
Results: Compared to good sleepers, subjects with insomnia showed two-fold values of TF (103 ± 11 vs 45 ± 14 pg/ml, $p<0.05$), and PAI-1 (28 ± 4 vs 12 ± 5 ng/ml, $p<0.05$). Self reported sleep duration of the night preceding the blood draw correlated significantly with PAI-1 (R = -0.55, $p<0.05$) and TF (R = -0.70, $p<0.05$), after adjusting for age and sex.
Conclusions: Subjects with chronic insomnia show heightened levels of prothrombotic markers. Enhanced blood coagulability in insomnia could be one pathophysiological mechanism linking insomnia to cardiovascular diseases.

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TISSUE FACTOR AND TISSUE FACTOR PATHWAY INHIBITOR IN DIABETIC FOOT SYNDROME

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Keywords: TF, TFPI, diabetic foot

Background/Aims: The diabetic foot syndrome is one of the most important complication of type 2 diabetes mellitus. In the pathogenesis of this disease hemostasis disorders play a major role associated with hypercoagulability. The purpose of this study was to evaluate the extrinsic pathway concerning the activation of coagulation system in patients with type 2 diabetes mellitus.
Materials and methods: The study was conducted on 74 patients with type 2 diabetes mellitus: 44 with diabetic foot syndrome (DF), (F/M 15/23), average ages 60 years, 30 with non-complicated diabetes (DM), (F/M 13/17), average ages 63 years. The control group consisted of 20 patients (F/M 11/9), average ages 53 years. Concentration of total TF, TFPI was performed by ELISA.
Results: In the study we observed a significantly higher concentration of TF in patients with DF in comparison to the control group and to patients with DM ($p<0.0002$ both). Patients with DF had also a significantly higher concentration of TFPI than the control group and patients with DM ($p=0.0001$, $p=0.001$ respectively).
Conclusion: In diabetic foot patients enormous high activation of extrinsic pathway of coagulation was observed.

Table

Assessed parameters [units]	DF (I) Me Q1/Q3 n=44	DM (II) Me Q1/Q3 n= 30	Control Group (III) Me Q1/Q3 n=20	*p*- Values
TF [pg/ml]	420.00 188.80/561.90	150.04 117.39/200.00	122.89 79.89/178.23	$p=0.0002^{Ivs.III}$ NS $^{IIvs.III}$ $p=0.0002^{Ivs.II}$
TFPI [ng/ml]	160.20 84.90/277.75	72.20 63.30/97.62	48.20 36.93/59.07	$p=0.0001^{Ivs.III}$ NS $^{IIvs.III}$ $p=0.001^{Ivs.II}$

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PLASMA CLOTTING IN SLOW STREAM

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Keywords: plasma, clotting, stream

The importance of information on clotting is obvious as thrombosis is often a reason of death. Clinic laboratory data are obtained in constant condition of measurement, but there is a wide variety of coagulation conditions in an organism. Such important parameters as blood pressure and stream are not reproduced at all. We studied the influence on stream on plasma coagulation. The samples of blood were obtained from 30 volunteers. Plasma was placed into the special measurement equipment where it was moved by hydraulic pressure (245, 343, and 441 N/m2). Flow parameters were registered. Clotting time of unmoving sample was the control. Average volume velocity was 37+ 16 (100%), 57+23 (154%), and 79+29 (214%) (mcL/sec). Average linear velocity was 5.2+2.3, 8.1+3.3 and 11.2+4.1 (mm/sec). Average share rate was 14.0+6.0, 21.5+8.7, and 30.0+10.9 (sec-1). They were about the same in venules and small veins. The control clotting time was 274+45 sec (100%). The stop time of the plasma stream as the result of coagulation was 180+21 (66%), 145+16 (53%) and 135+11 (49%) (sec). Stream parameters were unchanged during the most part of this period and extremely reduced in the last part. This one was 22+1 (12%), 12+1 (8%) and 12+1 (9%). (100% is the total duration of flow before stopping). We suppose that the first period, i.e. the time of stable stream, regards thrombin generation and the last period reflects properly fibrin generation in fluid when viscosity of the plasma was increasing. So it was shown that clotting time of moving plasma was shorter than the non moving one and that the slowing down of stream owing to clotting was only in the last 10% of the whole coagulation time.

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VASCULAR ENDOTHELIUM ACTIVATION IN MORBID OBESITY

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Keywords: tPA, sel E, sTM, morbid obesity

Background/Aims: Epidemiologic data show the great number of patients suffering from morbid obesity. The reason of death is myocardial infarction or stroke depending upon hypercoagulability of blood. There are a few studies in morbid obesity dealing with function of vascular endothelium engaged in haemostatic process. The aim of this study was the evaluation of endothelium function with known blood plasma markers.

Material and methods: The study was performed on 28 obese patients mean aged 38.54 ± 9.11 with body mass index (BMI) 48.5 ± 5.56 (F/M 18/10). The control group consisted of 30 healthy volunteers sex and age-matched. In venous blood plasma using ELISA (enzyme linked immunoassay) with kits of Roche the following parameters were estimated: tPA:Ag (antigen of tissue plasminogen activator), sel E (selectin E) and sTM (soluble thrombomodulin).

Results: Results are shown in the table below. We detected in morbid obesity significantly higher than in controls assessed parameters of endothelial cells activity.

Conclusions: Our results indicate an activation of vascular endothelium in obese patients, an important reason of hypercoagulability.

Table

		Obesity n = 28	Controls n = 30	
Assessed parameters	Units	M (Q_1; Q_3)	x ± SD	P
tPA:Ag	ng/ml	12,68 (9,21; 13,99)	4,94 (3,71; 5,485)	< 0,0001
sel E	ng/ml	78,165 (48,94; 119,62	21,085 (9,895; 24,585)	< 0,0001
sTM	ng/ml	2,05 ± 0,54	1,85 (1,675; 2,245)	< 0,001

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P22

HEMORHEOLOGY AND PROPENSITY TO THROMBOSIS IN PATIENTS WITH HYPERLIPOPROTEIDEMIA AND HIPOLIPIDEMIC THERAPY

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Keywords: hemorheology, hyperlipoproteidemia, hipolipidemic therapy

Aims: To study rheological properties of blood (RBP) in patients with hyperlipoproteidemia (HLP) and influence of therapy with simvastatin and fenofibrate on hemorheology in these patients.

Materials and methods: Research has been made at the Department of Therapy of Faculty of Advanced Medical Training, Russian State Medical University (Moscow) under the supervision of Professor P. Dzhanashija.

50 patients with HLP and CAD, AH, NIDDM were inspected. HLP IIb, IV n= 29, age 54.05+2.1 and HLP IIa n= 21, age 55.8 +1.4. Levels of TC, TG, HDL-C, LDL-C and other biochemical indices (ALT, AST, KFK) were determined in parallel before and after of 12 weeks after beginning of treatment with simvastatin in doses 20 mg/day and micronized fenofibrate in doses 200 mg/day.

The parameters RPB− yield stress, apparent viscosity, erythrocytes cohesion factor (ECF), fast aggregation time (T1), slow aggregation time (T2), hydrodynamic strength of aggregates (HSA), were studied on coaxial-cylinder aggregometer-viscosimeter modified by P. Dzhanashija, which combines aggregometer and viscosimeter, allows to reduce blood samples and time of determination of RPB (**Figure**). Erythrocyte deformability factor (EDF) was determined using laser digital deformometer of erythrocytes (LDDE-7, Russia) based on a principle of nephelometry.

Results: Initially parameters of RPB were worse in patients with HLP IV (r=0.7, $p<0.01$ for yield point and TG). EDF was decreased in all types of HLP, especially in patients HLP type IV ($p<0.05$) (**Table**). RPB parameters improvement in micronized fenofibrate group were strongly pronounced in HLP IV vs. HLP IIb ($p<0.05$ for yield point and ECF) (**Table**). In HLP IIa simvastatin positive influence on RPB appears after 12 weeks. Positive influence of micronized fenofibrate vs. simvastatin on EDF: after 12 weeks EDF rise on 70% ($p<0.01$) and 23% ($p<0.05$).

Discussion: Increased time of RBC aggregation (T1, T2) and bad RBC deformability may lead to thrombosis in microcirculatory vessels especially in patients with type IV HLP.

Conclusions: Hypolipidemic therapy improves microcirculation.

Figure

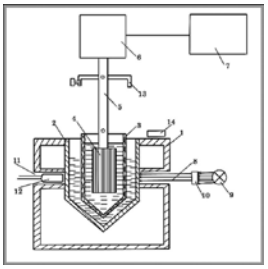

Table: Rheological properties of blood and HLP types

RPB	Norma	HLP types		
		IIa	IIb	IV
yield stress, N/m^2	0,035	0,071±0,01*	0,078±0,02*	0,091±0,01**
apparent viscosity, mPa•s	20,40	28,383±0,03	30,124±0,09	31,753±0,03*
ECF	0,51	1,459±0,26*	1,534±0,41*	1,726±0,09*
T1, sec.	11,55	7,182±0,92	7,213±0,95	5,723±0,82*
T2, sec.	32,68	21,471±2,01	20,001±2,31	19,149±1,1*
HSA, sec.$^{-1}$	52,11	97,839±6,13*	96,527±8,74*	102,835±9,2*
EDF	0,21	0,131±0,01	0,135±0,02	0,114±0,02*
			*p<0,05	**p<0,01

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P181

PLATELET ACTIVATION IN LIVER CIRRHOSIS: CONTRIBUTION TO DEVELOPMENT OF PORTAL VEIN THROMBOSIS AND HEPATOCELLULAR CARCINOMA

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Keywords: liver cirrhosis, HCC, PVT, platelet activation

Background: Liver cirrhosis (LC) is one of the most important health problems according to current gastroenterology literature. Hepatocellular carcinoma (HCC) and portal vein thrombosis (PVT) commonly develop in patients with LC.

Aims: To assess platelet activation and its possible contribution to the pathogenesis of LC, HCC and PVT.

Patients and methods: Forty-five patients with LC caused by dual schistosomiasis and viral hepatitis infections were enrolled in the study, 15 had LC only, 15 were complicated with HCC, and 15 were complicated with PVT, in addition to 15 healthy controls. Platelet morphological parameters including platelet count, platelet crit, mean platelet volume (MPV) and platelet distribution width (PDW), as well as platelet activation as evidenced by measuring soluble platelet selectin (Sp-selectin) level and the release of beta-thromboglobulin (β-TG), transforming growth factor β-1 (TGF- β1) and platelet derived growth factor-AA (PDGF-AA) were evaluated.

Results: The platelet count, platelet crit and MPV were significantly decreased while PDW was significantly increased in all LC patients in comparison to controls. Sp-selectin, β-TG, TGF-β1and PDGF-AA revealed significant increase in all diseased groups when compared to control group. Patients complicated with HCC or PVT demonstrated significant increase in the aforementioned parameters in comparison to patients with LC only. Patients with PVT demonstrated significant increase versus HCC patients.

Conclusions: Platelet activation is a prominent feature in LC and its serious complications HCC and PVT. This activation can play an important role in the pathogenesis of LC, HCC and PVT in patients with mixed schistosomiasis and viral hepatitis infections. Such patients need careful medical attention and effective treatment. Stabilization of the activated platelets and the dual suppression of PDGF and TGF- β1 could be new therapeutic strategies against LC and its sequelae.

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P337

TF AND TFPI IN CHRONIC OBTURATIVE PULMONARY DISEASE (COPD)

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Keywords: TF, TFPI, chronic obturative pulmonary disease

Beckground/Aims: Pulmonary thrombosis often appears to complicate the course of patients with chronic obturative pulmonary disease (COPD). There is now a large body of evidence to indicate the inflammation present in patients with COPD may activate the coagulation of blood. Very few is known about the role of tissue factor (TF) pathway in activation of coagulation system in COPD.
The aim of study was to evaluate TF and tissue factor pathway inhibitor (TFPI) in patients suffering from COPD.
Material and methods: The study was performed in venous blood plasma of 66 patients with COPD in different stages of disease according to GOLD 2006, aged mean 60.4 years (F/M 18/48). The control group consisted of 25 healthy volunteers (non-smokers). Using ELISA (enzyme – linked immunoasorbent assay) total TF and TFPI concentrations in blood plasma were determined.
Results: Results are shown in the table below.
In patients with COPD statistically significant higher than in controls concentrations of TF and TFPI were observed.
Conclusions: In patients suffering from COPD activation of TF depending pathway coagulation of blood is present.

Table

		COPD n = 66	Controls n = 25	
Assessed parameters	Units	Me (Q_1; Q_3)		P
TF	pg/ml	118,29 (76,00; 166,27)	83,08 (55,55; 119,09)	0,03
TFPI	ng/ml	120,17 (91,88; 165,97	96,12 (72,56; 106,92)	0,004

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P390

VENO-OCCLUSIVE DISEASE IN PEDIATRIC PATIENTS AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION - RELEVANCE OF ACTIVATED COAGULATION AND FIBRINOLYSIS MARKERS AND NATURAL ANTICOAGULANTS

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Keywords: VOD, HSCT, children

Background/Aims: Veno-occlusive disease (VOD) is a life threatening complication after hematopoietic stem cell transplantation (HSCT). Its prediction, precise diagnosis and treatment are subjects of various investigations but remain unclear until now. Our goal was to investigate the relevance of activated coagulation and fibrinolysis markers, as well as natural anticoagulants, for the development of VOD in paediatric patients underwent HSCT.
Materials and methods: We investigate parameters in 47 paediatric patients, 22 with neuroblastoma, 17 with leukemias and 10 with lymphomas. The study was prospective and values of antithrombin (AT), protein C (PC), fibrinogen (FI), thrombin-antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2) D-dimer were measured. Each parameter was determined before conditioning regimen (baseline -7 day), on HSC infusion day (day 0), and on +7, +14 and +30 day after HSCT. Patients were monitored for the occurence of VOD.
Results: In our group VOD occured in 10 patients, et median post HSCT day 17.5 (range- day 2 to day 28). In the VOD group, on the baseline day, levels of FI were significantly lower and there were no differences in parameters levels at day 0 between the two groups. On day +7 we found relevant difference in levels of F1+2 (higher in VOD group). From day +14 and thereafter, levels of all parametres except TAT were relevantly different between the two groups of patients (higher levels of F1+2 and D-dimer and lower levels of FI, AT and PC). TAT levels were not different between the two groups of patients at any check point day.
Conclusions: Levels of F1+2 were increased and AT, PC and FI decreased before the clinical onset of VOD. Noticeable differences in early post-transplant period may have predictive value in VOD onset, thus making the investigated parameters of great value in routinely monitoring pediatric patients after HSCT.

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P467

EFFECT OF MICROPARTICLES FROM DENGUE VIRUS INFECTED PATIENTS ON THE COAGULATION PROFILE OF NORMAL ROTATIONAL THROMBOELASTOGRAM

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Keywords: microparticles, dengue, thromboelastography

Background: During the febrile period of dengue infection the virus replicates into the mononuclear cells. In this febrile period pro-inflammatory cytokines are increased and pro-coagulant and fibrinolisis activation are present. An increase in D-dimers is a common finding. We hypothesized that plasma microparticles (MP) contribute to this coagulopathy.
Materials and methods: We studied the effect of circulating microparticles (MP) from 10 febrile, (plasma virus positive) dengue cases on the thromboelastogram (Rotem, Pentapharm, Germany) of normal PPP+ Ca++, and compared it with MP from normal donors and with commercial thromboplastin (Lab Stago, Asnièrs, France). MP were prepared from ultracentrifuged (100x103 g for 2 hours at 4°C) EDTA platelet poor plasma obtained at 22°C. The bottom with the MP was re-suspended in buffered saline (100ul/ml ultracentrifuged PPP) and preserved at 40°C. Flow cytometry visualized CD41 and CD11 positive MP in the preparations. Two different concentrations of MP were tested in each case.
Results: MP substituted thromboplastin in the thromboelastographic pattern (EXTEM), but less active for the clot formation (CT and CTF prolonged) and slower in the clotting kinetics(ALP); clot stiffness (MCT) and 30 minutes lyses remained the same. When we doubled the amount of MP, the CFT and ALP improved. The number of MP were not determined during this experiment.
Conclusions: Although circulating WBC and platelet were low in dengue cases (4.9 to 1.3 x 106/ml and 164 to 5 x106/ml respectively) we could clearly demonstrate by this method that their MP activate the coagulation cascade, but no significant difference could be observed in the behavior of the MP from the normal controls. More cases shall be analyzed by this method quantifying number and origin of their MP to evaluate its sensitivity for these type of studies.

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New anticoagulants

Thursday 8th July, 2010

☺ P600

INHIBITION OF COAGULATION FACTOR XI BY ANTISENSE OLIGONUCLEOTIDES: A NOVEL EFFECTIVE ANTITHROMBOTIC STRATEGY WITH LOWERED BLEEDING RISK

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Keywords: coagulation factor XI, antisense oligonucleotides, antithrombotic treatment

Background/Aims: Existing anticoagulants effectively inhibit the activity of coagulation factors of the extrinsic and common pathway but have substantial limitations and can cause severe bleeding complications. Intrinsic coagulation factor XI (FXI) appears to be an attractive target for anticoagulant therapy as recent work points to an important role for FXI in thrombosis but a relatively minor role in normal hemostasis. Antisense oligonucleotides (ASO) are novel therapeutic agents that can powerfully and selectively inhibit protein synthesis. We studied the effect of anti-factor XI antisense treatment as a novel therapeutic approach for the treatment of thrombosis.
Materials and methods: Selective second generation ASOs targeting FXI were developed and characterized for their efficacy and safety in various murine models of thrombosis and bleeding. The effect of FXI antisense therapy was compared to currently used anticoagulants warfarin and enoxaparin, and plasma-derived FXI concentrate was evaluated for its potential use as an antidote for FXI antisense treatment.
Results: Systemic treatment of mice with FXI ASO led to specific, potent and dose-dependent reduction of hepatic FXI mRNA levels with corresponding reductions in plasma levels of FXI protein and activity. Furthermore FXI antisense treatment produced potent and dose-dependent antithrombotic activity in various venous and arterial thrombosis models, comparable to warfarin or enoxaparin. However, unlike warfarin or enoxaparin, FXI inhibition did not cause bleeding. Co-administration of FXI ASO with enoxaparin or the anti-platelet drug clopidogrel produced improved anti-thrombotic activity without increased bleeding. Finally, plasma-derived FXI concentrate was shown to effectively and rapidly reverse the anticoagulant effect of FXI antisense therapy.
Conclusions: These results support the concept that inhibition of FXI through antisense therapy might serve as a new and effective strategy for the treatment and prevention of thromboembolic disease with improved specificity and safety.

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☺ P485

SUCCESSFUL REMOVAL OF DABIGATRAN IN FLOWING BLOOD WITH AN ACTIVATED CHARCOAL HEMOPERFUSION COLUMN IN AN *IN VITRO* TEST SYSTEM

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Keywords: dabigatran, antidote, direct thrombin inhibitor

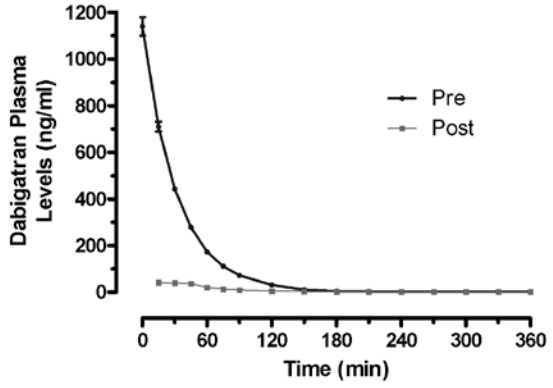
Background/Aims: Dabigatran etexilate is an oral DTI showing efficacy and safety in prevention of stroke in patients with atrial fibrillation and in VTE treatment. A general limitation of anticoagulant use, particularly chronic treatment, is the lack of rapidly acting antidotes to reverse effects in emergency situations. Dabigatran has low plasma protein binding and could be dialysed and removed from patient blood by charcoal hemoperfusion. This was tested in vitro using the Adsorba™ cartridge, which contains specially activated carbon allowing hemoperfusion and is clinically available for use in drug and toxin intoxifications.

Materials and methods: Dabigatran was added to 5L bovine whole blood (sodium citrate) achieving supratherapeutic concentrations (1000ng/mL) and was pumped through tubing (flow rate 150-350 mL/min) across a Gambro Adsorba™ cartridge (n=3 experiments). Blood pre- and post-Adsorba™ cartridge was serially sampled at 15-30 min intervals over 6hrs. Blood was centrifuged and frozen. Dabigatran was measured using standard LC-MS/MS methods. Effects of dabigatran on clotting in bovine blood were performed using TT, aPTT and ECT.

Results: Initial dabigatran levels in bovine blood were 1140 ± 40 ng/ml. As blood was pumped across the Adsorba cartridge, circulating dabigatran was almost completely removed, i.e. at 15min dabigatran pre-filter was 710 ng/ml and were reduced to 42 ng/ml post-filter (**Figure**). Dabigatran plasma levels in the 5 L volume decreased exponentially by ~85% reduction when 1.8 plasma volumes were treated after 60 min and >95% reduction when 3.6 plasma volumes were treated after 120min. Dabigatran also prolonged clotting in bovine blood in a concentration-dependent manner.

Conclusions: This demonstrates dabigatran can be removed from whole blood in vitro by adsorption across an activated charcoal column (Adsorba™ Cartridge). This may be a method to remove dabigatran rapidly in blood in emergency situations; further testing is required in vivo before this can be recommended for clinical use.

Figure



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☺ P509

SINGLE INTRAVENOUS ADMINISTRATION OF TB-402 FOR THE PROPHYLAXIS OF VTE AFTER TOTAL KNEE REPLACEMENT SURGERY

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Keywords: TB-402, anticoagulant, prophylaxis

Background/Aims: TB-402 is a human monoclonal antibody that partially inhibits Factor VIII. Its half-life (±3 weeks) may allow for prolonged prophylaxis of venous thromboembolism (VTE) following a single administration. The aims of this study were to evaluate the efficacy and safety of a single intravenous administration of TB-402 for the prevention of VTE in patients undergoing total knee replacement (TKR).

Patients and methods: This was a phase II, multicenter, dose-escalating, randomised, enoxaparin-controlled open-label trial. All patients received enoxaparin 40mg pre-operatively and were randomized post-operatively in a sequential cohort design to one of three doses of TB-402 (0.3mg/kg, 0.6mg/kg or 1.2mg/kg) or enoxaparin 40mg (3:1; n=75 per group). TB-402 was administered as an intravenous bolus 18–24 hours after TKR. Enoxaparin 40mg sc q24h was administered for at least 10 days.

The primary efficacy endpoint was the composite of asymptomatic DVT as detected by bilateral venography and symptomatic VTE by Day 7–11. The primary safety endpoint was the number of patients with major or clinically relevant non-major bleeding from randomisation until the end of the study at 3 months. All outcomes were adjudicated by a blinded independent central adjudication committee.

Results: 316 patients were randomized. Total VTE was lower in each of the three TB-402 groups compared to the enoxaparin group (**Table**). A comparison of the pooled TB-402 groups vs the enoxaparin group demonstrated a statistically significant reduction in the incidence of total VTE [47/218;22%(95%CI:17–28) vs 30/77;39%(95%CI:29–50) $p<0.05$].

Major or clinically relevant non-major bleeding was observed in 3/75(4.0%), 4/74(5.4%), 7/87(8.0%) and 3/79(3.8%) patients for TB-402 0.3mg/kg, 0.6mg/kg, 1.2mg/kg and enoxaparin, respectively (p=NS).

Conclusions: In this phase II trial, TB-402 demonstrated superior antithrombotic activity as compared to enoxaparin 40mg. The incidence of major and clinically relevant non-major bleeding was similar in patients randomised to enoxaparin 40mg or to TB-402 0.3mg/kg or 0.6mg/kg.

Table: Single intravenous administration of TB-402 for the prophylaxis of VTE after total knee replacement surgery

			TB-402 0.3 mg/kg	TB-402 0.6 mg/kg	TB-402 1.2 mg/kg	Enoxaparin 40 mg
Efficacy			**N= 72**	**N= 67**	**N=79**	**N=77**
	Total VTE	**n (%)**	**12 (16.7%)**	**16 (23.9%)**	**19 (24.1%)**	**30 (39.0%)**
		[95 C.I.]	**[9.8-26.9]**	**[15.3-35.3]**	**[16.0-34.5]**	**[28.8-50.1]**
	Major VTE	**n (%)**	**1 (1.4%)**	**0 (0%)**	**1 (1.3%)**	**3 (3.9%)**
Safety			**N= 75**	**N= 74**	**N=87**	**N=79**
	Major Bleeding	**n (%)**	**0 (0%)**	**1 (1.4%)**	**4 (4.6%)**	**0 (0%)**
	CRNMB	**n (%)**	**3 (4.0%)**	**3 (4.1%)**	**3 (3.4%)**	**3 (3.8%)**

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☺ P486

BERIPLEX P/N REVERSES BLEEDING IN AN ACUTE RENAL INJURY MODEL AFTER DABIGATRAN OVERDOSE IN RABBITS

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Keywords: dabigatran, antidote, direct thrombin inhibitor

Background/Aims: Clinically relevant bleeding while on antithrombotic treatment with oral anticoagulants are of concern for patients and physicians. The need for an appropriate strategy to reverse anticoagulation is crucial for patients requiring emergency procedures. Dabigatran etexilate is a new oral, reversible direct thrombin inhibitor showing efficacy and safety in the prevention of stroke in patients with atrial fibrillation and VTE treatment. The effect of Beriplex® was tested in a rabbit trauma bleeding model as a potential method to reverse dabigatran after overdosing.

Materials and methods: Rabbits were anesthetized using isoflurane, mechanically ventilated and monitored. Animals were allocated to placebo (n=8); II dabigatran 0.4 mg/kg iv (n=5) alone; or combined with Beriplex P/N 20 IU/kg (n=5), 35 IU/kg (n=5) or 50 IU/kg (n=5). Time to hemostasis (TH) and volume of blood loss (BL) were observed up to 30 min following a standardized kidney incision injury. Blood samples were taken to monitor dabigatran plasma levels and thrombin generation.

Results: Dabigatran increased BL and TH to 29.3±13.7 mL and 23.7±11 min, respectively. There was a dose-dependent reversal with Beriplex P/N of dabigatran-induced bleeding. Beriplex P/N 20 IU/kg had no influence on BL or TH (34±22.91 mL and 21.3±7.8 min), however, the doses of 35 IU/kg and 50 IU/kg decreased BL (10.3±3.1 mL and 5.5±1.3 mL) and TH (11.6±1.7min and 7.6±1.7min). Peak plasma levels achieved with dabigatran were ~900 ng/ml. Beriplex had no effect on plasma levels. TGA showed almost complete inhibition of thrombin generation with the bleeding dose of dabigatran used.

Conclusions: This study demonstrated Beriplex® inhibits dabigatran-induced bleeding in a rapid, dose-dependent manner. The highest dose of 50 IU/kg Beriplex P/N reduced bleeding to near normal values. These data suggest that Beriplex could potentially be used as an antidote for the direct thrombin inhibitor dabigatran in case of severe bleeding complications.

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☺ P366

SAFETY AND EFFICACY OF EDOXABAN IN PATIENTS UNDERGOING HIP FRACTURE SURGERY

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Background/Aims: Edoxaban (the free base of DU-176b) is an oral, once-daily direct factor Xa inhibitor in advanced clinical development for the prevention and treatment of thromboembolic events. The objective of this study was to investigate the safety and efficacy of edoxaban in patients undergoing hip fracture surgery (HFS).

Materials and methods: In a multicenter, randomized, open-label Phase 3 trial, Japanese patients aged ≥20 years undergoing HFS were randomized in a 2:1 ratio to edoxaban 30 mg qd or the active control, subcutaneous enoxaparin sodium 2,000 IU (enoxaparin) every 12 hours (Japanese standard of care). Edoxaban was started within 6 to 24 hours and enoxaparin was started within 24 to 36 hour after surgery, both were continued for 11 to 14 days. Venography of both legs was conducted within 24 hrs after the last dose of the study medication. Primary endpoints were bleeding events. Secondary endpoints included thromboembolic events and adverse events.

Results: A total of 92 patients were randomized and 76 completed the study. Baseline characteristics were similar between the treatment groups, although creatinine clearance was lower in the edoxaban group (63.7±22.4 mL/min) than in the enoxaparin group (73.2±30.8 mL/min). The incidence of major and clinically relevant non-major bleeding was 3.4% (2/59, 95% CI, 0.9-11.5) in the edoxaban group and 6.9% (2/29, 95% CI, 1.9-22.0) in the enoxaparin group. There was one episode of major bleeding in each group. The incidence of thromboembolic events was 6.5% (3/46, 95% CI, 2.2-17.5) in the edoxaban group and 3.7% (1/27, 95% CI, 0.7-18.3) in the enoxaparin group. All the thromboembolic events were asymptomatic distal DVT. The incidence of adverse events was similar between the treatment groups.

Conclusions: Edoxaban 30 mg qd demonstrated similar safety and efficacy to enoxaparin in Japanese patients undergoing HFS.

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☺ P505

RIVAROXABAN CALIBRATORS AND CONTROL SETS MEASURING RIVAROXABAN PLASMA CONCENTRATIONS USING THE PROTHROMBIN TIME

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Keywords: factor xa inhibitors, rivaroxaban, therapeutic drug monitoring

Background: Rivaroxaban is an oral, direct factor Xa inhibitor which is marketed for the prevention of venous thromboembolism and for the prevention and treatment of arterial and venous thromboembolism. No routine coagulation monitoring is required but rivaroxaban plasma concentrations might be required in some cases, *i.e.* severe overdose or compliance. Variation in response sensitivity of prothrombin time (PT) reagents to rivaroxaban is well described in the literature and the international normalized ratio correction of results can not be used to correct for this variation.

Aims: This multicentre study evaluated rivaroxaban calibrators and controls and the PT for the measurement of rivaroxaban plasma concentrations. The interlaboratory precision of rivaroxaban plasma concentration measurement was also evaluated.

Methods: 20 centres were provided with sets of rivaroxaban calibrators (50, 41, 219, and 430 ng/mL) and rivaroxaban pooled human plasma controls (19, 160, and 643 ng/mL). The evaluation was performed over 10 consecutive days by each laboratory using its own PT reagent as well as STA Neoplastine CI Plus, Diagnostica Stago. A rivaroxaban calibration curve was produced daily. The day-to-day precision was evaluated by testing in duplicate three plasma controls. The control was diluted and re-tested if the level was above the highest concentration of the calibration curve.

Results: A large interlaboratory variation (in seconds) was shown for the controls when local PT reagents were used, and their coefficient of variation (CV) was 14 to 30%; but the results were more consistent when using the same reagent with a CV of <6% (with undiluted samples). Expressed in ng/ml, a smaller interlaboratory variation was observed (CV ranging from 2% for the highest to 7.5% for the lowest). In addition, the CV for the 41 ng/ml calibrator with the central reagent was 4.4%, and the results were reliable for concentrations >40 ng/ml and up to 600 ng/ml.

Conclusion: Such measurements may be of assistance when determination of rivaroxaban plasma concentrations is required.

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ASSESSMENT OF PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTION BETWEEN APIXABAN AND ENOXAPARIN IN HEALTHY SUBJECTS

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Keywords: apixaban, LMWH interaction, anti-Xa activity

Background/Aims: Apixaban is an oral, direct, selective, reversible inhibitor of coagulation factor Xa being developed for prevention and treatment of thromboembolic diseases. This study assessed the effect of single-dose enoxaparin on the pharmacokinetics (PK) and pharmacodynamics (PD) of apixaban following coadministration and administration 6 hours apart.

Materials and methods: This was an open-label, randomized, 4-treatment, 4-period, 4-sequence, single-dose crossover study in healthy subjects. Subjects were randomized to receive each of the following: single-dose apixaban 5 mg orally, single-dose enoxaparin 40 mg subcutaneously, single-dose apixaban (5 mg) and enoxaparin (40 mg) concomitantly, and single-dose apixaban (5 mg) followed by single-dose enoxaparin (40 mg) 6 hours later. Apixaban PK and anti-Xa activity parameters were derived from plasma concentration and anti-Xa activity versus time, respectively. Prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT) were also measured.

Results: Twenty subjects (26–45 y) were randomized; 18 completed all 4 treatments. The PK of apixaban 5 mg was not affected by enoxaparin 40 mg; both Cmax and AUC 90% CI were within the prespecified equivalence interval of 80% to 125% following coadministration with enoxaparin. Maximum anti-Xa activity was 1.4 U/mL for 5 mg apixaban and 0.4 U/mL for 40 mg enoxaparin when administered alone. Maximum anti-Xa activity was 1.9 U/mL when the 2 agents were coadministered, which is approximately additive. When the 2 agents were administered 6 hours apart, a 15% increase in peak anti-Xa activity (1.56 U/mL) was observed compared with apixaban alone. Small increases in PT/INR and aPTT were observed following coadministration. No bleeding-related adverse events were reported.

Conclusions: Coadministration was well tolerated in healthy subjects. Enoxaparin did not affect apixaban PK. An additive effect on anti-Xa activity was observed after coadministration of enoxaparin and apixaban; administration 6 hours apart resulted in similar peak activity to apixaban alone.

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GENERIC VERSIONS OF ARGATROBAN CAN BE DIFFERENTIATED FROM BRANDED ARGATROBAN IN THE THROMBIN GENERATION ASSAYS

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Keywords: argatroban, generic, thrombin generation

Beside the direct inhibition of thrombin Argatroban is capable of inhibiting the generation of tissue factor mediated protease such as thrombin and Xa. Subsequently the formation of fibrin is also decreased. Since differences in the IC50 values in generic and branded argatrobans have been noted for thrombin inhibition. We hypothesized that differences in these agents for their relative effects on thrombin generation may also be observed. To test this hypothesis, we used a TGA kit (TECHNOTHROMBINÒ TGA, Technoclone, Vienna, Austria) to measure the kinetic of thrombin generation in human blood plasma supplemented with different argatroban preparations. Each of the argatroban preparation was supplemented to platelet poor plasma (PPP) and platelet rich plasma (PRP) at a concentration of range of 0.6 to 5 mg/ml. Various activators containing tissue factor and phospholipids at different concentration were used to generate thrombin. The generation of thrombin was measured using florescence, which was proportional to the amount of thrombin generated. The generic argatroban preparations represented slovastan, gartban, and argaron. Branded argatroban in comparison to generic products showed significant differences in the inhibition of the generated thrombin. In various activation systems the order of inhibitory potency was argatroban> gartban> slovastan in the PPP system. All of the generic products showed the stronger thrombin generation inhibition in the PPP in the comparison to branded argatroban. On the other hand in the PRP systems there was a much wider difference among the generic argatrobans and order of potency was slovastan> gartban> argaron. The results obtained the generic argatroban were comparable to branded argatroban. Interestingly in the fibrinokinetic assays wider differences were noted between generic and branded products. The rank order was gartban> slovastan > argaran> branded argatroban. These observations underscore the differences between the generic and branded argatroban and suggest that beside thrombin inhibitory assays argatroban can be differentiated in other biologic assays.

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DABIGATRAN INHIBITS STAPHYLOCCUS AUREUS COAGULASE

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Keywords: staphylocoagulase, thrombin inhibitor, dabigatran

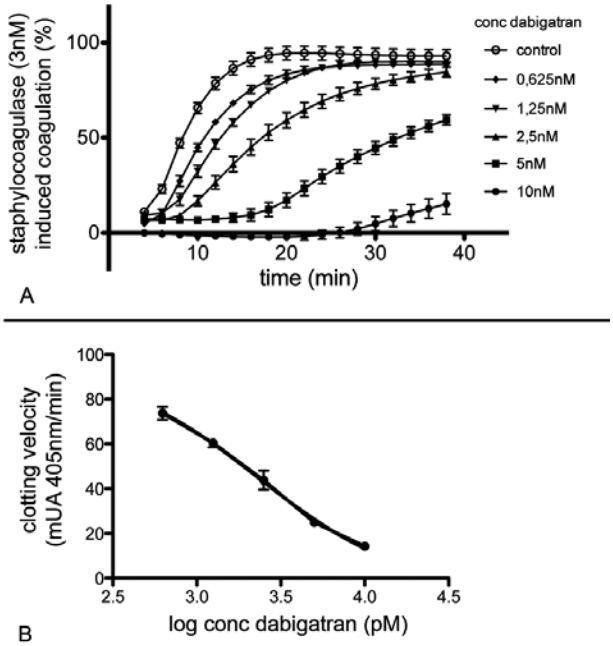
Background/Aims: Staphylocoagulase directly binds and activates thrombin, bypassing the physiological coagulation cascade. Detection of coagulase activity of staphylococci is routinely used to distinguish S. Aureus from less pathogenic coagulase negative staphylococci, but the role of coagulase in staphylococcal infective potential remains unclear. We investigated if the new direct thrombin inhibitor (DTI) dabigatran inhibits staphylocoagulase and if the expanding clinical use of DTI's could potentially influence routine laboratory staphylococcal classification.

Coagulation of citrated human plasma by purified coagulase (3nM) in the presence of varying concentrations of dabigatran was measured by monitoring optical density. We compared the pharmacokinetics of the inhibition of alpha-thrombin and of a mixture of staphylocoagulase and prothrombin by dabigatran using the conversion rate of the S-2238 chromogene substrate. We added varying concentrations of dabigatran to citrated rabbit plasma routinely used as coagulase tube test reagent and assessed clotting 2, 6 and 24 hours after addition of one colony of 5 different clinical strains of S. Aureus.

Results: Dabigatran inhibited coagulation of human plasma induced by staphylocoagulase (3nM) in a concentration-dependent way with an IC50 of 2,3nM (95% CI 1,2 - 4,5nM). Dabigatran inhibited amidase-activity of staphylothrombin (Ki 8,6 ± 1,3 nM) and alpha-thrombin (Ki 10,9 ± 1,6 nM) to a comparable extent. At a concentration of 10nM, dabigatran delayed positive tube testing from 2 to 6 hours in 4 out of 5 cases, and the presence of 100nM dabigatran delayed tube test positivity beyond 6 hours in 1 out of 5 tube tests.

Conclusions: Dabigatran inhibits staphylocoagulase-induced clotting of human as well as rabbit plasma. A pharmacological coagulase-inhibitor could facilitate further research on the controversial role of staphylocoagulase as a virulence factor in staphylococcal infections. Since rabbit plasma is routinely used for staphylococcal differentiation, anticoagulation carryover could potentially delay tube testing in patients with S. Aureus bacteremia, but this requires further prospective evaluation.

Figure Dabigatran inhibits staphylocoagulase induced clotting of human plasma (A) with IC50 of 2,3 nM at coagulase-concentration of 3nm (B).



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EGFP-EGF1 PROTEIN-CONJUGATED PEG-PLA NANOPARTICLES AS A NOVEL DRUG DELIVERY SYSTEM TARGETING TISSUE FACTOR

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Keywords: antithrombotic, nano-targeting, tissue factor

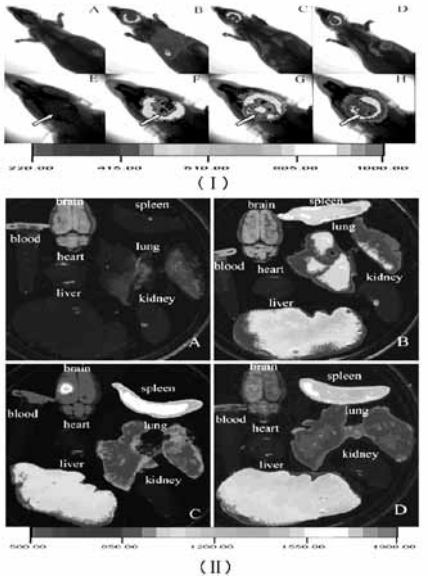
Background: *Nano-targeting* is an innovative strategy for anti-thrombotic therapy. It is generally accepted that the initial event in thrombus formation is the exposure of cell-surface protein—tissue factor (TF). Recent evidences supported the roles of the EGF1 domain peptide of FVIIa in TF binding and the TF/FVIIa complex formation. These intriguing results inspired us to develop a novel anti-thrombotic drug delivery system, EGFP-EGF1 conjugated nanoparticles—EGFP-EGF1-NP, which has specific affinity to TF via its natural ligand—EGF1 and nanoparticles made of biodegradable polymers can serve as an ideal carrier for therapeutic agents.

Materials and methods: EGFP-EGF1 fusion protein was expressed in E.coli BL21 cells, and then the purified protein was thiolated and conjugated to the malemide covering on the pegylated nanoparticles to form the EGFP-EGF1-NP. The EGFP-EGF1-NP was characterized in terms of morphology and size. Targeting feature of EGFP-EGF1-NP was qualitatively and quantitatively evaluated by combining EGFP-EGF1-NP with the TF-expressing cells *in vitro*, and its *in vivo* localization in rat tissues was examined by using *in-vivo* multispectral fluorescent imaging. The *in vitro* cytotoxicity of the nanoparticle system was also investigated.

Results: The SDS-PAGE showed 36 kDa fusion protein EGFP-EGF1 was highly expressed. TEM photograph confirmed that EGFP-EGF1 protein was conjugated to the surface of PEG-PLA nanoparticles and EGFP-EGF1-NPs were generally spherical and of regular shape, the size of EGFP-EGF1-NP was no more than 110 nm, which is believed to be favorable to drug transport. The resulting nanoparticle system exhibited minimal cytoxicity. The EGFP-EGF1–NP showed significantly higher binding ability with TF-expressing cells than EGFP-NP, due to the targeting process of TF induced by EGF1. *In vivo* multispectral fluorescent imaging demonstrated that EGFP-EGF1-NP had high specificity and sensitivity in targeting thrombi **(Figure)**.

Conclusions: Our study demonstrated that EGFP-EGF1-NP is a promising TF-targeting drug delivery system for thrombolytic treatment.

Figure Distribution of EGFP-EGF1-NP and EGFP-NP *in vivo*



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FIRST HUMAN STUDY WITH EP217609, A NEW SYNTHETIC PARENTERAL NEUTRALIZABLE DUAL ACTION ANTICOAGULANT

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Keywords: anti-Xa, thrombin inhibitor, anticoagulant

EP217609 is a new synthetic parenteral dual action anticoagulant combining an indirect factor Xa inhibitor (antithrombin binding pentasaccharide) and a direct thrombin active site inhibitor (peptidomimetic). In addition, EP217609 can be neutralized by avidin, an egg-derived glycoprotein, which binds with high affinity and specificity to the biotin moiety of EP217609.
This phase I study assessed the pharmacokinetics (PK), pharmacodynamics (PD), safety and tolerability of a single intravenous bolus of EP217609 (1, 3, 10 mg) and an equimolar dose of EP42675 (2.6 mg) in 40 healthy male subjects (8 active and 2 placebo per group). EP42675 is the non-biotinylated form of EP217609, and has completed Phase I.
Plasma and urinary concentrations were measured by anti-Xa and anti-IIa specific bioassays. PD was assessed by activated clotting time (ACT), thromboelastometry, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), ecarin clotting time (ECT), and thrombin generation test (TGT). The EP217609 PK showed a dose-dependent increase in exposure. EP217609 was partially eliminated by the kidney as unchanged drug. There was a dose-dependent increase in ACT, TT, ECT, PT, aPTT and TGT lag time. Maximum anticoagulant effect was reached within 5 minutes after bolus injection and lasted for up to 3 days. The PK and PD profiles of 3 mg of EP217609 and 2.6 mg of EP42675 were similar. EP217609 was well tolerated and no change in liver function tests was observed over the 10-day study period. EP217609 showed a predictable PK/PD profile with a low inter-subject variability.

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REVERSIBLE ACTIONS OF ANKAFERD ON EPCR AND PAI-1 INSIDE VASCULAR ENDOTHELIAL CELLS WITH AND WITHOUT LPS

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Keywords: ABS, EPCR, PAI-1,HUVEC

Backround/Aims: Ankaferd Blood Stopper (ABS), historically used as a hemostatic agent in Turkey, comprises a mixture of the plants Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica. Endothelial Protein C Receptor (EPCR) is involved in hemostatic, vascular and immunological actions. Likewise, plasminogen activator inhibitor-1 (PAI-1) is an important biological mediator for fibrinolysis, infection, obesity and wound healing. The aim of this study was to investigate the intracellular effects of ABS on endothelium and immune response investigating possible changes in EPCR and PAI-1 without and with LPS-challenge.
Methods: 10µL and 100µL ABS is given to HUVECs in 5minute (min.), 25min., and 50min. time periods with and without 10µg/mL lipopolysaccharide (LPS) for one hour to observe ABS-effects on HUVECs. Total RNAs were isolated from HUVECs and EPCR ve PAI-1 mRNA expression levels were investigated with qPCR.
Results: It was microscopically observed that cells arose from surface and adhered to each other after the ABS application to the HUVECs. Also, after 24 hours cells returned the normal growth and physiology. It suggests that the adhesive cellular functions of ABS may be reversible. 10µl ABS has negative effect on EPCR expression and it decreases PAI-1 expression. Moreover, the effects increases with 100µl ABS. EPCR and PAI-1 expression increased by time with LPS and 10µl ABS. Expressions were very low during the first hour when LPS and 100µl ABS were given but at the end of 24 hour, EPCR and PAI-1 expression increased similar to LPS and 10µl ABS experiment.
Discussion: ABS affected EPCR and PAI-1 expressions without and with LPS-challenge inside HUVECs.
Conclusions: We observed that Ankaferd has reversible actions depend on dose and concentration on EPCR and PAI-1 inside vascular endothelial cells in the model of HUVEC. ABS might have a role on several cell mechanisms like coagulation.

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COMPARISON OF THE EFFECT OF EDOXABAN AND FONDAPARINUX ON THE INHIBITION OF THROMBIN GENERATION (*IN-VITRO* STUDY)

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Keywords: edoxaban, fondaparinux, thrombin generation

Background/Aims: Edoxaban is a new oral direct inhibitor of Factor Xa (FXa) in clinical development for prevention and treatment of thromboembolic disorders. Fondaparinux is an approved indirect FXa inhibitor. The objective of the study is to compare the effect of edoxaban (direct anti-FXa) to fondaparinux (indirect anti-FXa), on thrombin generation (TG) using the calibrated automated thrombogram (CAT). The studied parameters include lag time (LT), time to peak (TTP), endogenous thrombin potential (ETP), Peak and mean velocity rate index (mVRI).
Pooled citrated platelet poor plasma (PPP) from healthy subjects (PNP, Cryopep, France) was spiked with increasing concentrations of edoxaban (up to 3,6µM) or fondaparinux (up to 1,2µM). TG was measured by the CAT method using 5 pM of tissue factor and 4 µM of phospholipids (Normal PPP reagent, Stago, France). The experiments were repeated on 6 different days.
Results: Fondaparinux had a similar effect on ETP, LT, and TTP but twice as active on Peak and mVRI. Edoxaban had a similar effect across TG parameters, but demonstrated less activity on ETP. Based on molar concentrations, edoxaban was about 3-fold more active than fondaparinux across TG parameters with the exception of ETP (**Table**). Steeper slopes of concentration-effect curves for Peak and mVRI were observed for edoxaban versus fondaparinux. Across both drugs, LT and TTP were less reproducible than Peak which appears to be a good parameter for comparison with an acceptable coefficient of variation.
Conclusions: Edoxaban and fondaparinux demonstrated concentration dependent inhibition of TG by CAT method. Edoxaban, on a molar basis, was more active than fondaparinux with the exception of ETP. This difference may be related to the broader inhibition activity of edoxaban compared to fondaparinux which does not inhibit FXa complexed with FVa on phospholipids. Peak and mVRI appear to be the most sensitive parameters to edoxaban.

Experiments conducted on 6 different days with PPP	Concentrations needed to:				
	Double time		Inhibit 50%		
	LT	TTP	ETP	Peak	mVRI
Edoxaban (µM)	0,16 ± 0,04	0,13 ± 0,05	0,84 ± 0,32	0,07 ± 0,02	0,03 ± 0,01
Fondaparinux (µM)	0,58 ± 0,31	0,52 ± 0,18	0,49 ± 0,07	0,24 ± 0,03	0,18 ± 0,03

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A NOVEL SULFATED LOW MOLECULAR WEIGHT LIGNIN INHIBITS THROMBIN IN A SPECIFIC AND ALLOSTERIC MANNER TO INDUCE POTENT ANTITHROMBOTIC EFFECT

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Keywords: anticoagulants, allosteric inhibition, drug discovery

Thrombotic disorders afflict a large number of people. Thrombotic disorders are also 3-fold more likely in people with cancer. Anticoagulants are the mainstay of treatment and prevention of thromboembolic disorders. Yet, current anticoagulation therapy (heparins, coumarins and hirudins) is beset with a large number of adverse reactions. We reasoned that to reduce the problems associated with the current therapy, molecules radically different from all the current agents should be discovered. Our line of search has led to the discovery of chemo-enzymatically synthesized low molecular weight lignins (LMWLs), which possess extremely interesting anticoagulant properties and a novel mechanism of action. Sulfated LMWLs prolong prothrombin time and activated partial thromboplastin time at concentrations similar to low molecular weight heparins (LMWHs). Whole blood clotting studies using thromboelastography and hemostasis analysis system also reveal high potency of our new anticoagulants. Yet, the anticoagulant action of LMWLs is dramatically different from that of LMWHs. Wheras LMWHs are indirect inhibitors of coagulation enzymes, the new anticoagulants inhibit these enzymes in a direct manner. Direct inhibition arises from an allosteric disruption of enzyme's catalytic apparatus. Whereas majority of sulfated LMWLs potently inhibit thrombin, factor Xa, factor IXa and factor XIa, one designed sulphated LMWL specifically inhibits thrombin with an IC50 value of 10-20 nM. Competitive binding studies show that the sulfated LMWLs interact with exosite II of thrombin, a site not typically known to be associated with inhibition. Studies using A549 lung and HepG2 liver cell lines show no induction of toxicity by the sulfated DHPs at concentrations as high as 50 mg/L. In summary, the chemically synthesized LMWLs possessing novel allosteric mechanism of inhibition are radically different structures from all the known anticoagulants and are likely to yield new agent for antithrombotic use.

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COMPARISON OF RIVAROXABAN IMPACT ON THROMBIN GENERATION AND WHOLE BLOOD THROMBOELASTOMETRY

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Background/Aims: Routine monitoring of rivaroxaban, a specific direct factor Xa inhibitor, is not required. However it might be necessary in specific groups of patients. We evaluated the pertinence of thrombin generation (TG) test and minimal tissue factor triggered thromboelastometry (minTF-TEM) to detect rivaroxaban antithrombotic effect.
Materials and methods: Normal citrated plasma was pooled from healthy volunteers (n=5) and spiked with rivaroxaban at different concentrations (0, 41, 219 and 430 ng/ml). TG was assessed with Calibrated Automated Thrombogram® (Stago, France) in platelet poor plasma (PPP) using PPP-reagent, Platelet Rich Plasma (PRP), Innovin (1/200). The following parameters were analysed: lagtime, time to peak (ttPeak), Peak, endogenous thrombin potential (ETP) and mean rate index (MRI). minTF-TEM was performed using Rotem® instrument (TEM htl. Gmbh, Munich, Germany). Whole blood was mixed with diluted thromboplastin (Innovin) and CaCl2 0.2 M. Parameters analysed were: clotting time (CT), clot formation time (CFT), α angle and maximum clot firmness (MCF).
Results: A rivaroxaban concentration of 41 ng/ml was sufficient to double lag-time, ttPeak and to reduce by half the peak in PPP. However, a greater concentration (219 ng/ml) was required for the same effect in PRP. In whole blood, the same concentration of rivaroxaban was required for doubling CT. Only a discrete reduction of α-angle (10%) and MCF (5%) was observed with the highest studied concentration (430 ng/ml).
Conclusions: TG and minTF-TEM are sensitive assays to detect rivaroxaban antithrombotic effect at prophylactic or therapeutic concentrations. Greater concentrations in PRP are required to reach the similar TG inhibition obtained in PPP. ETP is the most accurate TG parameter. The rivaroxaban inhibitory effect on TG kinetics in PRP was comparable to that observed in minTF-TEM. The clinical relevance of these data is being evaluated.

Table: Rivaroxaban concentrations (ng/ml) inhibiting PPP and PRP TG test and whole blood MinTF-TEM

TG parameters					
	2-fold lagtime	2-fold ttPeak	IC50 ETP	IC50 Peak	IC50 MRI
PPP	41	41	219	41	25
PRP	219	219	430	219	125
MinTF-TEM parameters					
whole blood	2-fold CT	1.25-fold CFT	90% α	95% MCF	
	219	219	430	430	

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ENHANCED SUPRESSION OF THROMBIN GENERATION BY RIVAROXABAN COMPARED TO FRAGMIN AFTER HIGH RISK ORTHOPAEDIC PROCEDURES

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Total hip/knee replacement surgeries are associated with an increased risk of venous thromboembolism, consequently post-operative thromboprophylaxis is now standard treatment. This study was undertaken to: (1) assess the impact of elective hip/knee replacement surgery on thrombin generation (TG) test, (FVIII, D-dimer, fibrinogen and VWF:Ag levels; (2) compare the prophylactic anticoagulant effect of fragmin and rivaroxaban on TG test, 24 hours after surgery. All haemostatic variables were assessed in plasma samples from 50 patients taken: immediately pre-operatively; peri-operatively at the time of joint insertion; and 24 hours post-operatively. Prophylaxis, once a day, with fragmin (5000 units s.c.) or rivaroxaban (10mg orally), starting 6-8 hours post-operatively, was administered in 24 (13 knees, 11 hips) and 26 patients (13 knees, 13 hips) respectively. TG parameters (expressed as median and IQR) increased from pre-operative to peri-operative period (ETP pre- 1275nM.min [1070-1511] vs. peri- 1403 [1218-1573], p=0.001; peak thrombin pre-150nM [124-175] vs. peri- 194nM [164-220], p<0.0001). Patients who received Rivaroxaban showed a greater reduction in TG between peri-operative and post-operative periods than those on fragmin (rivaroxaban: ETP peri- 1466nM.min [1260-1601] vs. post- 1100nM.min [867-1324], p<0.0001; peak thrombin peri- 197nM [167-208] vs. post- 82nM [56-99], p<0.0001; Fragmin: ETP peri- 1345nM.min [1172-1431] vs. post- 1209nM.min [904-1414], p=0.03 and peak thrombin peri- 180nM [155-232] vs. post- 172nM [128-224], p=0.41). FVIII, fibrinogen and VWF:Ag decreased during surgery (~25%) and increased 24 hrs after (~35% FVIII and VWF-Ag and 9.5% fibrinogen). D-dimer increased during and after surgery (pre- 0.7 vs. peri-1.2 vs. post- 2.9 [mg/L FEU], p<0.0001). There were no post-operative clinical bleeding episodes, however one patient in the fragmin group developed deep vein thrombosis. During the peri-operative period of hip/knee replacement surgery there are increases in TG, D-dimer and consumption of FVIII, fibrinogen and VWF:Ag. rivaroxaban inhibits TG more effectively than fragmin 24 hrs after surgery.

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ENOXAPARIN DOES NOT DECREASE TOTAL MICROPARTICLES TISSUE FACTOR ACTIVITY IN AN EXPERIMENTAL MODEL OF EARLY VENOUS THROMBOSIS IN MICE

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Keywords: enoxaparin, microparticles, experimental surgery

Background/Aims: Microparticles (MPs) are small fragments of cell membrane shed from platelets, leukocytes and endothelial cells. They are rich in tissue factor (TF) and other pro-coagulant factors, facilitating and magnifying coagulation in the presence of thrombus. In our model of Inferior vena cava (IVC) ligation in mice, we evaluated MP associated tissue factor activity (MPTFA) after injection of a low-molecular-weight heparin (LMWH) enoxaparin or saline after the initiation of venous thrombosis.
Materials and methods: IVC ligation was performed on C57BL/6 mice (n =40). Animals were euthanized on day 2, three hours after single drug (6mg/Kg of enoxaparin sq, dose chosen based on basal metabolism of mice) or saline injection to assess MP (200ul/PPP) production and MPTFA, quantitated using a chromogenic assay (pM). Another group of mice (n=40) underwent the same IVC ligation and received either enoxaparin or saline on daily basis (multiple doses). After sacrifice on day 2, MP were extracted and MPTFA and MP Tissue Factor Pathway Inhibitor Activity (MPTFPIA) evaluated. Results were expressed on ratios (r) of enoxaparin/ saline outcome.
Results: Enoxaparin reduced MPs (r=0.59, p<0.05), but did not decrease MPTFA (r=1.44 single dose, r=1.40 multiple dose) or increase MPTFPIA (r=1.0), in the concentration of 160,000 MP (200 ul/ PPP). With different MP concentrations, from 80,000 to 10,000, a strong negative correlation between MPTFA and MPTFPIA was observed (R=-0.95).
Conclusions: Enoxaparin reduced total MP numbers but did not decrease MPTFA or increase MPTFPIA in a concentration of 160.000 MP (200 ul/ PPP). It appears to modulate TF and TFPI activity on MP, with possible impact in venous thrombogenesis.

Table: Single and Multiple doses of enoxaparin/ saline total MP, MPTFA and MPTFPIA ratios

Enoxaparin/ Saline Ratio	Total MP ratio	MPTFA 160,000 MP 200ul/PPP	MPTFPIA 160,000 MP 200ul/PPP	p - value
Single dose	0.59 *	1.44	-	p<0.05 *
Multiple dose	-	1.40	1.0 *	NS *

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DIFFERENT CHARACTERISTICS OF DIRECT FACTOR XA INHIBITORS: IN VITRO COMPARATIVE STUDIES OF RIVAROXABAN AND APIXABAN

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Keywords: rivaroxaban, apixaban, clotting times

Background/Aims: Rivaroxaban and apixaban are selective, reversible, structurally different, direct factor Xa inhibitors in late-stage clinical development for the prevention and treatment of venous and arterial thrombosis. Animal studies have demonstrated venous and arterial antithrombotic efficacy with these agents. This study characterizes and compares these agents in in vitro functional assays.
Methods: factor Xa activity and rate constants (kon/koff) were assessed by measuring the amidolytic activity of purified factor Xa. Prothrombinase activity was measured in a reconstituted prothrombinase complex with prothrombin as substrate and measuring the amidolytic activity of the generated thrombin. Clotting times and thrombin generation (TG) were measured using commercially available kits. Tissue factor (TF)-mediated platelet aggregation was measured in defibrinated plasma.
Results: Rivaroxaban and apixaban showed similar characteristics: affinity for free factor Xa (Ki 0.4 nM and 0.6 nM, respectively); association (kon 1.7 x 107 M–1 s–1and 0.88 x 107 M–1 s–1, respectively) and dissociation (koff 5 x 10–3 s–1 and 2.4 x 10–3 s–1, respectively) rate constants; and inhibition of prothrombinase-bound factor Xa (IC50 2.1 nM and 2.7 nM, respectively). However, in human plasma-based systems, these agents showed different potencies. Although similar plasma protein binding has been reported for apixaban and rivaroxaban (87% and 92–95%, respectively), higher molar concentrations of apixaban were required, compared with rivaroxaban, to inhibit TG (IC50, peak TG 0.20 μM and 0.06 μM; endogenous thrombin potential 4.96 μM and 1.48 μM, respectively) and for TF-mediated platelet aggregation (IC50 0.51 μM and 0.06 μM, respectively). In addition, the concentrations needed to double clotting times in different assays were three- to eightfold higher for apixaban than for rivaroxaban.
Conclusions: Although they have a similar affinity to factor Xa, structurally different factor Xa inhibitors may differ in their antihaemostatic potency.

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EVIDENCE THAT CURRENT REVERSIBLE THROMBIN INHIBITORS SHOW PLEIOTROPIC INHIBITORY EFFECTS ESPECIALLY FOR THE INTRINSIC ROUTE OF COAGULATION ACTIVATION

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Keywords: reversible thrombin inhibitors, factor XII, pleitropy

Background/Aims: Current evidence suggests that low potency of the human factor XII system is related to risk of venous thromboembolism (VTE). The C46T promoter polymorphism coding for low factor XII is overrepresented in patient groups with VTE. Current medication with reversible thrombin inhibitors (rDTIs) shows in the therapeutic range an inhibition in the APTT which is much stronger than for the PT. We asked the question whether pleiotropic effects underlie this observation since the inhibitors are used at levels around 100x the inhibition constant for thrombin.
Methods: We developed two chromogenic factor Xa generation tests using either extrinsic (eXa-test) or intrinsic (iXa-test) activation. Excess hirudin excluded effects of thrombin. We validated this strategy in prothrombin deficient plasma.
Results: In the eXa-test we observed pleiotropic effects, but at concentrations above the therapeutic range and with the order of potency of argatroban> melagatran> dabigatran, similar to the order of effects in the prothrombin time test. Both inhibition of factor Xa and factor VIIa were involved. In the iXa-test we observed a delay in factor Xa formation in the therapeutic plasma range with the potency order of dabigatran> melagatran > argatroban similar to the sequence in the APTT. Detailed analysis shows the effect to involve different specific coagulation factors in the chain factor XII, kallikrein, XI and IX, for each compound. The magnitude of the effect was comparable to that of rivaroxaban in its therapeutic plasma range.
Conclusions: We demonstrated clear pleiotropic effects of rDTIs, focussed on the intrinsic route of coagulation. The question is whether this partial inhibition of the factor XII-contact route resembles reduced factor XII levels and carries a component of VTE risk, or influences the benefit/risk ratio otherwise. Clinical evaluation of rDTIs not exerting effects on intrinsic clotting may provide further answers in the future.

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MONITORING DIRECT AND INDIRECT FACTOR XA INHIBITORS WITH A NEW LIQUID HEPARIN ASSAY

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Keywords: heparin, monitoring, LMWH

Background: Monitoring heparin activity in patients under treatment is critical in keeping the patient risk-free from bleeding or thromboembolism. HemosIL Liquid Heparin (LH) (Instrumentation Laboratory, NY) has proven to be a very convenient and accurate assay for monitoring both unfractionated and different types of low -molecular-weight heparins in patients. This kit is a ready to use liquid-based, Xa-dependent chromogenic assay, with no external antithrombin. In conjunction with HemosIL Heparin Calibrators (Instrumentation Laboratory, NY), it allows rapid simultaneous detection of different commercial heparins in a wide activity range (0-2.0 IU/mL) using a single calibration curve. Currently, a number of new anticoagulant drugs are emerging, and a simple and straightforward procedure for their detection will be extremely useful for monitoring patients. While being potentially useful for detection of both, indirect and direct factor Xa inhibitors, HemosIL LH Assay is reporting anti-FXa activity in international units (IU) of heparin per 1 mL of plasma, although some drugs are required to be measured in drug-specific units. To address that, we report here a simple procedure for adapting the LH Assay to measure various FXa inhibitors in plasma samples in drug-specific units using HemosIL LH Kit and HemosIL Heparin Calibrators.
Methods: The performance of this procedure was evaluated on different ACL analyzer platforms. Instruments were calibrated using HemosIL Heparin Calibrators and HemosIL LH Kit. Normal pooled plasma was spiked with different concentrations of the drug, and anti-FXa activity in spiked samples was measured using the HemosIL LH Assay. The applicability of the LH Assay was evaluated for each drug by correlation between the test results and drug dilution levels. The ratio between obtained IU and drug-specific units were also calculated. The mean ratio of at least three concentration points in the assay range was used as a conversion factor. Obtained conversion factor was then incorporated into a copy of the LH test. This test was either calibrated separately using drug-specific spiked calibrators, or calibration curve was imported from the original test. Method comparison was performed to compare recovery of the spiked and clinical samples of assayed drug using the modified application versus the recovery obtained with the LH test, calibrated with spiked drug-specific calibrator.
Results: The conversion factor for each assayed drug, including fondaparinux and Orgaran, was determined and incorporated into a copy of LH test. Obtained recovery values of spiked samples were within 10% of the target values. Method comparison using spiked and patient samples revealed close correlation of recovery results obtained with drug-specific calibration curve and with imported HemosIL Calibrator calibration curve with conversion factor.
Conclusions: We developed a simple procedure for adopting HemosIL LH Test to determine FXa inhibitor drugs in patient samples using HemosIL Heparin Calibrator calibration curve on ACL analyzers. This procedure includes establishing the relationship between heparin IU and drug-specific units, followed by slight modification of the LH Test.

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EFFECT OF RIVAROXABAN ON CLOTTING ASSAYS FOR COAGULATION FACTORS MEASUREMENT

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Background/Aims: In contrast to antithrombin-dependent specific inhibitors (i.e. fondaparinux), rivaroxaban, a direct specific FXa inhibitor, prolongs both prothrombin time (PT) and activated partial thromboplastin time (aPTT) in a concentration-dependent manner. We evaluated the rivaroxaban influence on coagulation factors dosage based on PT and aPTT determinations.
Materials and methods: Normal platelet poor plasma (PPP) was obtained from healthy volunteers (n=5). PT, aPTT and clotting factor activities were determined using conventionally assays on STA-R II instrument (Stago, France). aPTT reagent was from trinity biotech plc, Ireland. Thromborel S and clotting factor depleted plasmas were from Siemens Healthcare (Marburg, Germany). Clotting tests were performed in control and spiked plasmas with increased rivaroxaban concentrations (0, 41, 219 and 430ng/ml). Clotting factor determinations were performed in plasma diluted in Owren Buffer.
Results: Spiking normal plasma with increased quantities of rivaroxaban induced a significant concentration-dependent decrease of all clotting factors rates. At low PPP dilution (1/10) the differences between the observed and expected levels of clotting factors were significantly higher for FXII, FXI, FIX and FVIII compared to FVII, FX, FV and FII ones. At high PPP dilution (1/80), this difference was less than 5% for PT-based clotting assays but remained over 20 % for aPTT-based clotting assays.
Conclusions: Rivaroxaban induces a significant underestimation of coagulation factors activities when assessed by PT- and aPTT-based clotting assays. This effect is partially reversed by plasma dilution up to 1/80. This impact should be taken into account when clotting factors dosage is needed in rivaroxaban treated patients.

Table: Difference between observed and expected activities of clotting factors induced by increasing rivaroxaban concentrations. Ratio expressed in % for a low PPP dilution:(1/10)

	Rivaroxaban concentration ng/ml		
	41	219	430
FVIII%	24	41	52
FIX%	23	45	60
FXI%	22	42	59
FXII%	19	33	43
FII%	11	17	21
FV%	13	24	40
FVII%	10	17	24
FX%	6	18	32

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KNOWLEDGEMENT OF LACKING REVERSAL ANTICOAGULATION PRODUCED BY THE NEW ORAL ANTICOAGULANTS, CONDITION PATIENT PREFERENCES IN LONG TERM ANTICOAGULANT TREATMENT

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Keywords: dabigatran, warfarin, bleeding

Background/Aims: No difference in bleeding episodes, in relation to warfarin, was found in new oral anticoagulants (NOA) trials. Despite this, oral anticoagulation reversal is a common clinical situation in real life: accidental overdose, dramatic significant bleeding, head trauma, bleeding in surgical site, etc. From a literature review, looking for clinical evidence in handling these episodes when NOA were used, we assess the patient's decision when they know this information and have experience with vitamin K Antagonist (VKA) treatment.
Methods: In a simulated clinical trial comparing VKA with NOA, we have attempted to recruit patients receiving VKA for at least 6 months.
Results: 1. Literature review: No guidelines for bleeding reversal were reported in 9 clinical trials of these drugs, despite finding 435 and 1116 major or significant bleeding episodes, from 24854 treated patients. Several drugs, blood derived products or dialysis are proposed for anticoagulation reversion produced by NOA, based on drug pharmacokinetics, in vitro, animal or ex-vivo experimentation. recombinant factor VIIa did not reverse the anticoagulant effects of melagatran in healthy subjects. 2. Simulated Clinical Trial: Eighty-three patients were contacted. Everyone was informed that there was no antidote not even experience to reverse the effect of NOA. Five had a previous history of clinical significant (4) or major bleeding (1) episode, all of them besides other 35 patients (48 %) recognized that this circumstance was the main reason for declining to participate, but only16 patients (19 %) agree to participate.
Conclusions: There is no clinical evidence for reverting action of the NOA. NOA discontinuation, until the drug anticoagulant effect disappears, replacing the lost blood if necessary, is the only solution provided. The patient preference evaluating this risk/comfort benefit, after an understandable explanation of this circumstance, especially if they have no experience with anticoagulant treatment, is essential before drug prescription.

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THE ULTRA-LOW-MOLECULAR-WEIGHT HEPARIN (ULMWH) SEMULOPARIN FOR PREVENTION OF VENOUS THROMBOEMBOLISM (VTE) AFTER HIP FRACTURE SURGERY

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Background/Aims: Semuloparin is a new ULMWH under development for VTE prevention in cancer patients, and also in patients undergoing major abdominal or orthopaedic surgery. This study aimed to compare the efficacy and safety of semuloparin versus enoxaparin after hip fracture surgery.
Materials and methods: In this multinational, double-blind, double-dummy study, patients were randomised to receive semuloparin 20 mg or enoxaparin 40 mg once-daily for 7–10 days. Bilateral ascending contrast venography was performed between day 7 and 11. Symptomatic VTE and death were recorded.
Results: Of 1003 patients randomised, 753 (75%) were evaluable for the primary efficacy analysis. The occurrence of the primary efficacy endpoint of any VTE and all-cause death was lower in the semuloparin group than in the enoxaparin group, but the difference did not reach statistical significance (**Table**). The rates of major and non-major bleeding were slightly higher in the semuloparin group (**Table**). No case of drug-induced liver injury was identified. Thrombocytopenia rates were similar in the 2 groups.
Conclusions: In patients undergoing hip fracture surgery, trends in favor of semuloparin 20 mg once-daily for the incidence of VTE and death, and in favor of enoxaparin 40 mg once-daily for the incidence of bleeding events were observed.

Table

Endpoints	Semuloparin % (n/N)	Enoxaparin % (n/N)	Odds ratio (95% exact confidence interval)
VTE or all-cause death	**17.7** (68/384)	**22.0** (81/369)	0.77 (0.53–1.12)
Major bleeding	**1.0** (5/488)	**0.6** (3/499)	1.71 (0.39–8.73)
Clinically-relevant non-major bleeding	**1.0** (5/488)	**0.2** (1/499)	5.16 (0.71–122.9)
Any clinically-relevant bleeding	**2.0** (10/488)	**0.8** (4/499)	2.59 (0.83-9.57)

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Fibrinolysis and thrombolysis

Thursday 8th July, 2010

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THE ROLE OF THE G455A POLYMORPHISM ON FIBRINOGEN B-CHAIN GENE IN ADVANCED ATHEROSCLEROSIS: EFFECTS ON FIBRINOGEN, D-DIMERS AND PLASMINOGEN LEVELS

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Background: Genetic polymorphism G455A on fibrinogen β-chain gene has been associated with increased fibrinogen levels in healthy individuals. However, the impact of this polymorphism on the coagulation cascade in patients with coronary artery disease (CAD) is unknown. In the present study we examined the impact of this polymorphism on fibrinogen levels, D-dimers levels and plasminogen levels.
Methods: The study population consisted of 398 subjects, 253 of which angiographically documented for CAD. The G455A polymorphism was detected by polymerase chain reaction (PCR) and appropriate restriction enzymes. Fibrinogen levels were measured by immunonephelometry, while plasminogen and D-dimers levels were measured by standard coagulometry techniques.
Results: The genotype distribution was GG: 51.4%, GA: 37.9% and AA: 10.7% for patients with CAD, while GG: 54.5%, GA: 35.8% and AA: 9.7% for controls. Patients with CAD had significantly higher fibrinogen levels (mg/dl) than controls (434.7±132.7 vs 384.7±103.7, p=0.0002). In addition, in patients with CAD fibrinogen levels were importantly higher in the 455AA homozygotes vs 455G carriers (561.6±127.3 vs 442.6±132.2, p<0.0001), while no similar difference occurred in controls (420.9±143.9 AA vs 380.6±98.5 GG+GA, p=NS).
Moreover, D-dimers levels (mg/L) were significantly higher in CAD patients than controls (415.02±201.9 vs 332.8±199.4, p<0.0001). However, no significant difference was observed for 455G carriers vs 455AA homozygotes for both CAD patients (394.8±189.6 vs 489.6±318.4, p=NS) and controls (368.1±286.9 vs 406.9±258.2, p=NS).
Finally, CAD patients and controls had no significant difference in plasminogen levels (u/ml) (119.2±79.7 vs 113.9±22.9, p=NS). Although, CAD 455AA homozygotes had higher plasminogen levels compared to 455G carriers (116.9±10.6 vs 109.5±17.2, p=NS) this difference did not reach any statistical significance, while similar results were observed in controls for 455AA homozygotes vs 455G carriers (116.7±15.9 vs 113.6±23.7, p=NS).
Conclusions: Our findings showed that genetic polymorphism G455A on fibrinogen β-chain gene strongly affects fibrinogen levels. However, this polymorphism fails to affect D-dimers levels as well as plasminogen levels in patients with stable coronary artery disease.

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PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) 4G/5G POLYMORPHISM AND ENDOMETRIAL CANCER. INFLUENCE OF PAI-1 POLYMORPHISM ON PAI-1 PROTEIN AND MRNA EXPRESSION

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Keywords: Fibrinolysis, PAI-1 polymorphism, endometrial cancer

Background/Aims: Plasminogen activator inhibitors-1 (PAI-1), the major inhibitor of the plasminogen activation system, inactivates plasminogen activators but also plays an important role in signal transduction, cell adherence, and migration. Changes in PAI-1 biosynthesis are usually preceded by changes in its gene transcription. We have previously reported that the PAI-1 4G/5G polymorphism seems to be associated with tissue PAI-1 levels and tumor severity in breast cancer. Moreover, high tissue PAI-1 levels have been related with increased risk of progression in endometrial cancer. The aim of the present study was to evaluate the PAI-1 4G/5G polymorphism in a group of women with and without endometrial cancer and to analyze the influence of this polymorphism in PAI-1 expression in endometrial tissue.
Materials and methods: In 508 women (155 patients with endometriosis and 353 controls) PAI-1 4G/5G polymorphism was determined by PCR amplification using allele-specific primers. Quantitative real-time RT-PCR assay was used to quantify PAI-1 mRNA and PAI-1 protein levels were quantified by ELISA.
Results: The frequencies of the PAI-1 4G allele and 4G4G genotype were significantly higher in patients than in controls (P=0.011 and P=0.010, respectively). The population was in Hardy-Weinberg equilibrium. Control women carrying the 4G4G genotype had higher endometrial PAI-1 mRNA (P=0.014) and protein (P=0.026) levels than those with the 5G/5G genotype. A significant increase in PAI-1 mRNA and protein was observed in endometrial cancer in comparison with the endometrial control tissue (P<0.001).
Conclusions: The frequency of the PAI-1 4G allele was significantly higher in women with endometrial cancer than in control women. PAI-1 levels in endometrial tissue seem to be associated with PAI-1 4G/5G polymorphism in controls. Further studies with a greater number of patients are needed to clarity the role of PAI-1 4G/5G polymorphism in endometrial cancer. (FIS PI080185, Red RECAVA RD06/0014/0004, Beca Fibrinolisis FETH 2009, Spain).

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ENDOVASCULAR THROMBOLYSIS IN ACUTE MESENTERIC VEIN THROMBOSIS. A 3-YEAR FOLLOW-UP WITH THE RATE OF SHORT AND LONG-TERM SEQUAELAE IN 32 PATIENTS

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Background: Mesenteric vein thrombosis (MVT) is a rare, often lethal, entity that accounts for 10-15% of all cases of mesenteric ischemia. Current indications for surgery in patients with acute MVT include peritonitis, bowel infarction, hemodynamic instability. In all other cases, anticoagulation is of choice. At variance with anticoagulation, thrombolysis leads to a rapid re-opening of vessels, with immediate tissue reperfusion.
Aims: To evaluate mortality and the development of portal hypertension in patients with MVT treated with percutaneous transhepatic thrombolysis and mechanical thrombectomy, and then warfarin, compared with warfarin alone.
Methods: We have prospectively followed-up for 3 years 32 patients with acute MVT documented by CT-scan. None of them had indications for surgical interventions.
After 1-week low-molecular-weight-heparin therapy (LMWH), 14 patients (controls) received warfarin (INR 2-3). The other 18 patients (treated group) underwent percutaneous-transhepatic thrombolysis and mechanical thrombectomy before starting warfarin. Mean age, sex ratio, risk factors, the American Society of Anesthesiologist score on admission, localization of thrombosis and the duration of symptoms were similar in both groups.
Results: 30-d mortality rate was similar in the two groups: because of co-morbid conditions (sepsis, pneumonia, myocardial infarction), 3 patients (16.6%) died in the treated group and 2 (14.2%) in the control group (p=0.998). Bowel resection was needed in 1 patient (5.5%) in the treated group (bleeding from a recent colo-rectal anatomosis) and in 5 patients (35.7%) in the control group (bowel ischemia with peritoneal signs) (p=0.022). A significant difference (p=0.043) was also found as to development of portal hypertension (7/14 patients in the control group, 50%; 2/18 in the group receiving thrombolysis, 11.1%).
Conclusions: In spite of the technical complexity and the bleeding risk, when administered promptly, percutaneous transhepatic thrombolysis and mechanical thrombectomy is a valuable mean of preventing bowel ischemia and long-term portal hypertension in patients with MVT.

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VENOUS THROMBUS RESOLUTION IS ACCELERATED BY TREATMENT WITH RNASE

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Background: Extracellular RNA is a procoagulant factor. Preventive administration of RNase has led to reduced thrombus sizes in arterial thrombosis induced by FeCl3 injury in rats. The aim of the present study was to investigate the therapeutic effect of RNase on venous thrombus resolution in a murine model of stagnant flow venous thrombosis.
Methods: Thrombosis was induced in the infrarenal vena cava of BALB/c mice (n=40, 18-20g, 6-8 weeks) by creating a venous stenosis with a silk suture. After ligation, mice were injected i.v daily with 100µL RNase-A (n=20; 0.5mg/mL) or saline (control; n=20) until sacrifice. Thrombi were harvested on days 3, 7, 14 and 28 after surgery for analysis (5 animals per time point).
Results: Thrombus cross-sectional area analysis demonstrated a significant decrease in thrombus area by day 7 after surgery in animals treated with RNase compared with controls. Immunohistochemical staining using antibodies against F4/80 for detecting thrombus macrophages, and isolectin B4 for detecting microvessels revealed an increased number of macrophages and microvessels in RNase–treated thrombi by day 7.
Conclusions: Administration of RNase leads to accelerated venous thrombus resolution. The data suggest that RNase may facilitate leukocyte transmigration and angiogenesis, which are crucial components of the venous thrombus resolution process.

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RELATION BETWEEN THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR AND HEMOSTATIC ALTERATIONS IN PATIENTS WITH CHRONIC LIVER DISEASE AND PORTAL VEIN THROMBOSIS

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Keywords: TAFI, CLD, PVT

Background: The thrombin activatable fibrinolysis inhibitor (TAFI) is a newly discovered inhibitor which down regulates fibrinolysis following its activation by thrombin.
Aims: To evaluate TAFI level and its relationship to some important hemostatic parameters in chronic liver disease (CLD) patients in a trial to clarify the role of TAFI in hemostatic alterations frequently encountered in CLD.
Patients and methods: The study included 35 CLD patients (15 Child B and 20 Child C), 15 patients out of them were complicated by portal vein thrombosis (PVT), in addition to 15 healthy controls. Prothrombin time (PT), partial thromboplastin time (PTT), TAFI, tissue factor (TF), prothrombin frgment 1+2 (PF1+2), thrombomodulin (TM), protein C (PC), protein S (PS), thrombus precursor protein (TpP) and D-dimer were assessed.
Results: PT and PTT were significantly prolonged in all CLD patients in comparison to controls. TF, PF1+2, TM, TpP and D-dimer were significantly increased while PC and PS were significantly decreased in CLD patients with and without PVT when compared to controls and also in PVT patients compared to patients without thrombosis. A significant reduction in TAFI level was detected in CLD patients with and without PVT in comparison to controls, however significantly higher values were noticed in patients complicated by PVT when compared to those without thrombosis. Correlation analysis demonstrated a strong correlation between TAFI and each of PT, PTT, PC, PS, and D-dimer in PVT group.
Conclusions: TAFI plays a crucial role in regulation of coagulation and fibrinolysis. Reduced TAFI level in patients with CLD could result in up regulation of fibrinolysis. High TF level, associated with decreased natural anticoagulants namely PC and PS accompanied by a higher TAFI level and its increased activation by thrombin could play a role in the development of PVT as a complication of CLD.

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P18

EFFICACY AND SAFETY OF RECOMBINANT STREPTOKINASE SUPPOSITORIES IN THE THROMBOSIS AND HEMORRHOIDAL FLUXION

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Keywords: recombinant streptokinase suppositories, hemorrhoidal crisis, clinical trials

Background/Aims: The hemorrhoidal disease constitutes a health problem, where a medical treatment could be beneficial before resorting to aggressive procedures. The recombinant streptokinase (SK) has shown favorable results in animal models of rectal inflammation and in humans. A phase II-III, multicenter, randomized, double blind, placebo controlled clinical trial was carried out to determine the efficacy and safety of SKr suppositories in the treatment of patients with hemorrhoidal fluxion and thrombosis.
Patients and methods: Eighty patients over 18 years who gave their consent to participate were included. They were randomly distributed in 4 treatment groups: I-Placebo, II-Sodium salicylate, III-SK 100 000 IU and IV-SK 200 000 IU per suppository. The corresponding product was administrated by the rectal route every 6 hours up to 4 administrations. The patients were hospitalized for 24 hours and the evaluations were carried out at 24 hours and at 3, 5 and 20 days after the inclusion. An adaptive design using a Bayesian sequential analysis was performed.
Results: The efficacy of the SK suppository (200 000 IU) was demonstrated for healing of the haemorrhoidal fluxion and thrombosis at the 5th day (37% significant difference with respect to placebo); time for healing was significantly shorter in this group IV (SK–200 000 IU) with respect to the rest of the groups. Likewise, response evaluation at the 5th day showed the superiority of the SK suppository (200 000 IU) with statistically significant differences with respect to the placebo, salicylate and SK–100 000 IU groups. In the internal and mixed haemorrhoids grades III and IV, the higher SK dose showed advantage of healing with respect to placebo (68% difference) and superiority with respect to placebo and to salicylate groups concerning total response (52 and 40% differences, respectively).
Conclusions: The Recombinant Streptokinase suppository was effective, safe and tolerable for healing of the haemorrhoidal crisis.

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P536

THE CORRELATION OF THE MUTATION STATUS PAI 1 4G/5G WITH PLASMATIC ACTIVITY PAI-1 AND TPA

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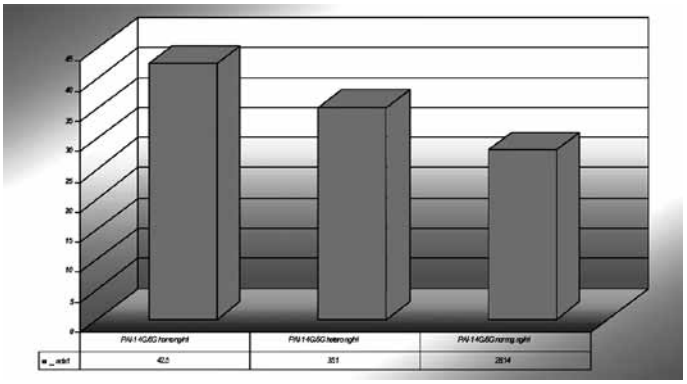
Background: The inhibitor of plasminogen activator I (PAI-1) is a glycoprotein with molecular weight of 50 kD. The molecule of PAI-1 is a member of serine protease inhibitors family (serpins) with homology as antithrombin III, PAI-2, alfa-2-antiplasmin, C1-inhibitor and alfa-1 protease inhibitor.
A common 4G/5G single nucleotide insertion/deletion polymorphism in the promoter region of PAI 1 gene is associated with higher genetic risk of arterial thrombosis. The patophysiology this mutation is in potentiation of gene expression for PAI-1 that are associated with a high plasma levels of PAI-1. The result of this process is a higher production of t-PA- PAI-1 complexes which leads to reduced activity of fibrinolytic system. This process causes a higher risk for atherosclerosis.
Materials and methods: The detection of PAI-1 mutation was perform with standard izolated DNA. PCR reaction with restriction by endonuclease was used. The cleavage of PCR product (98 pb or 99 pb) by endonuslease Bsl I provid specific fragments 77 pb for 5G allele and 98 for 4G allele. The levels of t-PA and PAI-1 were be detected by ELISA kits (Technoclone GmbH Austria).
Results: The levels of t-PA, PAI-1 and genetic variant PAI-1 4G/5G were detected in group of 71 patients. Although the homozygous form of PAI-1 mutation 4G/4G were of significantly higher plasmatic level of PAI-1, the plasmatic level of PAI-1 has high variability.
Conclusions: The genetic risk by mutation PAI-1 4G/5G represents only one of a number of factors that influence fibrinolytic potential. Above all in patients with acute manifestations of atherosclerosis the plasmatic level of PAI-1 minimally influenced by genetic mutation of PAI-1 gene and unambiguosly predominate the acquired factors. From this point of view is indispensable the combined detection of genetic variant PAI-1 it with the plasmatic levels PAI-1 and t-PA.

Acknowledgements: Supported by the Grant IGA of Min. of Health Czech Republic IGA NS 10319-3/2009 86-14 and NR 9282-3/2007 86-12.

Figure



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P623

EFFECTS OF THE HORMONES OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS ON SOME FACTORS OF THE FIBRINOLYTIC SYSTEM IN RATS

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Keywords: thyroid axis, plasminogen, fibrinolysis

Background/Aims: Multiple clinical studies demonstrate diverse effects of hormones of thyroid hormonal axis on fibrinolytic system. Aim of the study was to investigate the effects of the hormones of the hypothalamic-pituitary-thyroid axis on some basic parameters of fibrinolysis in rats.
Materials and methods: Five groups of Wistar rats (n≡13) were treated by thyreotropin releasing hormone (TRH) (0.06 mg/kg b.m.), thyreotropin (TSH) (1IU/kg b.m.), liothyroninum (T3) (0.08 mg/kg b.m.), levothyroxinum (T4) (0.08 mg/kg b.m.) and saline s.c. for three consecutive days. Fibrinolysis parameters were evaluated in citrated plasma. Fibrinogen, plasminogen and α-2-antiplasmin activity were measured by chromogenic methods. Tissue-type plasminogen activator (TPA) antigen, plasminogen activator inhibitor type-1 (PAI-1) antigen and PAI-1 activity were estimated by ELISA methods.
Results: Fibrinogen was decreased significantly ($p<0.001$) after application of TRH, TSH, T3 and T4. All the four hormones increased both plasminogen and TPA ($p<0.001$). PAI-1 antigen and activity, and α-2-antiplasmin activity were significantly decreased in all experimental groups.
Discussion: The decreased fibrinogen content after TRH, TSH, T3 and T4 application may be a sign of a suppressed fibrinogen synthesis, though this finding is in controversy with the established anabolic effects of the thyroid hormones. The increased plasminogen and TPA indicate stimulation of fibrinolysis. On the contrary, both basic fibrinolysis inhibitors PAI-1 as activity and concentration, and alpha-2-antiplasmin are reduced, and this is in harmony with the stimulated fibrinolytic activity found.
Conclusions: The hypothalamic-pituitary-thyroid axis is involved in the regulation of the fibrinolytic system in rats by stimulating plasminogen and its main activator TPA and by a suppression of two basic fibrinolytic inhibitors PAI-1 and α-2-antiplasmin.

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P137

TAFI, PAI-1, T-PA AND F1+2 IN TYPE 2 DIABETIC PATIENTS

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Keywords: fibrinolysis, diabetes type 2, patients

Background/Aims: The aim of the study was to investigate the relations among plasminogen activator inibitor 1 (PAI-1), thrombin-activatable fibrinolysis inhibitor (TAFI), tissue plasminogen activator (t-PA), prothrombin fragments 1+2 (F1+2), glycemic control, hypertension, BMI and medication in DM 2 patients with normo- and microalbuminuria.
Patients and methods: Forty-two normoalbuminuric (NAU), 42 microalbuminuric (MAU) DM type 2 patients and 42 blood donors as control group were enrolled. TAFI, PAI-1, t-PA and F1+2 were assessed by ELISA in all subjects.
Results: TAFI was significantly increased in the MAU group, PAI-1 and F1+2 were significantly increased in both groups, but t-PA was not elevated in either group compared to controls. There were positive correlations in the NAU: TAFI and fibrinogen (r=0.65, p=0.02), PAI-1 and triglycerides (r=0.67, p=0.01), in the MAU: TAFI and F1+2 (r=0.48, p=0.02), TAFI and systolic blood pressure (r=0.53, p=0.01), PAI-1 and BMI (r=0.43, $p<0.05$).
Conclusion: We found decreased fibrinolysis in DM type 2 presenting with increased PAI-1 in both NAU and MAU as well as the increased TAFI in MAU. ACE inhibitors, statins and oral antidiabetics (OADs) led to increased t-PA, while beta blockers had TAFI-lowering effect. OADs improve hypofibrinolytic state in DM type 2 by lowering both TAFI and PAI-1. We confirmed the hypercoagulable state in patients with DM type 2 with higher F1+2.

Acknowledgements: This work was supported by grant VEGA 1/0018/10.

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☺ P352

CHARACTERISATION OF PLATELET AGGREGATION INDUCED BY HUMAN CERVICAL CARCINOMA CELLS

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Background/Aims: Tumor cell-induced platelet aggregation is a crucial step for the development of hematogenous metastases. However, molecular mechanisms implicated in tumor cell-induced platelet aggregation in cervical carcinoma are unclear. This study was designed to investigate the process of cervical carcinoma cell (HeLa)-induced aggregation of human platelets.
Materials and methods: Fresh blood was anticoagulated with 3.8% (wt/vol) trisodium citrate. Platelets were isolated from platelet-rich plasma by two-step centrifugation in a Tris/EDTA buffer solution. HeLa cells were grown in monolayers in plastic dishes and maintained at 37°C in culture medium 199 supplemented with 10% fetal bovine serum and 50 mg/liter kanamycin sulfate. HeLa cells were isolated from culture medium by centrifugation and resuspension in phosphate buffer saline (PBS). The process of cell aggregation was measured at 37°C by light transmission using an analyzer of platelet aggregation.
Results: We obtained that HeLa cells dose-dependently induced aggregation of washed platelets, resuspended in PBS. Therefore it can be assumed that plasma proteins, particularly exogenous fibrinogen, are not required for this process. However, the calcium ions were essential. As platelet-tumor cell interaction may occur due to lectin-like receptor binding to glycosylated proteins, we investigated HeLa-induced platelet aggregation in the presence of sugars. It was shown that N-acetyl-D-glucosamin (GlcNAc) inhibited platelet aggregation in response to HeLa, while α-methyl-D-mannoside and lactose were without effect. These data indicated that platelet-HeLa cell interaction is carbohydrate dependent and GlcNAc-specific. Furthermore, using various cell metabolic activity inhibitors (neomycin sulfate, D609, aristolochic acid, indometacin, N-ethylmaleimide, glutathione and others) we obtained that phospholipase C, phospholipase A2 and cyclooxygenase activity as well as cellular sulfhydryls are essential in HeLa-induced platelet aggregation.
Conclusions: Our results indicate that HeLa cells are able to induce platelet aggregation in the absence of plasma proteins. This process is carbohydrate dependent, GlcNAc-specific and induces phospholipase C, phospholipase A2 and cyclooxygenase activation.

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☺ P512

PREDICTION OF VENOUS THROMBOSIS IN CANCER PATIENTS USING A MICROPARTICLE BASED CLOTTING TEST

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Keywords: cancer, venous thrombosis, microparticles

Background: Although in patients with cancer the risk of venous thromboembolism (VTE) is increased, the incidence is too low to routinely give prophylactic treatment. Procoagulant microparticles (MPs), especially tissue factor (TF)-bearing MPs, contribute to the risk of VTE in cancer patients. In the present study, we assessed the MP-associated procoagulant activity using a functional assay, the fibrin generation test (FGT), to identify cancer patients prone to develop VTE.
Methods: As an ongoing study, plasma was collected from cancer patients, mainly with stage III or IV pancreatic, gastro-intestinal, breast or lung cancer. The MP-associated procoagulant activity was determined via the FGT with the addition of an inhibitory antibody to factor VII. The prolongation of the clotting time in the presence of anti-factor VII is a measure for the contribution of TF-bearing MPs to the clotting time. Patients were followed up for 6 months.
Results: 100 patients were included, of which 77 have completed follow-up until now. The first 43 patients were used to establish a cut-off value of the FGT. Receiver operating characteristics showed that a prolongation of the clotting time of 13% after addition of anti-factor VII, was the optimal cut-off value. In the entire group, 8 of 77 patients (10%) developed VTE, of which 7 could have been predicted by the FGT. Using this cut-off value, 23 patients (30%) had a FGT-result above the cut-off (positive test) and 54 patients had a FGT-result below the cut-off (negative test). The prevalence of VTE was 30% in the FGT-positive patients and 2% in the FGT-negative patients (sensitivity 88%, specificity 77%).
Conclusions: The FGT seems an excellent predictor for VTE in cancer patients. The next step will be to test the efficacy of prophylactic anticoagulants in patients with cancer and a high thrombosis risk based on the FGT.

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☺ = bestposter

☺ P572

INCIDENT CANCER IN PATIENTS ON VITAMIN K ANTAGONISTS: A POPULATION-BASED STUDY

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Keywords: cancer, warfarin, population

Background/Aims: Whether the long-term use of vitamin K antagonists (VKA) may decrease the development of cancer is uncertain.
Methods: We conducted a population-based study on all cancer- and thromboembolism-free residents of Health Area 16 of the Veneto Region (Italy) aged 65 to 90 years in the period January 1, 1996 to December 31, 2002. The period of observation ended December 31, 2007 or with the reaching of the 90th birthday, diagnosis of malignant disease, death or emigration. All subjects on chronic use of warfarin or acenocoumarol were identified as VKA exposed group, whereas the remaining subjects served as control group. The study endpoints was the development of a previously undiagnosed malignant disease (ICD-9-CM) and the incidence of cancer-related mortality.
Results: Of a total of 76,008 subjects, 3,231 were classified as being chronically exposed to VKA, whereas the remaining 72,777 were controls. The whole cohort was followed-up for a mean of 8.2 (±3.2) years. After adjusting for age, sex and time-to-event the hazard ratio (HR) of newly diagnosed cancer in VKA exposed group as compared to control group was 0.88 (95% CI, 0.80 to 0.98; $p<0.05$). Among specific tumors, the decrease in the risk of malignant diseases was significant in prostate cancer, 0.69 (95% CI, 0.50 to 0.97; $p<0.05$) and nearly significant in renal and urinary tract cancers, 0.80 (95% CI, 0.60 to 1.06) and respiratory tract cancers, 0.78 (95% CI, 0.57 to 1.07). The adjusted HR of cancer-related and overall mortality were 1.07 (95% CI, 0.92 to 1.24) and 1.12 (95% CI, 1.05 to 1.19) respectively.
Conclusions: The long-term use of vitamin K antagonists is likely to decrease the overall risk of cancer, among specific tumors that of the prostate is significantly spared.

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☺ P659

LONG TERM CLINICAL OUTCOMES OF CANCER ASSOCIATED VENOUS THROMBOEMBOLISM: FINDINGS FROM THE MASTER REGISTRY

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Background: The clinical characteristics of venous thromboembolism (VTE) have been reported to be different in cancer and in non cancer patients included in randomised clinical trials. However, sparse information is available on the long-term clinical outcomes of VTE in a large cohort of unselected cancer patients.
Aims: To prospectively evaluate whether long-term clinical outcomes of VTE are different in cancer and non cancer patients.
Patients and methods: MASTER is a multicenter registry of consecutively recruited patients with symptomatic, objectively confirmed, acute VTE. Patients were followed-up at 6, 12 and 24 months for many major clinical outcomes.
Results: From January 2002 to October 2004, 2,119 patients with acute VTE were enrolled in the registry. For the aim of this study, we restricted the analysis to the 1,883 patients followed with at least one visit at 6, 12 or 24 months; 343 patients (19.3%) had cancer. The excess of risk for death or recurrence of VTE were estimated in a Cox model and expressed as Hazard Ratio (HR) between patients affected and not affected by cancer. The risk of death (HR= 6.84;95CI% 4.32-10.83; $p<0.0001$) and recurrence of VTE (HR=1.62; 95CI% 0.98-2.68; $p=0.056$) was higher in cancer patients. We analysed the cumulative incidence of the following major outcomes as Odds Ratio (OR) between patients with cancer and without cancer: post-thrombotic syndrome (25.3% versus 5.9%; OR: 5.39;95CI% 3.92-7.41; $p<0.0001$), acute myocardial infarction (1.7% versus 0.5%; OR: 3.17;95CI% 1.09-9.21; $p=0.02$), ischemic stroke (0.6% versus 0.4%; OR: 1.39;95CI% 0.28-6.95; $p=0.68$), any bleeding (5.5% versus 1.8%; OR: 3.1;95CI% 1.72-5.58; $p<0.0001$), inferior vena cava (IVC) filter implantation (7.2% versus 4.3%; OR: 1.69;95CI% 1.06-2.71; $p=0.02$), long term treatment with low-molecular-weight heparin (25.9% versus 14%; OR: 2.14;95CI% 1.62-2.82; $p<0.0001$).
Conclusions: During the two year follow-up, the risk of death and recurrence of VTE was higher in patients with cancer than in patients without cancer. In addition, post-thrombotic syndrome, myocardial infarction, and bleeding were more common in cancer patients.

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PROGNOSTIC RELEVANCE OF ASYMPTOMATIC VENOUS THROMBOEMBOLISM IN PATIENTS WITH CANCER

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Keywords: asymptomatic venous thromboembolism, cancer

Background: The association between cancer and symptomatic venous thromboembolism (VTE) is well documented. In particular, symptomatic VTE is associated with an increased mortality in cancer patients. Advances in computed tomography have led to the detection of asymptomatic VTE on routine cancer staging scans. However, the clinical impact of VTE in cancer patients remains to be established.

Methods: We performed a multicenter, retrospective cohort study evaluating mortality at six months in a group of 60 consecutive cancer patients with asymptomatic VTE, in a group of 120 consecutive cancer patients with symptomatic VTE diagnosed on routine staging, and in a group of 60 consecutive cancer patients in which VTE was objectively excluded.

Results: Age and gender were similar in the three groups. There were no differences among groups in cancer site and stage (96.6%, 93%, and 96.6% of patients had cancer in stage III or IV, p=NS). All patients with symptomatic or asymptomatic VTE where treated with low molecular or unfractionated heparin. Length of treatment was similar. Mortality at six months was similar in cancer patients with symptomatic or asymptomatic VTE (47.5% and 45%, p=NS) whereas mortality at 6 months was significantly higher in each of the two groups when compared to patients without VTE (p=0.007 and p=0.036, respectively).

Conclusions: Our results suggest that cancer patients with asymptomatic or symptomatic VTE have a similar mortality rate at six months. These results highlight the prognostic relevance of asymptomatic VTE in these patients.

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BETAINE DOWNREGULATES TISSUE FACTOR EXPRESSION IN HUVEC AND TUMOR CELLS

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Background: Betaine, a methyl donor in the methionine cycle, has been implicated in the etiology of cancer. Some studies have shown the potential importance of betaine in carcinogenesis, for example betaine may reduce the risk of breast cancer in a manner similar to that of folate. It is used in clinical practice to lower homocysteine levels with a potential benefit for cardiovascular health.

Tissue factor (TF) is expressed by tumour-associated endothelial and inflammatory cells, as well as by cancer cells themselves, and, in addition to its procoagulant activity, is considered a hallmark of cancer progression, contributing to a variety of processes, such as growth, metastasis formation and tumor angiogenesis.

Aims: To evaluate the effect of betaine on TF expression in human monocytes (MN), human umbilical vein endothelial cells (HUVEC) and the metastatic breast carcinoma cell-line MCF7.

Methods: MCF7 cells and HUVEC were grown until confluency. MN were obtained from healthy donors. Cells were then incubated with or without betaine at 37°C. Procoagulant activity was assessed by a one-stage clotting assay. TF antigen cellular expression was determined by flow-cytometry and TF mRNA by RT-PCR.

Results: Betaine decreased the constitutive TF activity of MCF7 in a dose-dependent way. The decrease was accompanied by a downregulation of TF antigen as well as of mRNA.

Similar results could be observed when endotoxin-stimulated HUVEC were cultured in the presence of betaine. Surprisingly, TF expression by MN exposed to endotoxin, as well as to TNF-alpha or IL-1beta was unaffected by betaine.

Inhibitory antibodies demonstrated that the activity was solely attributable to TF, which was expressed by the different cells at various degrees.

Conclusions: These data support the hypothesis that betaine, by downregulating TF, could exert a beneficial effect in thrombotic disorders where enhanced host and/or tumor cell procoagulant activity may play a role.

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P298

VENOUS THROMBOSIS IN THE SETTING OF METASTATIC PANCREATIC CARCINOMA

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Keywords: venous thromboembolism, pancreatic carcinoma, chemotherapy, cisplaten

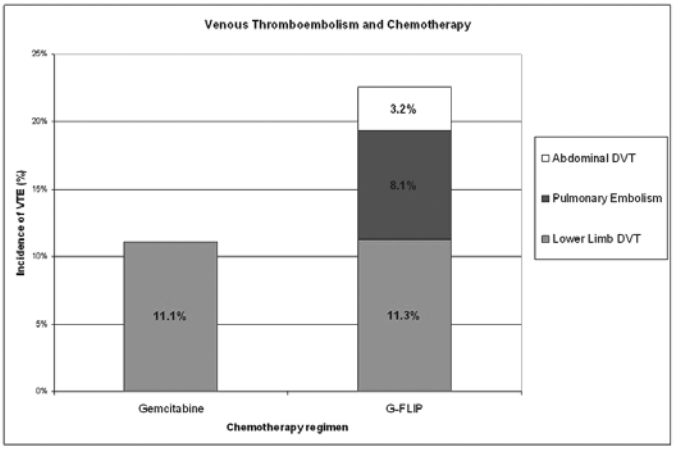
Background: Venous thromboembolism (VTE) is a significant concern in cancers that generate an intrinsic hypercoagulable state. Pancreatic carcinoma is associated with cancer cell activation of platelets and expression of procoagulant factors. Also, some chemotherapy drugs further increase the risk of VTE. In this study, we investigated risk factors for VTE in the setting of metastatic pancreatic carcinoma treated with gemcitabine and/or the G-FLIP regimen (gemcitabine, 5-flourouricil, leucovorin, irinotecan and cisplatin). We identified an enhanced risk of VTE associated with G-FLIP.

Methods: The electronic patient database at the William Osler Health Centre in Brampton, Ontario was searched for patients with metastatic pancreatic carcinoma treated with gemcitabine and/or G-FLIP since January 1, 2004. Patients undergoing chemotherapy at the time of data collection were excluded. In total, 94 patients fitting inclusion criteria were identified. Medical records were searched for smoking history, insertion of central venous access lines, chemotherapy treatments and incidence of thrombosis.

Results: Of 94 patients reviewed, 30 (31.9%) had at least one detected thrombotic event excluding central line-related deep vein thrombosis (DVT). Of 62 patients receiving G-FLIP, 22.6% experienced lower limb DVT, abdominal DVT or pulmonary embolism (PE) during or shortly after treatment (see Fig. 1). Of 45 patients on gemcitabine, only 11.1% experienced these modalities of thrombosis. This demonstrates a substantial increase in thrombosis risk for G-FLIP. Particularly, PE risk appears markedly elevated. Also, of 56 patients with known central lines for G-FLIP administration, 21.4% experienced associated upper limb DVT.

Conclusions: The overall rate of thrombosis demonstrated in this study is higher than that in most previous investigations of thrombosis in pancreatic carcinoma. Notably, all patients in this study had advanced metastatic disease and received chemotherapy. These data also demonstrate a significantly increased risk of VTE for patients treated with multi-agent, platinum-based chemotherapy.

Figure: Incidence of VTE with gemcitabine and G-FLIP



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DOWNREGULATION OF TISSUE FACTOR EXPRESSION IN HUMAN BREAST CARCINOMA CELLS BY INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM

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Background/Aims: The renin-angiotensin system (RAS), whose receptors have been found on surface of human tumor cells, has been associated to proliferation of tumors, and plays a role in cell migration. Moreover, its inhibition may protect against cancer. Tissue factor (TF), often expressed by tumor cells, for its involvement in tumor growth, angiogenesis, and metastasis is considered a hallmark of cancer progression. Having shown that RAS system is involved in the downregulation of TF expression in human stimulated monocytes (Napoleone et al. CircRes 2000), the aim of our study was to evaluate whether RAS blockade modulates TF constitutive expression by the metastatic breast carcinoma MDA-MB-231 cell-line.
Methods: MDA-MB-231 were incubated with the different reagents at 37°C. TF activity was assessed by one stage clotting time, TF antigen by ELISA and TF mRNA levels by real time PCR. Angiotensin receptor (AT1) was detected by flow-cytometry and angiotensin-II levels were measured by EIA.
Results: The strong constitutive TF activity and antigen expressed by MDA-MB-231 were significantly reduced in a dose-dependent manner by captopril and enalapril. Since flow cytometry assays showed the presence of the AT1 on MDA-MB-231 membrane, we tested whether blockade of AT1 could affect the procoagulant potential of the cells. Losartan, a competitive inhibitor of AT1, reduced TF activity and antigen to a degree similar to that exerted by ACE inhibitors. Captopril and Losartan largely downregulated the strong constitutive expression of TF mRNA. Similar results were observed when an anti-AT1 antibody was used instead of losartan. Abs against angiotensin-II, which was present in cell conditioned medium could reduce TF activity and antigen.
Conclusions: These results could, at least in part, contribute to explain the supposed effects of ACE inhibitors and AT1 receptor antagonists in some types of malignancy, and offer new clues to support their use for tumor control.

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IMPACT OF LOW MOLECULAR WEIGHT HEPARIN ON SERUM VEGF IN BREAST CANCER PATIENTS

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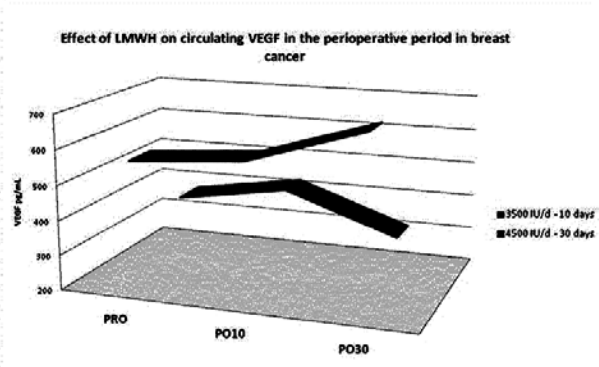
Keywords: VEGF, LMWH

Background: VEGF has been found to implicate tumor growth and spread by promoting angiogenesis. Anti-VEGF agents are in current use, but their anti-healing properties do not allow to administer them during the first post-operative (post-op) period. Low-molecular-weight heparins (LMWH) have been shown to be able to modulate the activities of angiogenic growth factors and thus a role in the prognosis of cancer patients.
Aims To evaluate the impact of a LMWH on the post-op levels of serum VEGF (sVEGF) in breast cancer patients who underwent therapeutic surgical treatment.
Patients and methods: 32 consecutive breast cancer patients comprised the study population. 16 were given peri-operative thromboprophylaxis with 3,500 IU of a LMWH in a daily basis for 10 days (group I), whilst the other 16 were given 4,500 IU of the same LMWH for 30 days (group II). In all patients the levels of sVEGF were evaluated at days 0 (the day before the operation), 10th post-op and 30th post-op using a commercial ELISA kit. The one-way ANOVA and t-test were used for statistical analysis.
Results: A statistical significant difference in the levels of sVEGF was detected at the 30th post-op day between the two groups: group I, mean sVEGF value = 691±478 pg/ml vs. group II, mean sVEGF value = 314±212 pg/ml ($p < 0.05$).
Conclusions: In breast cancer patients, an increased and prolonged peri-operative thromboprophylaxis with a LMWH (4,500 IU/day for 30 days) may give a significant oncological advantage during the early post-op period.



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CANCER-RELATED DISSEMINATED INTRAVASCULAR COAGULATION: FIRST LINE THERAPY WITH PLASMA-DERIVED PROTEIN C

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Keywords: disseminated intravascular coagulation, cancer, plasma-derived protein C

Background: Cancer-related disseminated intravascular coagulation (DIC) is a rare but life-threatening condition. Acute management is mainly based on administration of fresh-frozen plasma (FFP) and antithrombin (AT) with the aim of restoring coagulation factors deficiency. The protein C (PC) pathway is a modulator of the coagulation as well as the inflammatory system; PC deficiency leads to increased activation of the coagulation system, resulting in thrombin generation. Some clinical trials have demonstrated the efficacy of administering activated recombinant (a-r) PC in adult patients with sepsis-related coagulopathy, but increasing the risk of major bleeding. Plasma-derived PC has a self-limiting process in determining anticoagulation thus it seems more suitable than a-rPC in patients at high risk for bleeding, such as cancer patients.
Aims: To describe the efficacy and safety of PC concentrate to restore physiological values in adult cancer patients with overt DIC. Study Design: Not controlled clinical trial (NCCT).
Materials and methods: Adult cancer patients affected by DIC, having PC plasma concentration less than 50%, were treated with PC concentrate (Ceprotin®, Baxter) as an adjusted bolus of 30 to 50 UI/Kg/die to restore normal PC values (70-120%). Clinical outcomes (bleeding, thrombosis and mortality) were recorded up to a follow-up of 28 days from the initial diagnosis of DIC. PC activity, WBC, platelets, D-dimer, fibrinogen, PT, aPTT, AT and DIC score were measured after 12, 24, 48, 7 and 10 days.
Results: Twenty-two patients were included over a period of 3 years; among them 16 had solid cancer and 6 had haematological cancer. All patients had advanced/metastatic neoplasm. PC concentrate normalized PC activity in all patients within 48h and remained upper the lower normal value for the following days. Baseline PC levels were lower in non-survivors than in survivors although this difference was non-significant. During the study period, there was a significant increase of platelets, fibrinogen, PT, AT, and a significant decrease of D-dimer, aPTT and DIC score (**Table**). No bleeding or thrombosis were observed; mortality at 28 days was 35%.
Conclusions: Our investigation shows that PC concentrate is safe and normalizes laboratory variables in cancer patients with overt DIC.

Table: Changes in laboratory findings obtained from all patients during the study period (mean ± SD)

	Baseline	24 h	48 h	72 h	7th day	14th day
PC (%)	27.3 ± 7.1	71 ± 15.6*	85.9 ± 12.5*	91.2 ± 11.6 *	92.2 ± 13.4 *	99.1 ± 13.5 *
WBC ($\times 10^9$/L)	8.2 ± 3.1	7.8 ± 2.2	6.5 ± 1.9	6.7 ± 1.5	7.3 ± 1.5	8.1 ± 0.6
Platelet ($\times 10^9$/L)	49.3 ± 20.4	51.2 ± 19.4	71.2 ± 33.4	91.7 ± 41.1	113.4 ± 65.1	154.8 ± 109.2*
d-Dimer (µg/L)	2.133.6 ± 1.643	2.366 ± 1.561	1.230 ± 1.045 *	800.2 ± 686 *	350 ± 225*	541 ± 246*
Fibrinogen (g/L)	2.1 ± 1.4	2.8 ± 1.1	3.6 ± 1.5	4.4 ± 1.4*	4.5 ± 1.2*	4.2 ± 1.3
PT (%)	46.4 ± 11.5	46.2 ± 12.1	51.8 ± 13.8	63.3 ± 15.2	65.4 ± 0.9*	69.7 ± 14.3 *
aPTT (s)	40.1 ± 13.4	34.8 ± 7.6	35.4 ± 6.1	33.4 ± 6.1	32.9 ± 7.5	31.2 ± 3.6*
AT (%)	54.2 ± 12.2	61.6 ± 23.3	73.4 ± 21.4	77.7 ± 22.2 *	80.6 ± 16.5 *	87.1 ± 18.5 *
DIC score	6.26 ± 1.12	5.38 ± 1.42	4.26 ± 0.96	3.16 ± 0.98 *	2.97 ± 0.87 *	2.21 ± 1.43 *

Legenda: *$P<0.05$ vs baseline

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DOES WARFARIN INCREASE DOXORUBICIN-INDUCED CYTOTOXICTY ON TUMOR CELLS? AN *IN VITRO* STUDY

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Keywords: cytotoxicity,warfarin,doxorubicin

Previous results from our laboratory gave evidence that warfarin treatment may enhance the toxic effect of radiation in leukemic cell. Doxorubicin (DOX) is an antitumor drug commonly used as single and in combination with other chemotherapeutic agents, for treatment of wide range of human malignancies. This study investigated combinations of DOX with warfarin for their cytotoxicity against leukemic cells following a 72 h exposure in vitro. Cell proliferation and apoptosis assays with various concentrations of warfarin with or without DOX (1 μM) were done on two leukemic cancer lines, human chronic myelogenous leukemic K562 cells and promyelocytic leukemic HL-60 cells, and on normal human peripheral blood mononuclear cells (PBMC). The warfarin at the lower concentrations (<50μM) with DOX treatment also induced in additive manner apoptosis in K562 and HL-60 cells. Combined treatment also attenuated cell proliferation in both cell lines. Presence of 100 μM N- acetylcysteine, as antioxidant, in the cell culture did not impair the cytotoxicity of DOX and warfarin. The present results indicate that warfarin treatment may enhance the toxic effect of DOX in leukemic cells. The mechanism by which warfarin potentiate this cytotoxicity is unclear, but it may not be directly due to toxic damage induced by warfarin-generated free radicals, since co-treatment with N- acetylcysteine was unable to prevent this toxicity.

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RADIOTHERAPY AND CIRCULATING PROCOAGULANT MICROPARTICLES IN PATIENTS WITH PROSTATE CANCER

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Keywords: microparticles, prostate cancer, radiotherapy

Background: Circulating microparticles (MPs) are small fragments of the plasma membranes released by apoptotic or activated cells. MPs are generated from different types of cells-platelets, endothelium, leukocytes, tumor cells. Recent date show that MPs may play an important role in inflammation, thrombosis and cancer progression.

Aim: The purpose of the study was to evaluate the influence of radiotherapy on microparticles generation in patients with prostate cancer.

Materials and methods: Plasma samples were collected from 40 patients with localised prostate cancer, mean age of patients was 67.7 years, and 30 age - matched healthy male controls. In cancer group blood samples were collected before, during and 1 month after radiation therapy. Procoagulant activity of circulating MPs, concentrations of TF and TFPI antigens were measured by immunoenzymatic method (ELISA, American Diagnostica)

Results: The mean value of MPs activity (9.56±4.34nM) and TFPI concentration (130.76±26.10 ng/ml) were significantly (p<0.05) higher in patients with prostate cancer (before radiotherapy) in comparison to control group (respectively: 7.94±1.55nM, 87.54±15.67ng/ml) whereas there was no difference in mean concentration of TF between cancer and control group (78.38±19.87 vs 77.09±17.18 pg/ml).

The influence of radiotherapy on MPs generation, TF and TFPI concentrations are summarised in the **Table**.

Conclusions: Radiotherapy cause a transient increase in MPs generation. These preliminary data suggest a role for circulating MPs in intravascular coagulation activation during radiation therapy of patients with prostate cancer.

Table: The effect of radiotherapy on MPs generation.

Parameter	Before radiotherapy Means ±SD	During radiotherapy Means ±SD	1 month after radiotherapy Means ±SD
MPs [nM]	9,56 ± 4,34	17,30 ± 7,57*	11,40 ± 3,12
TF [pg/ml]	78,38 ± 19,87	66,34 ± 23,10*	83,08 ± 25,42
TFPI [ng/ml]	130,76 ± 26,10	101,06 ± 17,78*	111,85 ± 25,06

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PREVALENCE OF FACTOR V LEIDEN, PROTHROMBIN G20210A, METYLENETETRAHYROFOLATE REDUCTASE C677T POLYMORPHISMS IN CANCER PATIENTS WITH AND WITHOUT THROMBOSIS

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Keywords: cancer, thrombosis, FVL

Background/Aims: Venous thromboembolism (VTE) is a well-recognized complication of cancer; however its pathogenesis is not entirely established. We have reported the frequencies of several polymorphisms in thrombophilia like factor V Leiden (FVL), prothrombin (PT) G20210A, metylenetetrahyrofolate reductase (MTHFR) C677T, dihydrofolate reductase 19-bp deletion, intron F G79A of protein Z, endothelial protein C receptor 23 bp insertion, PAI 4G/5G in cancer patients with and without VTE. The frequencies of FVL, PT G20210A, and MTHFR C677T polymorphisms in cancer patients with and without VTE were re-evaluated in the present study including new cases.

Patients and methods: The study consisted of 77 cancer patients who developed thrombosis (group 1) and 209 cancer patients without VTE (group 2). Genomic DNA isolation was performed from peripheral venous blood and FVL, PT G20210A and MTHFRC677T polymorphisms were determined by using commercially available LightCycler kits. The frequencies of the variables were tested by chi-square or two-sided Fischer exact test.

Results: The prevalence of FVL was significantly greater in group 1 compared with group 2 (35.1 % vs. 2.9 %, p<0.0001). No differences were seen in genotypes and allele frequencies of MTHFR C677T and PT G20210A between two groups (p> 0.05).

Conclusions: FVL testing is probably necessary in cancer patients with thrombosis in regions with high prevalence of this mutation like Turkey.

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HUMAN VON WILLEBRAND FACTOR (VWF) ROLE IN THE BIOLOGY OF HUMAN AND MOUSE TUMOR CELLS *IN VITRO*

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Keywords: VWF, tumor cells, viability, cell adhesion

Background/Aims: Some observations in experimental models had suggested that VWF can modulate the tumoral metastatic potential. The aim of this study was to explore whether human VWF affects the cell adhesion and viability of human (MCF-7) and mouse (F3II) breast and pulmonary (B16-F0) tumor cells.

Materials and methods: Haemate-P (H) and sonicated H (SH; 20% amplitude, 3 minutes), were evaluated for VWF:Ag (10 IUmL-1, 8.6 IUmL-1, respectively) and VWF:CB (9.7 IUmL-1, 1.4 IUmL-1, respectively). Cells were incubated with H*; SH*; and vehicle (*VWF 0.5, 1 and 3 IUmL-1). Cellular proliferation was assessed using a colorimetric nonradioactive proliferation assay (MTS), and cell adhesion assay was evaluated by cell counts with Trypan Blue exclusion. GPIb mRNA was evaluated by RT-PCR and automated sequencing. VWF-binding to tumor cells was analyzed by flow cytometry (FC, median fluorescence intensity- MFI) and Cell-ELISA.

Results: H induced cell death in a dose-dependent manner in F3II (10-30% cell death) and B16-F0 (10-20% cell death) cell lines. SH increased 1.2 to 1.5 folds the cell death regard to H. When cells were seeded in presence of H, MCF-7 and F3II were unable to adhere, while B16-F0 adhered to H in a dose-dependent manner (1.8 to 2.2 fold increase). MCF-7, F3II and B16-F0 expressed GPIb mRNA. After H treatment (1 IUmL-1), MCF-7 and F3II revealed VWF bound (positive control MFI= 19; MFI= 13), but B16-F0 (MFI= 1.2) did not; Cell-ELISA results confirmed these findings.

Conclusions: Although all cell lines expressed GP1b, only F3II and MCF-7 showed VWF-binding but the last one failed in the cell death test in presence of H. VWF, regardless of function (H vs. SH), seems to be involved in the F3II cell line responses.

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ASSOCIATION BETWEEN INCREASED TUMOR NECROSIS FACTOR-ALPHA LEVELS AND IMPAIRED FUNCTIONALITY OF THE PROTEIN C ANTICOAGULANT PATHWAY IN PATIENTS WITH METASTATIC COLORECTAL CANCER

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Keywords: colorectal cancer, protein c abnormalities, tumor necrosis factor-α

Background/Aims: Tumor cells and/or tumor-associated macrophages may produce inflammatory cytokines, such as TNF-α. Such release is actively involved in the induction of a pro-thrombotic status. In particular, increased TNF-α levels have been associated with changes in the activated protein C (APC) system, with a decrease in the ability to generate APC.
Patients and methods: TNF-α levels (by immunoassay) and abnormalities in the APC system (by a novel assay specifically designed to globally evaluate the functionality of the PC anticoagulant pathway) were evaluated in 45 metastatic CRC patients (17 females, 28 males, aged 60±9 years) undergoing chemotherapy to investigate their possible association. Thromboembolic events (VTE) were recorded during follow-up.
Results: TNF-α levels were increased [3.2 pg/ml (1.9–6.6)] and APC functionality was decreased [74 PICI% (71–80)] in mCRC patients compared to age and sex-matched controls (both $p<0.001$). An inverse correlation was observed between TNF-α and APC impairment in mCRC (Rho= 0.336, $p=0.02$). Multivariate regression analysis including functionality of the PC anticoagulant pathway as the dependent variable and age, sex, ECOG, platelet counts, hemoglobin, BMI, concurrent treatments and TNF-α levels as the predictor variables showed that an increase of TNF-α [β= -0.332, $p=0.029$] was the only independent predictor for APC abnormalities during chemotherapy. Nine of 45 mCRC patients experienced VTE during chemotherapy. Survival analysis of patients stratified on the basis of pre-treatment serum TNF-α levels demonstrated a worst cumulative event-free survival (56%) of mCRC patients in the upper (>6.6 pg/ml) compared to those in the lower quartiles (86%, Log-rank 2.3, $p=0.02$).
Conclusions: The results obtained suggest that the host inflammatory response to cancer cells and/or tumor-derived cytokines could be responsible for an impairment of the APC system and a switch toward a pro-thrombotic state, which might predispose to the occurrence of VTE in mCRC patients undergoing chemotherapy.

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THE ONCOLOGY TREATMENT OF PATIENTS WHO USE ORAL ANTICOAGULANTS IS CONNECTED WITH HIGH RISK OF BLEEDING COMPLICATIONS

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Keywords: oral anticoagulants, cancer patients, bleeding

Background: The data about risk for bleeding complications during anticoagulation in cancer patients with different oncology treatment are conflicting.
Aims: To investigate the rate of bleeding in the course of oral anticoagulants, during treatment of malignant diseases, we conducted a retrospective study including 75 patients on stable anticoagulation prior to commencing their different oncology treatment.
Methods: All patients were treated according to the consiliar decision, made based on the localization and pathohistological findings of the malignant disease. During their treatment the regular laboratory monitoring of INR was done. Every dose of oral anticoagulants, INR changes, as well as the size and localization of bleeding were recorded.
Results: During all the malignancy treatment 22 (30%) of patients were overanticoagulated. In 15 (20%) patients it was associated with bleeding, while 3 (4%) of them had to be transfused with fresh frozen plasma to stop the major bleeding. Most bleeding complications occurred in the group of patients treated with chemotherapy or with analgesics in the group with advanced disease. None of the bleeding complications were observed in patients treated with irradiation and surgery alone, where the bridging of oral anticoagulants with low-molecular-weight heparin was done before surgery.
Conclusions: The oncology treatment of patients who take oral anticoagulants was connected with high risk for bleeding especially if chemotherapy as a therapeutic options was used. Therefore physicians should be aware of this risk and carefully monitor the intensity of anticoagulant therapy, especially during the first treatment weeks when the risk of bleeding is greatest.

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CIRCULATING ENDOTHELIAL CELLS, MICROPARTICLES AND MARKERS OF ANGIOGENESIS AND INFLAMMATION IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER.

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Keywords: circulating endothelial cells, microparticles, lung cancer, angiogenesis

Background/Aims: Circulating endothelial cells (CECs) and microparticles (MPs) are modulated in a variety of diseases including cancer, and are promising surrogate biomarkers in oncology. The aim of this study is to quantify the CECs and MPs in patients with newly diagnosed, stage IIIB and IV non-small cell lung cancer (NSCLC). We have also analyzed their relationship with inflammatory and angiogenesis markers.
Methods: We studied 15 stage IIIB and 24 stage IV NSCLC patients, before first line treatment based on taxane and platinum chemotherapy. Forty normal subjects were evaluated as controls. Isolation and quantification of CECs were carried out by an immunomagnetic technique. Flow cytometry of plasma was used for detection of total phosphatidylserine-positive MPs, labeled with FITC-Annexin V. Circulating markers of inflammation: interleukin-6 (IL-6) and sialic acid (SA), and angiogenesis: vascular endothelial growth factor (VEGF) and VEGF-receptor 1 (VEGF-R1) were quantified by commercial assays
Results: Levels of CECs were significantly increased in patients (111±78 cells/mL) as compared to controls (10±5 cell/mL; $p<0.0001$). Levels of MPs were significantly higher in patients (3527 ±3649 MPs/μL) than in controls (1176±742 MPs/μL; $p<0.05$). Levels of IL-6 and SA were significantly higher in patients (13.1±13.2 pg/ml and 91.1±21.6 mg/dL) than in controls (0.9±0.9 pg/ml and 53.7±7.8 mg /dL, respectively; $p<0.05$). Levels of VEGF-R1 and VEGF were significantly higher in patients (93.3±17.8 pg/mL and 469± 388 pg/mL) than in controls (71.1±17.0 pg/mL and 158±61 pg/mL, respectively; $p<0.05$). The Pearson correlation between CECs and MPs was positive and statistically significant (r=0.40, $p<0.001$). A positive and significant correlation was also found between levels of CECs and SA, IL-6 and VEGF (r=0.63, $p<0.001$; r=0.62, $p<0.001$; r= 0.43, $p<0.001$, respectively).
Conclusions: CECs and MPs, as well as several markers of inflammation and angiogenesis, are elevated in patients with advanced NSCLC .

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RELATIONSHIP BETWEEN SP-SELECTIN AND INFLAMMATION INDICES (IL-6, AND CRP) IN COLORECTAL CANCER.

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Background/Aims: Platelets are an important element in the thrombotic process, inflammation and cancer progression. Considering the increased platelet activation and induction of immune response in neoplastic disease the question arises whether platelet activation and inflammatory process are mutually related. In order to test out this hypothesis we assessed the plasma level of sP-selectin, IL-6 and CRP in colorectal cancer patients, and tried to find the correlation between these parameters.
Material and methods: The study included 42 CRC patients (25 men, 17 women) and 38 healthy subjects (21 men and 17 women)-group C. The CRC patients were divided into two groups according to the TNM classification. sP-selectin and IL-6 concentration were measured using immunoenzymatic method, CRP level was measured by a turbidymetric immunoassay.
Results: Soluble P-selectin, CRP and IL-6 concentrations were significantly increased as compared to the control group ($p<0.001$). Plasma levels of sP-selectin, CRP and IL-6 were higher in group B (with metastases) than in group A (without metastases) ($p<0.001$). The analysis showed a positive correlation between IL-6 and CRP (r=0.7638; $p<0.01$) and between sP-selectin and IL-6 in (r=0.5633; $p<0.03$). We also observed correlation between sP-selectin and platelet count in both study groups.
Conclusions: We observed enhanced platelet activation and inflammation response in patients with colorectal cancer. The inflammatory process and platelet activation progress along with colorectal cancer advancement. Our data seem to confirm the relationship of platelet activation with inflammatory response in colorectal cancer. sP-selectin and indexes of inflammation-IL-6 and CRP may be useful markers for diagnosis and applying anti-inflammatory therapy in patients with advanced cancer.

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EPIDEMIOLOGY OF CANCER-RELATED THROMBOSIS AT THE NATIONAL CANCER INSTITUTE, BOGOTÁ COLOMBIA

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Keywords: thrombosis, cancer

Study objective was to establish the frequency of thromboembolism among patients at the Colombian National Cancer Institute (NCI) from 2007 to 2009. One hundred and seventy-four case histories were reviewed. Median age was 59. Percentage of cancer related thrombosis: breast cancer, 25.8%; cervical cancer, 14.9%; pancreatic cancer, 1.14%; central nervous system tumors, 0.57% . For men, the thrombosis-neoplastic relation: prostate cancer, 22%; digestive tract, 18.3% (gastric cancer, 10.4%; colon cancer, 8.3%); germinal tumors, 10.4%.
Cancer stage at which diagnosis of thromboembolism occurred: while undergoing curative treatment, 29.78%; disease under control, 13.93%; metastasis, 17.72%; undergoing diagnosis, 9.61%; relapse, 23.41%.
Median number of days from tumor diagnosis to thromboembolic development was 93. Anticoagulation was suspended by 17.1% of patients; from this group, 2 patients suffered recurrent thrombosis. Median number of days on anticoagulants among group of patients who suspended anticoagulants, 275.
In our study, distribution of thromboembolic events proved to be different from that mentioned in the literature, i.e., greater frequency among cases of breast and cervical cancer and very low frequency for pancreatic and central nervous system cancer. We documented thrombosis related to cervical cancer, which is not reported on in the literature as a thromboembolic risk factor. It is necessary to carry out further studies which will lead to establishing associations with the pathologies evaluated.

Table: Data Base Thrombosis and Cancer 2007-2009

Variable	Result
Age (Median)	59
Gender Male Female	 26,4% 73,6%
Neoplasia Classification	
Breast Cervical Prostate Lung Gastric Pancreatic Central nervous system	25,8% 14,9% 6,32% 4,02% 2,87% 0,14% 1,14%
Treatment	
Dalteparine Warfarin	90% 10%
Disease Stage	
Under control Metastasis In curative treatment Undergoing diagnosis Relapse	13,9% 17,7% 29,7% 9,6% 23,4%
Related Factors	
Postoperative	16,5%
Recurrent thrombosis	2,3%
Bleeding	4,8%
Suspension of anticoagulants	17%
Time from diagnosis to thrombosis (median)	93 días

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P125

DOES COLORECTAL CANCER CLINICAL ADVANCEMENT AFFECT ADHESION MOLECULES (SP-SELECTIN, SESELECTIN AND SICAM-1) CONCENTRATION?

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Background: Adhesion molecules take place in physiological and pathological processes. They are involved in inflammatory reaction and play an important role in tumor invasion and neoplastic spread.
Aims: The aim of the study was to assess the correlation between platelet and endothelial cell activation in the progression of colorectal cancer.
Materials and methods: We measured the level of sP-selectin-marker of platelet activation and levels of sE-selectin and sICAM-1-markers of endothelial cell activation. The study involved 44 CRC patients and 34 healthy subjects. The patients were divided according to TNM classification. sP-selectin, sE-selectin and sICAM-1 concentration were measured in plasma using enzyme-linked immunosorbent assay.
Results: Plasma level of all three adhesion molecules was significantly higher in CRC patients than in control group ($p<0.001$). There was no correlation between sP-selectin and sE-selectin, but we found a positive correlation between sE-selectin and sICAM-1 in all CRC patients groups. The highest level of sE-selection and sICAM-1 were observed in patients with liver metastasis. We suggest that plasma concentration of sE-selectin and sICAM-1 may indicate tumor progression and liver metastasis.

Table Plasma values of sP- selectin, sE- selectin and sICAM-1 in controls and in patients with colorectal cancer according to TNM classification.

	Stage I, II (n= 24)	Stage III (n= 12)	Stage IV (n= 8)	Controls (n= 34)
sP- selectin (ng/ml)	67.90±44.52*	51.84±23.35	53.14±44.22	45.07±24.71
sE- selectin (ng/ml)	43.60±21.55^	44.38±22.82	59.14±25.13*	29.03±15.55
sICAM-1 (ng/ml)	215.80±50.74	216.36±30.14	260.85±56.94*	201.69±24.71

* Statistically significant differences ($p<0.001$) vs. Control

^differences between stage I/II and stage IV ($p<0.05$)

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P557

PULMONARY EMBOLISM IN CANCER PATIENTS: AN INDIAN STUDY

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Keywords: pulmonary embolism, cancer, India

Background/Aims: Pulmonary embolism (PE) has been considered to be rare in Asian population and is underreported in patients with malignancy. The purpose of our study is to determine the characteristics of PE in cancer patients in the Indian population and compare them with western studies.
Methods: Retrospective analysis of 38 diagnosed PE patients admitted in a tertiary oncology centre was performed. Demographic data, primary malignancy site, staging (by TNM classification), histopathologic correlation of malignancy, co-morbid factors and treatment administered for PE (medical and surgical) along with their outcomes were recorded**.**
Results: Thirty-eight patients diagnosed as having PE from clinical data and radiologic evaluation was analyzed. Isolated PE was found in 57.8% patients and was associated with DVT in 42.2%. Males constituted 44.7% of study population. The commonest site of malignancy was gynecologic tumors in 23.6% of study patients while the commonest histopathologic variant was adenocarcinoma in 31.5%. Clinically, 5 patients (13.1%) were asymptomatic and were detected incidentally by staging CT scan. Amongst the symptomatic patients, intermittent episodes of dyspnoea was the commonest presenting symptom seen in 50% of patients. The commonest echocardiographic finding was RV dysfunction seen in 53% patients. Anticoagulation was the commonest treatment modality offered. IVC filter was inserted in 3(7.8%) patients. Eleven patients (29%) expired due to PE and the remaining survived the acute episode.
Conclusions: A high index of suspicion, vigilance and dedicated assessment for pulmonary embolism is recommended while evaluating cancer patients even in the absence of DVT. Asymptomatic incidental pulmonary embolism should be suspected in all patients in advanced stage of malignancy especially involving gynaecological and gastrointestinal tract.

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P53

THROMBOPROPHYLAXIS WITH LOW-MOLECULAR-WEIGHT HEPARIN IN PATIENTS WITH CANCER UNDERGOING CHEMOTHERAPY

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Keywords: low-molecular-weight heparin, haemostasis, chemotherapy

Aims: To study the effect of different low-molecular-weight heparins (enoxaparin, nadroparin, dalteparin) on the haemostasis and the rate of thrombotic complications in patients with cancer undergoing chemotherapy.
Materials and methods: The haemostatic system was studied in 180 cancer patients undergoing chemotherapy. Most patients had stage IV cancer (88%). The 1st group (n=80) comprised patients with hypercoagulation determined on the basis of coagulation test without clinical manifestation of thrombosis. The patients were administered LMWH 2 hours prior to the beginning of chemotherapy, then all along the chemotherapy course (1-8 days, not longer than 14 days), and 1-2 days after the discontinuation of each chemotherapy. The patients (n=100) of the 2nd group (control) received no thromboprophylaxis.
Results: Pre-chemotherapy hypercoagulation with chronic intravascular coagulation is characteristic for cancer patients and there is a higher risk of VTE depending on the number of chemotherapies undergone: 50% of VTE develop after 3 to 4 chemotherapy courses. LMWH administration during chemotherapy decrease the activation of pro-coagulation (prolongation of APTT, decrease in prothrombin activity and of fibrinogen concentration), platelet aggregation, the level of intravascular coagulation markers (D-dimers, fibrin monomers soluble complex) and von Willebrand factor, contribute to the reconstitution of thrombin inhibitors (antithrombin III and protein C) and support the defensive role of fibrinolysis. VTE developed in 21% in the control group versus 4% in the group of cancer patients receiving LMWH thromboprophylaxis. The antifactor-Xa activity of LMWH was in the therapeutic range for thromboprophylaxis (0.2-0.4 IU/ml). There were no hemorrhagic complications.
Conclusions: LMWH administration in cancer patients undergoing chemotherapy were shown to be safe for thromboprophylaxis.

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Atrial fibrillation

Thursday 8th July, 2010

☺ P584

ENDOTHELIAL DAMAGE, ASSESSED BY VON WILLEBRAND FACTOR LEVELS, DETECTS HIGH RISK ATRIAL FIBRILLATION PATIENTS EVEN AFTER ORAL ANTICOAGULATION

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Keywords: atrial fibrillation, antithrombotic therapy, prognosis

Background/Aims: In non-anticoagulated atrial fibrillation (AF) patients, plasma von Willebrand factor levels (VWF, a marker of endothelial damage/dysfunction) has been correlated to stroke risk, and is predictive of vascular events. There are limited data on the prognostic role of biomarkers (including VWF) in AF patients taking oral anticoagulants (OAC). The aim of our study was to assess the prognostic value of VWF in a large cohort of chronic AF patients under oral anticoagulation.
Methods: We studied patients with chronic AF who were stabilised (for at least 6 months) on OAC (INRs 2.0-3.0). Stroke risk by CHADS2 score was recorded. VWF levels, were determined. Patients were followed-up for two years, and adverse events (thrombotic and vascular episodes, major haemorrhage and death) were recorded. We used a cut-off of 220 UI/mL for VWF (assessed by ROC curves) for adverse events.
Results: We included 816 patients (50% male; age 75±8 years) with median CHADS2 score of 2 (1-3) who were followed-up for (median) 708 days (range 18-1085). VWF levels were 171(131-230) UI/mL. On univariate analysis, VWF, CHADS2 score, ischaemic heart disease, hypercholesterolemia and smoking were associated with future thrombotic and vascular events (all $p<0.05$). After multivariate analysis, only high VWF levels remained significant (Cox regression HR 3.49, 95%CI 2.16-5.64; $p<0.001$). High levels of VWF were also predictive for major haemorrhage (HR 4.63, 95%CI 2.61-8.21, $p<0.001$). CHADS2 score, high VWF levels and chronic kidney failure were associated with death (all $p<0.05$) but on multivariate analysis, VWF levels and CHADS2 score were independently related to mortality (CHADS2 score HR 1.38, 95%CI 1.12-1.72, $p=0.003$ and VWF3.00, 95%CI 1.72-5.24, $p<0.001$).
Conclusions: Plasma VWF, indicating endothelial damage/dysfunction, is an independent risk factor for adverse events in anticoagulated AF patients. This biomarker may potentially be used to refine stroke and bleeding risk stratification in AF.

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☺ P683

A NATIONAL SURVEY OF THE MANAGEMENT OF ATRIAL FIBRILLATION WITH ANTITHROMBOTIC DRUGS IN ITALIAN PRIMARY CARE

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Aims: The aims of this study were to investigate trends in the incidence of diagnosed atrial fibrillation (AF), and to identify factors associated with the prescription of antithrombotics (ATs) and to identify the persistence of patients with oral anticoagulant (OAC) treatment in primary care.
Methods: We conducted a retrospective cohort study using data obtained from 400 Italian primary care physicians providing information to the Health Search/Thales Database from 2001 to 2004.
Results: The age-standardised incidence of AF was: 3.9–3.0 cases, and 3.6–3.0 cases per 1,000 person-years in males and females, respectively. During the study period, 2,016 (37.2%) patients had no prescription, 1,663 (30.7%) were prescribed an antiplatelet (AP) agent, 1,440 (26.6%) were prescribed an OAC and 301 (5.5%) had both prescriptions. The date of diagnosis ($p=0.0001$) affected the likelihood of receiving an OAC. AP, but not OAC, use significantly increased with a worsening stroke risk profile using the CHADS2 risk score. Older age increased the probability ($p<0.0001$) of receiving an AP, but not an OAC. Approximately 42% and 24% of patients persisted with OAC treatment at one and two years, respectively, the remainder interrupted or discontinued their treatment.
Conclusions: Underuse and discontinuation of OAC treatment is common in incident AF patients. Risk stratification only partially influences AT management.

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☺ P488

INCIDENCE OF THROMBOEMBOLIC AND BLEEDING COMPLICATIONS IN PATIENTS WITH ATRIAL FIBRILLATION TREATED WITH DIFFERENT ANTITHROMBOTIC REGIMENS

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Keywords: atrial fibrillation, thromboprophylaxis

Background: Atrial fibrillation (AF) is an important independent risk factor for ischemic stroke. The estimated annual rate of ischemic stroke among patients (pts) with AF not treated with antithrombotic therapy is about 5%.
Aim of the study: to compare the incidence of thromboembolic (TE) and bleeding complications in pts with AF treated with vitamin K antagonists (VKA) only and combination of VKA and ASA between two groups, receiving primary (PP) and the secondary (SP) thromboprophylaxis.
Materials and methods: We have investigated 251 patients, 155 patients receiving PP and 96 receiving SP, after ischemic stroke (IS), age range 38-84y, average 61 and 45-88, av. 66.5 in the primary and secondary prophylaxis group. The follow up was 9m-17y8m, total 881 pts/yr. VKA only group consisted of 158 pts and 93 pts received combined treatment –VKA +ASA 100mg daily. Target INR for both groups was 2-3. Prothrombin time-INR was determined from capillary blood, using Thrombotest reagent and coagulometer Thrombotrack, manufactured by Axis Shield, Norway.
Results: During the follow up 50 bleedings occurred, 31(0.11%/yr) and 19 (0.32%/yr) in the PP and SP group respectively. Regarding the therapeutic approach, in the VKA only group 31 episodes of bleeding occurred (0.04%/yr), 22(0.08%/yr) and 9 (0.02%/yr) in the PP and SP group respectively; in the VKA+ASA group there were 19 bleedings(0.02%/yr), 0.03%/yr and 0.02%/yr in the PP and SP group respectively. Major, minor and minimal bleeding occurred in 12 patients (0.01%/yr), 26 (0.03%/yr) and 11 (0.01%/yr) respectively. TE complications occurred in 8 cases, 4(0.01%/yr) in PP group and 4(0.01%/yr) in SP group, 2events (0.003%/yr) and 6 (0.007%/yr) in the VKA+ASA group and VKA only, respectively.
Conclusions: The rate of bleeding complications is similar in both investigated groups, and the VKA +ASA treatment is more efficient in terms of preventing TE events.

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☺ P140

USE OF STATINS AND RECURRENCE OF ATRIAL FIBRILLATION AFTER CATHETER ABLATION OR ELECTRICAL CARDIOVERSION: A SYSTEMATIC REVIEW AND A META-ANALYSIS OF THE LITERATURE

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Keywords: atrial fibrillation, statins, cardioversion

Background: Statins have important pleiotropic effects and have been shown to reduce vascular inflammation. Some evidences suggest that statins may have a role in the primary prevention of atrial fibrillation (AF), whereas little is know on the role of statins in patients with existing AF. We performed a meta-analysis of the literature to assess the effect of statins on the recurrence of AF after electrical cardioversion or ablation.

Methods: MEDLINE and EMBASE databases were searched up to January 2010. Relative risks (RR) and 95% confidence intervals (CIs) were then calculated and pooled using a random-effects model. For statistically significant treatment effects, we determined the absolute risk reduction and number-needed-to-treat for benefit (NNT) to prevent a recurrence. Statistical heterogeneity was evaluated through the use of I2 statistics.

Results: Sixteen studies were included in our systematic review. Statins did not reduce the risk of AF recurrence after ablation (4 studies including 750 patients; RR, 1.04; 95% CI, 0.85-1.28, p=0.71; I2 = 34%). Conversely, the use of statins was associated with a significantly reduced risk of AF recurrence after electrical cardioversion (12 studies including 1790 patients; RR, 0.78; 95% CI, 0.67-0.90, p=0.0003; I2 = 34%). The absolute risk reduction was 7.2% with a NNT of 14. This reduction was not statistically significant when the analysis was restricted to randomized controlled trials (RCTs) only (5 studies, 458 patients, RR, 0.76; 95% CI, 0.48-1.20).

Conclusions: Statins may lower the risk of AF recurrence after electrical cardioversion, but not ablation. However, this finding should be considered with caution, and larger RCTs are warranted to confirm our preliminary results.

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☺ P146

RESTORATION OF SINUS RHYTHM AFTER ELECTRICAL CARDIOVERSION IMPROVES ENDOTHELIAL FUNCTION IN PATIENTS WITH PERSISTENT ATRIAL FIBRILLATION

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Keywords: atrial fibrillation, endothelial dysfunction

Background/Aims: Atrial fibrillation (AF) is associated with endothelial dysfunction. Increased levels of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase contribute to endothelial dysfunction. We studied levels ADMA and L-arginine and their relation to maintenance of sinus rhythm after electrical cardioversion for AF, and the effects of angiotensin receptor blockade on these variables.

Methods: In a double blind, placebo-controlled study (Candesartan in the Prevention of Relapsing Atrial Fibrillation, CAPRAF), patients with persistent AF were randomised to candesartan 8 mg once daily or placebo for 3–6 weeks before and candesartan 16 mg once daily or placebo for 6 months after cardioversion. Plasma levels of L-arginine and ADMA were measured at baseline and at the end of the study, by use of an HPLC-method. The impact of ADMA and L-arginine levels and the L-arginine/ADMA ratio on rhythm outcome was analysed using Kaplan Meier analysis of quartiles, whereas analysis of covariance was used to analyse the impact of treatment with candesartan and rhythm outcome on these variables.

Results: Blood samples were available in 164 patients at baseline and in 98 at the end of study. Baseline levels of ADMA, L-arginine and L-arginine/ADMA ratio were not associated with rhythm outcome, and treatment with candesartan had no impact on the variables. Restoration and maintenance of sinus rhythm for 6 months after cardioversion showed no significant impact on L-arginine or ADMA levels (p=0.301 and p=0.076, respectively). However, an increased L-arginine/ADMA ratio was found in patients who remained in sinus rhythm (n=37) for 6 months when compared to patients with AF recurrence (n=61) (mean +11 vs. -4; p=0.006).

Conclusions: Increased L-arginine/ADMA ratio was found in patients still in sinus rhythm 6 months after cardioversion for persistent AF. Our findings suggest that sinus rhythm restoration and maintenance are associated with improved endothelial function.

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P147

ADMA LEVELS AS MEASURE OF ENDOTHELIAL DYSFUNCTION ARE INCREASED IN ELDERLY PATIENTS WITH ATRIAL FIBRILLATION

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Keywords: atrial fibrillation, endothelial dysfunction

Background/Aims: The prevalence of atrial fibrillation (AF) is increasing. Several independent risk factors for AF have been identified, however, the importance of endothelial dysfunction is still not clarified.
The aim of the present study was to evaluate the levels of L-arginine, the substrate for nitric oxide (NO) and the asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO-synthase, as related to the presence (or absence) of AF.

Material and methods: This is a case control study consisting of 75-year old subjects with permanent AF (n=62) and control subjects in sinus rhythm (n=124), matched for gender. Clinical data were obtained and fasting blood samples were collected at study entrance. EDTA-plasma was used for L-arginine and ADMA analyses, performed by an HPLC-method. Statistics: Group differences were compared by t-test and Chi-square was used for trend analysis through quartiles. Multiple regression models were performed for estimation of independency.

Results: Means (SD) are given. Levels of ADMA were elevated in AF vs controls (0.69 (0.13) vs 0.62 (0.12) umol/L, p<0.001) and the L-arginine/ADMA ratios were lower (114 (23) vs 124 (27), p=0.015), still significant after adjustment for relevant covariates (creatinine, hypertension, body mass index, diabetes, ischemic heart disease, LDL-cholesterol) (p=0.007 and p=0.037, respectively). When dividing the ADMA levels into quartiles there was a significant trend for having AF with increasing levels of ADMA (p=0.001) with a clear cut-off at the 25th percentile (<0.54umol/L), giving an OR for having AF of 7.16 (95% CI 2.43-21.09) (p<0.001) with higher levels. A similar inverse trend was seen for the L-arginine/ADMA ratio.

Conclusions: Elevated levels of ADMA are significantly predicting the presence of atrial fibrillation in the elderly, elucidating the importance of endothelial dysfunction in such patients.

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P128

PLATELET REACTIVITY AND ATRIAL FIBRILLATION

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Background: Relationships between platelet reactivity and atrial fibrillation (AF) have not been examined previously. Thus, platelet reactivity was compared with the incidence of sinus rhythm (SR) after a surveillance period exceeding two years after elective electrical cardioversion.

Methods: 33 patients having non-valvular AF subject to elective electrical cardioversion were included. Instantaneously before cardioversion determination of markers (see below) reflecting platelet reactivity was carried out. After an average of 26±8(SD) months an ECG was analyzed and platelet laboratory analysis repeated. A flow cytometry technique was used to determine platelet reactivity i.e. surface bound fibrinogen after stimulation. A thrombin receptor activating peptide (TRAP-6) (54 and 74 umol/L) and ADP (1.7 and 8.5 umol/L) were used as platelet agonists.

Results: As the study was terminated subjects with AF (n=18) had a tendency towards lower platelet reactivity. Compared with day 1 it reached significance (p=0.016) when using 1.7 umol/L ADP as a platelet agonist. For these patients responses towards the other agonists did not change significantly over time. On the contrary, after 26±8(SD) months subjects with SR (n=15) had significant lower platelet reactivity when stimulating with both agonists pairs. The p-values for the two TRAP-6 dilutions (1.7 and 8.5 umol/L) were p=0.042 and p=0.006. The corresponding figures for ADP (1.7 and 8.5 umol/L) proved to be p=0.042 and p=0.006, respectively.

Conclusions: After 26±8(SD) months patients returning to AF had higher platelet reactivity than those who remained in SR. The finding provides a connection between clot formation and arrhythmia.

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P563

ANTITHROMBOTIC PROPHYLAXIS IN ELDERLY PATIENTS ATRIAL FIBRILLATION ADMITTED TO INTERNAL MEDICINE WARDS IN ITALY: ADHERENCE TO GUIDELINES

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Keywords: atrial fibrillation, oral anticoagulants, vitamin K antagonists, guidelines, clinical audit

Background: Elderly patients with atrial fibrillation (AF) often fulfill the indication for vitamin K antagonists (VKA), but age, compliance, frailty, logistics and co-morbidities represent barriers to the adoption of VKA.
Aims: To verify adherence to guidelines (ACCP 2008) when indication to VKAs is present as assessed by the CHADS2 score at admission and discharge in a cohort of elderly patients.
Materials and methods: The project was a collaboration of the Italian Society of Internal Medicine and the Pharmacological Research Institute Mario Negri. During each of four selected weeks in 2008, 10 patients were recruited in any participating Centre and demographical and clinical information collected. Patients with AF (ICD9 427.31/32) and those treated with VKAs (ATC B01AA) or antiplateletet drugs (AP, ATC B01AC) were included in this analysis. The role for hemorrhage and cancer was also evaluated.
Results: 35 centres enrolled 1333 patients, of which 1156 were analysed. AF was diagnosed in 18.7%, of which 79.7% was > 75 years, 57.0% had hypertension, 25.7% diabetes, 26.2% heart failure, 0.8% previous stroke. In patients with CHADS2 2, VKA has been withdrawn in 19.6 % and started in 13.7%; among those discharged not on VKAs, 5/108 were admitted for bleeding and 1/44 bled during hospitalization. 31% of untreated and 16% of treated patients had cancer (OR = 0.43, *p* 0.03).
Conclusions: In elderly patients, clinical practice was found far away from guidelines. VKA treatment was largely underused and hospitalization did not have a large effect on adherence to guidelines.

Table

	All	VKA	AP	VKA+AP	No treatment
All		33.2 (34.7)	37.0 (30.6)	2.1 (1.8)	27.7 (32.9)
$CHADS_2 \geq 2$	68.9 (69.4)	30.2 (33.1)	40.1 (35.7)	2.5 (2.6)	27.2 (28.6)
$CHADS_2 = 1$	23.4 (25.2)	42.4 (39.3)	32.2 (17.9)	-	25.4 (42.9)
$CHADS_2 = 0$	5.9 (5.4)	28.6 (33.3)	21.4 (25.0)	7.1 (0)	42.9 (41.7)

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Miscellanea

Thursday 8th July, 2010

P160

INFLUENCE OF SHORT-TERM FISH EATING ON LIPID, FIBRINOLYTIC, AND RHEOLOGICAL PARAMETERS IN HEALTHY SUBJECTS

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Keywords: nutrition, fibrinolysis, rheology, intervention study

Background/Aims: Fish intake has long been indicated as a protective dietary factor for cardiovascular diseases, due to the beneficial effects of its content of omega-3 polyunsaturated fatty acids (EPA and DHA). However, the mechanisms underlying this protection have not been fully elucidated. Aim of this study was to evaluate the influence of short-term dietary intake of fish on biomarkers related to the atherosclerotic process.
Patients and methods: For a period of 10 weeks, 10 healthy subjects (6 males; 4 females) with a mean age of 48 years consumed during their main meals contents of about 300 g per week of tuna meat, each subject consuming 150g dorsal and 150g ventral muscle slides from two bluefin tunas (Thunnus thynnus). Lipid (total cholesterol, HDL-cholesterol, LDL-cholesterol and tryglicerides), fibrinolytic [clot lysing time (CLT), plasminogen inhibitor activator-1 (PAI-1), tissue factor pathway inhibitor (TAFI)], and haemorheological parameters [whole blood viscosity (WBV), plasma viscosity (PV), erythrocyte filtration rate (EF)], were determined in samples obtained at the beginning (T0) and at the end of the experimental period (T1).
Results: Lipid profile showed a significant improvement at the end of the dietary intervention, as seen by lower levels of total cholesterol [T1: (200.2 ± 49.5 mg/dL) vs. T0: (219.3 ± 48.2 mg/dL); p=0.01], LDL-cholesterol [T1: (125.8 ± 40.9 mg/dL) vs. T0: (140.2 ± 46.6 mg/dL); p=0.02], and triglycerides [T1: (83.7 ± 40.2 mg/dL) vs. T0: (112.1 ± 57.8 mg/dL); p=0.002]. With regard to haemorheological parameters, a significant (p<0.05) improvement of WBV at both highest and lowest shear rates was reported (WBV 94.500 sec-1: 4.3 ± 0.2 vs. 4.5 ± 0.4; WBV 0.512 sec-1: 20.1 ± 2.2 vs. 21.8 ± 2.5, for T1 and T0, respectively). Moreover, interestingly, as regarding fibrinolytic parameters, dietary intervention with fish reported a significant increase of CLT, [T1: (57.7 ± 9.5 min.) vs. T0: (47.1 ± 14.7 min.); p<0.05], possibly determined by the concomitant increase of PAI-1 [T1: (20.8 ± 15.9 mg/dL) vs. T0: (12.5 ± 10.5 mg/dL); p=0.01] and TAFI [T1: (13.7 ± 1.36 µg/mL) vs. T0: (11.6 ± 1.36 µg/mL); p=0.01] levels.
Conclusions: Dietary short-term intake of fish seems to impose favourable biochemical changes in healthy subjects, as showed by lipid and haemorheological parameters. An impaired fibrinolysis has been otherwise reported at the end of the dietary intervention.

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EFFECTS OF A PERSONALIZED PHYSICAL ACTIVITY PROGRAM ON CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND WEIGHT LOSS

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Aims: To assess the effect of a personalized physical activity program on weight and on circulating (CPC) and endothelial progenitor cells (EPC) in healthy subjects.
Materials and methods: Anthropometric measurements with body composition, cardiopulmonary test, a maximal stress exercise test with maximal oxygen uptake (VO2max), and a series of biochemical analyses were conducted before (T0) and after 3 months of physical activity (T1). CPC and EPC were determined by using flow cytometry and defined as CD34+, CD133+ and CD34+/CD133+ for CPC and CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+ for EPC.
Results: A total of 80 healthy overweight and obese subjects completed the program. The exercise program consisted of sessions of 45 minutes of aerobic exercise 3 times per week tailored according to the individual anaerobic threshold. At the end of the program, we divided the population into 2 groups, responders (group A) and non-responders (group B) according to the increase of VO2max. Group A reported significant reductions of weight by 3.1% (92.3 ± 17.1 vs. 89.4 ± 15.9 Kg; p<0.0001) and fat mass by 4.4% (32.2 ± 9.9 vs. 30.9 ± 9.5 Kg; p<0.0001) while group B showed a percentage of increase of fat mass by 1.5% at T1. In group A, a trend of increase at T1 for circulating levels of CPC and EPC was observed, reaching the statistical significance for all the three types of EPC. On the contrary, group B showed no significant increase in CPC and EPC. Furthermore, a significant correlation between decrease of fat mass and increase of CD133+/KDR+ EPC was reported in group A subjects (r= 0.50; p= 0.04).
Conclusions: Three months of physical activity significantly improved anthropometric measurements. A beneficial effect was observed regarding the increased number of EPCs in the responders' group, in relation to weight loss.

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DETERMINANTS OF THROMBOXANE BIOSYNTHESIS IN RHEUMATOID ARTHRITIS: ROLE OF RAGE AND OXIDANT STRESS

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Keywords: platelet, inflammation, oxidative stress

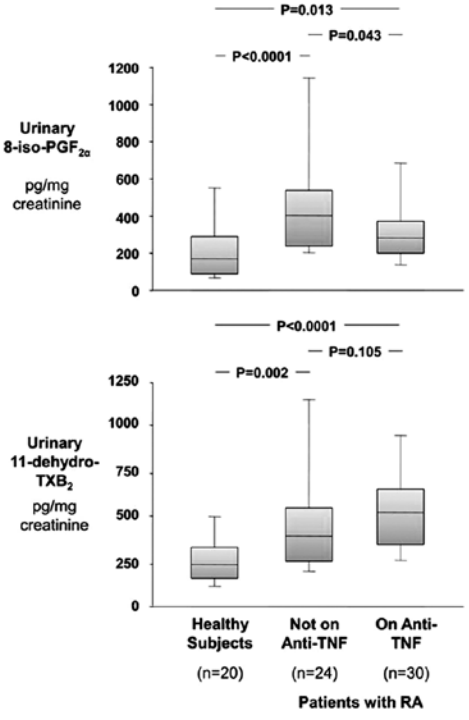
Background/Aims: The biosynthesis of thromboxane (TX) by platelets and other cells by inflammatory triggers may provide a molecular link between chronic inflammatory disease and atherothrombosis. In the present study, our aims were to evaluate: (i) the rate of TX biosynthesis in patients with rheumatoid arthritis (RA); (ii) the role of lipid peroxidation and receptor for advanced glycation end-products (RAGE) hyperactivation as determinants of altered TX biosynthesis in this setting, and (iii) possible modulation of these biochemical abnormalities by anti-tumor necrosis factor (TNF) agents.

Methods: Fifty-four patients with RA and 20 healthy subjects were recruited and a cross sectional comparison of urinary 11-dehydro-TXB2, 8-iso-PGF2a and plasma esRAGE levels was performed between patients and controls.

Results: Urinary 11-dehydro-TXB2 was significantly higher in RA patients than in healthy controls. Furthermore, urinary 8-iso-PGF2a and plasma esRAGE were higher and lower, respectively, in patients than in controls. A direct correlation was found between urinary 11-dehydro-TXB2 and 8-iso-PGF2a only in patients not on anti-TNF therapy. Conversely, patients on anti-TNF therapy showed significantly lower urinary 8-iso-PGF2a but not 11-dehydro-TXB2 than anti-TNF treated subjects, with esRAGE as the only independent predictor of 11-dehydro-TXB2 in this group of patients.

Conclusions: We provided biochemical evidence of enhanced TX biosynthesis in patients with RA, driven, at least in part, by lipid peroxidation. Treatment with anti-TNF agents may blunt isoprostane generation in the absence of significant effects on TX biosynthesis. We suggest that RAGE hyperactivity may escape TNF blockade thus contributing to persistent TX biosynthesis in this setting.

Figure: Urinary excretion of 8-iso-PGF2α (panel A) and 11-dehydro-TXB2 (panel A) in healthy subjects and in anti-TNF-treated and untreated patients with rheumatoid arthritis (RA)



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BIOLOGICAL EVALUATION OF SYNTHETIC LINEAR ANALOGUE PEPTIDES OF 1811-1818 LOOP OF THE A3SUBUNIT OF THE LIGHT CHAIN A3-C1-C2 OF FVIII

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Keywords: synthetic linear analogue peptides, 1811-1818 loop of the A3, inhibition of FVIII activation

Background: FVIII is synthesized as a multidomainal single-chained molecule (A1-A2-B-A3-C1-C2), with a molecular mass of ~300 kDa, circulates as a partial proteolyzed protein containing a heavychain (A1-A2-B domains) and a lightchain (A3-C1-C2 domains), which are held together by a metal ion dependent linkage. Recent studies have identified FVIII lightchain region Glu1811–Lys1818 as being involved in FIXa binding and in the assembly of the FX-activating FIXa/FVIIIa complex.

Aims: We have synthesized linear analogue peptides (AP) of the 1811-1818 loop of the A3 subunit of the lightchain of FVIIIa, in order to examine their anticoagulant activity.

Methods: The peptides were synthesized by the solid phase technique, using the 2-Chlorotrityl chloride resin, as a stationary phase, by the method of carbodiimides (DIC/HOBt), while for the peptide amides synthesis the Rink amide MBHA resin was used. The N-terminal amino groups of the peptides were either acetylated or left unprotected, while all the other protecting groups used during the synthetic procedure were removed. The reaction products were purified by reversed phase HPLC and identified by ESI-MS. Research protocol for checking the inhibition by AP: Incubation 30min in 37oC.
A) 300μl AP (1mg/ml in Owren-Koller buffer) +300μl NP, using as control 300μl OKbuffer +300μl NP. B) 300μl AP (1mg/ml in OKbuffer) +300μl pure recombinate FVIII (1u/ml in OKbuffer).

Conclusions: We tested the mixtures in IL coagulation instrument, measured the aPTT and the FVIII activity. The results was a prolongation of aPTT varying more >6sec for the mixture of analogue Ac-ETKTYFWK-NH2 and an important reduction of FVIII activity (> 40%).

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IMPACT OF THE WASHOUT PERIOD BETWEEN TWO COMBINED CONTRACEPTIVES ON HEPATIC ESTROGEN-SENSITIVE PROTEINS

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Keywords: Vaginal ring, coagulation, SHBG

Background: Combined contraception with ethinyl-estradiol (EE) by oral or non-oral route is associated with an increased risk of venous thromboembolism (VTE). Changes in coagulation and in SHBG (Sex Hormone Binding Globulin), a marker of estrogenicity, have been evaluated with regards to risk. They are proposed for assessing the hormonal impact of new contraceptives before epidemiological studies of the risk become available.

Materials and methods: Coagulation and SHBG changes were studied after use of a contraceptive vaginal ring (CVR) delivering Nestorone and EE (NES/EE 150/15μg/day) in women with or without previous hormonal use. Fibrinogen (Fg), factor VIII (FVIII), protein S (PS), SHBG assays were performed at baseline, after 132 to 252 days and >252 days in 93 women using a CVR: 60 were naïve (non-previous users), 33 using hormonal contraception were directly shifted to the CVR. Preliminary results. At baseline, as compared to naïve users, previous users had lower levels of PS (mean±SD 76.3±15.7 and 88.5±16.0% respectively, p=0.001) and higher Fg (306.5±66.8 and 262.3±63.1mg/dL, p=0.001), SHBG (150.2±64.7 and 58.8±26.2 nmol/L, p<0.0001) and FVIII (125.5±42.2 and 107.9±39.9%, p=0.03). After 132-252 days, in naïve users, there was a decrease of PS (p=0.002) and increases of Fg (p=0.002) and SHBG (p<0.001). Changes were similar after >252 days in naive users. In contrast, changes from baseline were not significant in previous users, except for SHBG at 132-252 days (p=0.01), indicating that the effect of previous use of EE-based contraceptives had already modified those proteins.

Conclusions: Prior immediate exposure to combined contraceptives has an important impact for the interpretation of changes induced by a new contraception. It may lead to misleading interpretation, namely an absence of biological effect. A wash-out of 6-8 weeks between 2 hormonal combined contraceptives is recommended for studying changes in coagulation parameters or other liver proteins.

Acknowledgements: Study supported by NICHD grant HHSN275200403377

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DECREASED IMMUNOGENICITY OF PURIFIED TOPICAL BOVINE THROMBIN PREPARATIONS

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Keywords: bovine thrombin, immunogenicity

It has been reported that severe coagulopathy following exposure to topical bovine thrombin may be attributed to the impurities in bovine thrombin preparations. Prepared through the activation of bovine prothrombin by thromboplastin, a crude thrombin preparation was further purified using ion-exchange chromatography and membrane filtration steps yielding thrombin 4A and 4B preparations.
The aim of this study was to compare the relative purity of these bovine thrombin preparations by virtue of the detection of bovine prothrombin-related antigens.
Bovine prothrombin was administered to three individual rabbits on days 0, 21, 42, 91, 123 and 151 using standard immunologic method. Blood was drawn on 30, 50, 105, 137 and 165 days and the pooled antisera from three rabbits were purified to obtain IgG using protein G affinity columns. Utilizing western blotting, serial diluted bovine crude thrombin, 4A and 4B preparations were probed using the prothrombin IgGs from each time point to explore prothrombin-related antigens in these preparations. Compared with the IgG collected on day 30, the prothrombin IgGs collected from day 50 to 165 showed a time-dependent increased detecting ability for the prothrombin antigens in the bovine thrombin preparations. The lowest amount of crude thrombin, 4A and 4B preparations that prothrombin IgG could detect was 0.125U, 10U and 20U, respectively. The rank order of the number of immunoreactive bands in three bovine thrombin preparations probed by the prothrombin IgGs collected from any given time point was consistent and reproducible: crude thrombin > thrombin 4A > thrombin 4B.
The results indicate that thrombin 4B represents the most purified thrombin preparation among three thrombin preparations studied.

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P455

MASTIC ADMINISTRATION DOES NOT INFLUENCE COAGULATION STATUS IN PATIENTS WITH DYSLIPIDEMIA

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Keywords: dyslipidemia, mastic, coagulation

Background/Aims: Dyslipidemia is a common metabolic disorder that has been tightly associated with the development of cardiovascular disease. It has been shown that mastic has anti-inflammatory properties and it is postulated that it may exert anti-coagulant actions as well. The role of mastic administration in dyslipidemic patients has never been investigated. The aim of this study is to assess coagulation status in patients with untreated dyslipidemia and to detect any possible changes after administration of mastic.
Methods: Consecutive non-hospitalized patients with dyslipidemia not requiring pharmacological intervention were randomized to mastic or placebo administration for a two month period. All patients were apparently healthy and did not have known cardiovascular, malignant or inflammatory diseases. Fibrinogen is considered a marker of coagulation status and an inflammatory marker as well, since it is an acute phase reaction protein. It was measured on fasting blood samples of all patients at baseline and 2 months later. Moreover, the endogenous thrombin potential of the patients in the two groups was assessed at baseline and after mastic or placebo administration. The patients that received pharmacological intervention (arm A) were compared with those who received placebo (arm B) at the end of the study.
Results: Out of the 26 dyslipidemic patients that have completed the follow-up period, 13 patients were under mastic treatment (arm A), while 10 received placebo (arm B). Baseline levels of fibrinogen and lipid profile in arm A and arm B did not differ significantly. Arm A patients demonstrated a slight reduction on fibrinogen levels [349 ± 65 mg/dl vs 322 ± 38 mg/dl p=0.089], while arm B patients demonstrated no effect on fibrinogen levels after two months [369 ± 56 mg/dl vs 365 ± 43mg/dl p=0.79]. Endogenous thrombin potential calculated as area under curve AUC showed no difference in either group of patients after administration of mastic or placebo.
Conclusions: The preliminary results of this ongoing study suggest that treatment with mastic fails to affect coagulation status in apparently healthy dyslipidemic patients. Results on a larger cohort of patients in the context of a prospective study are currently being processed and may elucidate the implication, if any, of this anti-inflammatory herb in the prevention of development of cardiovascular disease in patients with dyslipidemia

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ASSOCIATION BETWEEN EARLY MORNING BLOOD PRESSURE LEVEL AND BLOOD RHEOLOGY IN HEALTHY AND HYPERTENSIVE SUBJECTS

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Keywords: blood rheology, high blood pressure, cardiovascular risk

Aims: The purpose of the study was to investigate the association between morning blood pressure level and blood rheology in hypertensive and normotensive, practically healthy control subjects.
Methods: 57 patients with arterial hypertension (AH) (mean age±SD, 51.26±1.94; 30 men and 27 women) and 17 healthy controls (mean age±SD, 51±5.41; 9 men and 8 women) were included in the study. All the subjects underwent investigation of blood rheology and 24 hour ambulatory blood pressure measurements. Patients with cardiovascular, peripheral and cerebrovascular diseases, diabetes mellitus, smokers, pregnant, obese as well as those with secondary forms of hypertension were excluded from the study.
Results: Early morning average systolic, as well as diastolic blood pressures were significantly higher in patients with AH compared with healthy individuals (138.63±3.09 vs. 98.12±1.83, P=0.000 and 85.83±2.04 vs. 58.41±2.77, P=0.000, respectively). In hypertensive subjects early morning SBP, as well as DBP correlated with platelet aggregative activity (r=0.287, P=0.031 and r=0.490, P=0.000, respectively), erythrocyte aggregability (r=0.314, P=0.018 and r=0.292, P=0.027, respectively) and whole blood viscosity (r=0.284, P=0.032 and r=0.433, P=0.001, respectively). Furthermore, early morning DBP correlated with hematocrit level (r=0.317, P=0.016) and plasma viscosity (r=0.318, P=0.016). There was not any kind of correlation between early morning blood pressure levels and haemorheological data in healthy, non-hypertensive subjects.
Conclusions: Early morning blood pressure level, especially diastolic one, in hypertensive patients was associated with blood rheological disturbances. Our data indicate that as high is early morning diastolic blood pressure level, as increased is cardiovascular risk because of haemorheological disturbances in hypertensive population.

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EFFECTS OF A 1-YEAR DIETARY INTERVENTION WITH N-3 POLYUNSATURATED FATTY ACIDS-ENRICHED OLIVE OIL ON NONALCOHOLIC FATTY LIVER DISEASE PATIENTS: A PILOT STUDY

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Keywords: nonalcoholic fatty liver disease, nutrition, olive oil, primary prevention

Aims: Nonalcoholic fatty liver disease (NAFLD) is a worldwide diffuse medical condition. We recently demonstrated that n-3 long chain polyunsaturated fatty acids (n-3 PUFA) are able to restore insulin sensitivity and improve liver steatosis. However, no data concerning food containing or enriched with n-3 PUFA in patients with NAFLD are available. The aim of our study was to evaluate the effectiveness of long-term consumption of food enriched with n-3 PUFA in patients with NAFLD.
Methods: We enrolled 11 patients with clinical, laboratory, and ultrasonography features of NAFLD. They were planned for oral administration of 6.5 mL of olive oil enriched with n-3 PUFA (t-Omega3, tFarma s.r.l, Florence, Italy) for 12 months. Among the eligible patients, 6 (4 males, 2 females) assumed the treatment, while 5 (4 males, 1 female) were used as controls. Both groups underwent blood assay and ultrasound examination at baseline (T0), and after 12 months (T12) of follow-up.
Results: Consumption of olive oil enriched with n-3 PUFA demonstrated a significant improvement of liver echotexture and of Doppler Perfusion Index (DPI) after 12 months (T2: 0.19 ± 0.02 vs. T0: 0.15 ± 0.03; p<0.05), with some cases of normalized liver ultrasonography, whereas no significant changes were seen at the end of follow-up in controls. Moreover, patients who consumed the olive oil enriched with n-3 PUFA showed a significant amelioration of liver enzymes, AST (T2: 29.2 ± 3.4 vs. T0: 48.8 ± 7.6 U/L, p=0.02), ALT (T2: 56.3 ± 14.4 vs. T0: 87 ± 16.9 U/L, p=0.03), GGT (T2: 56.7 ± 11.4 vs. T0: 77.8 ± 16.4 U/L, p=0.03), and of triglycerides (T2: 132.8 ± 63.7 vs. T0: 164.5 ± 85.5 mg/dL, p=0.04) at a general linear model adjusted for age and gender. Interestingly, patients who underwent a nutritional treatment with olive oil enriched with n-3 PUFA reported to have a significant increase of adiponectin levels (T2: 1487.9 ± 96.7 vs. T0: 1143 ± 24.8 µg/mL, p=0.04) while no significant differences in controls have been observed (T2: 1254.9 ± 67.8 vs. T0: 1173.7 ± 78.7 µg/mL).
Conclusions: Long-term consumption of food enriched with n-3 PUFA in patients with NAFLD decreased circulating liver enzymes, triglycerides, with a significant improvement of adiponectin levels. These results suggest that dietary supplementation with n-3 PUFA may be helpful in patients with NAFLD.

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CROSS-REACTIVITY OF RABBIT ANTI-BOVINE THROMBIN IGGS WITH HUMAN ALPHA THROMBIN AND A RECOMBINANT VERSION OF HUMAN THROMBIN (RECOTHROMBIN)

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Keywords: recombinant thrombin, immunogenicity

It has been reported that patients exposed to topical bovine thrombin preparations may develop antibodies to bovine thrombin, factor V or various other proteins. In some cases these antibodies can cross-react with the corresponding human coagulation proteins. The present study was undertaken to determine if the antibodies induced by bovine thrombin antigens in rabbits could also cross-react with human a-thrombin and RecothromTM.
Bovine crude thrombin and its purified versions, thrombin 4A and 4B, were administered to individual groups of rabbits on 0, 21, 42, 91, 123 and 151 days. Blood was drawn from each rabbit on days 30, 50, 105, 137 and 165, respectively. The antiserum from each rabbit and the pooled antisera from individual groups were purified to obtain the IgGs. Utilizing western blotting method, the possible cross-reactivity of each IgG with human thrombin and RecothromTM was explored using serial diluted human α-thrombin (20µg, 10µg, and 5µg) and RecothromTM (10U, 5U, and 2.5U). No cross-reactivity with either human α-thrombin or RecothromTM was observed with both anti-bovine crude thrombin IgGs and thrombin 4B IgGs collected on day 30 and day 165. However, anti-bovine thrombin 4A IgGs showed cross-reactivity with both human α-thrombin and RecothromTM. Cross-reactivity of anti-bovine thrombin 4A IgGs with human α-thrombin and RecothromTM was augmented with time. The minimum concentration of 4A IgG required to exhibit cross-reactivity with human α-thrombin and RecothromTM varied considerably among individual rabbits.
These results demonstrate that rabbit anti-bovine thrombin 4A IgG could cross-react with both human a-thrombin and RecothromTM in a time/concentration-dependent manner.

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MODULATION OF PLATELET ACTIVATION RESPONSES BY HYPERICUM PERFORATUM EXTRACT

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Keywords: platelet activation, anti-platelet effect, hypericum perforatum

Background/Aims: Hypericum perforatum (St. John's wort) is presently one of the most consumed medicinal plants in the world due to its biological activities including antioxidant, anti-inflammatory and anti-depressive. Platelet activation plays a crucial role in cardiovascular diseases and the enhancement of platelet reactivity is a known mechanism that increases the risk of thrombosis. However, the eventual effect of this plant on platelet function had not been investigated. The aim of the present study was to evaluate the *in vitro* effect of the crude extract of Hypericum perforatum on platelet activation responses.
Materials and methods: Platelet activation responses, induced by physiological agonists, before and after treatment with HP, were evaluated using flow cytometry to assess biochemical markers, including: intracellular Ca2+ ([Ca2+]i, GPIIbIIIa activated, CD62 expression. The effect of Hypericum perforatum on platelet aggregation was studied, in platelet rich plasma, by turbidimetry.
Results: Changes in intraplatelet free Ca2+, activated GPIIbIIIa and CD62P expression induced by thrombin, reflected the inhibitory effect of Hypericum perforatum. Intracellular Ca2+ mobilization responses and activated GPIIbIIIa receptors expression were strongly reduced after Hypericum perforatum treatment, resulting in an inhibition higher than 50%. For these markers the inhibition observed was dependent on the concentration of the Hypericum perforatum. CD62P expression was less affected. On the other hand, Hypericum perforatum significantly inhibited platelet aggregation induced by collagen, ADP and epinephrine.
Conclusions: These results showed the inhibitory effect of Hypericum perforatum on platelet activation and emphasize the need for further studies. We believe that it could be recognized as a substance with anti-platelet properties and thus considered in the prevention of cardiovascular diseases.

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ASPECTS OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH METABOLIC SYNDROME AND HEPATIC DISEASE

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Keywords: metabolc sindrome, endothelial dysfunction

Background/Aims: Pathophysiologic concepts of obesity and atherosclerosis share similar pathways; inflammation constitutes a mechanistic link between them: adipokines released by adipose tissues induce insuline resistence, endothelial dysfunction, hypercoagulability and fatty acids and triglycerides storage alteration. The long term nutrient excess and unbalanced energy expenditure leads to fatty acid accumulation in the liver and altered functions, e.g.: synthesis, coagulation and glicorylation. When others hepatic aggression superposed like toxines, virus and fatty storage expression of endothelial markers is affected.
Aim of this study is to establish a correlation between some endothelial markers and liver injury in patients with metabolic syndrome.
Materials and methods: We investigated 60 patients diagnosticated with metabolic syndrome (MS): hypertension, dislipidemia and diabetes mellitus divided in three equal subgroups with hepatic pathology: ethanol, hepatic steatosis and viral infection (virus B or C). For these, we appreciate hepatic affection by biochemical test (transaminases, lipidic profile, glycemia) ultrasonografic methods, superior endoscopy and for some of them hepatic biopsy. We analised plasmatic levels of adipocitokines (leptin and adiponectin), homocysteine, coagulation fonction determined by von Willebrand factor (VWF) and fibrinogenemia as inflammation marker.
Results: In patients with ethanolic hepatitis we observed elevated values for VWF (65%) and homocysteinemia (80%); similary for fibrinogenemia (60%); adipocitokines were elevated only by leptin (40%) while adiponectin did not have significant variations. In patients with viral infection VWF was elevated to 85% patients, homocysteine - (60%) without oscillation for adipocitokines levels and fibrinogenemia was slowly elevated. In group with hepatic steatosis, homocysteine was elevated to 70% patients and for VWF and fibrinogenemia to 50% of them. Leptin is significant elevated (55% patients).
Conclusions:
1) Procoagulation status and homocystinemia levels were significantly elevated to patients with metabolic syndrome and other hepatic diseases.
2) Plasmatic values of adipocitokines was modified by leptin especially in steatosis and ethanolic comorbidity.
3) Fibrinogenemia like inflammation marker was elevated in all associations.

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ROLE OF ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF HYPERTENSION AND HEMOSTATIC COMPLICATIONS IN HEMODIALYSIS PATIENTS

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Keywords: hemodialysis, endothelial dysfunction, hypertension

Background: Hemodialysis (HD) patients face a markedly increased risk of vascular and hemostatic complications.
Aims: Assessing the role of endothelial dysfunction (ED) in the pathogenesis of hypertension and hemostatic complications in this population.
Methods: Thrombomodulin (TM), von Willebrand factor (VWF), endothelin-1 (ET-1), nitric oxide (NO) and thrombin-antithrombin complex (TAT) were measured in 30 HD patients (15 normotensive and 15 hypertensive patients) and 15 healthy controls.
Results: TM, VWF and ET-1 were significantly increased in all HD patients in comparison to controls. However, hypertensive HD patients showed significant increase in these parameters when compared to normotensive patients. NO was significantly increased in normotensive HD patients in comparison to both controls and hypertensive patients. This increase may counteract the vasoconstrictive effect of elevated ET-1 level and maintain the normal blood pressure. However, increased NO level inhibits platelet adhesion and aggregation thus predispose these patients to hemorrhagic complications. In contrast, hypertensive patients showed significant reduction in NO level in comparison to healthy controls and normotensive patients. This finding clarifies that altered NO/ET-1 balance could be involved in the pathogenesis of hypertension. As platelet aggregation plays a major role in thrombus formation, hypertensive HD patients with low NO levels are prone to thrombotic complications. TAT was significantly increased in normotensive and hypertensive HD patients when compared to healthy controls. The increase was also significant on comparing hypertensive to normotensive HD patients. A significant positive correlation between TAT and each of vWF and ET-1 together with a significant inverse correlation between TAT and NO were detected in hypertensive patients. These correlations demonstrate the involvement of ED in the development of thrombotic tendency.
Conclusions: ED plays a role in the pathogenesis of hypertension and hemostatic complications in HD patients. Modulation of endothelial function may offer a novel strategy to reduce these serious complications.

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CYSTATIN C IS A RISK FACTOR FOR VENOUS THROMBOEMBOLISM – THE TROMSØ STUDY

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Keywords: cystatin-C, venous thromboembolism

Background: Cystatin C is a marker of renal function. Recent epidemiological studies have shown that cystatin C is superior to creatinine and glomerular filtration rate (GFR) to predict cardiovascular morbidity and mortality. The aim of our study was to evaluate cystatin C as a risk factor of venous thromboembolsim (VTE) and to compare the predictive value of cystatin C with estimated GFR (eGFR) for VTE in a population-based prospective study.
Methods: Serum creatinine and cystatin C were measured in 6651 men and women, aged 25-84 years, who participated in the Tromsø Study in 1994-95. Incident VTE-events were collected from the date of inclusion through the end of follow-up, September 1, 2007. Cox-regression models were used to calculate hazard ratios (HR) with 95% confidence interval (CI) for VTE.
Results: There were 216 incident VTE-events during a median of 12.3 years of follow-up. Cystatin C levels were significantly associated with increased risk of VTE. Subjects with cystatin C ≥0.95 mg/L (upper quartile) had a 2-fold increased risk of VTE compared to those ≤0.76 mg/L (lower quartile) (HR 1.97, 95% CI: 1.29-3.02, p for trend across quartiles <0.001) in multivariable analysis adjusted for age, sex, body mass index, diabetes and hypertension. eGFR was inversely associated with VTE (multivariable HR per 20 ml/min/1.73m2 decrease in eGFR 1.21, 95% CI: 1.03-1.42). There were no significant differences in risk of VTE within predefined stages of renal disease.
Conclusions: In our study, cystatin C was a significant risk factor for VTE. Our findings suggest that cystatin C levels are also superior to eGFR to predict VTE in a general population.

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☺ P587

(APO-) LIPOPROTEIN LEVELS AND THE RISK OF VENOUS THROMBOEMBOLISM. RESULTS FROM A PROSPECTIVE POPULATION BASED COHORT STUDY

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Background: Recent studies indicated that cardiovascular risk factors may also predispose for venous thromboembolism (VTE). Data on the relation between lipid profile and VTE risk are mainly based on classical lipoproteins, such as total cholesterol (TC), triglycerides, high- (HDL) and low-density lipoprotein (LDL). We assessed whether various (apo)lipoproteins and their ratios, which are in general stronger predictors of arterial thromboembolism than classical lipoproteins, are also associated with VTE.
Methods: In 1997, all inhabitants of the city of Groningen, aged 28-75 years (n=85,421) were invited to participate in the PREVEND study designed to prospectively investigate cardiovascular and renal mortality and morbidity. Of responding subjects a cohort of 8,592 subjects was selected that completed extensive screening at our outpatient clinic. Data were collected on cardiovascular risk factors including fasting lipid profile. Only symptomatic and objectively verified VTE were considered. Association between lipid profile and VTE was assessed by Cox-models.
Results: Of 8,592 subjects (mean age±SD, 49±13 years; 50% male), 129 experienced VTE (51% idiopathic) during a mean follow-up period of 8.6±1.8 years. None of all various lipid parameters (i.e., TC, LDL, HDL, triglycerides, apolipoprotein (apo)-A1, apo-A2, apo-B, apo-E, lipoprotein (a), and ratios of apo-B/apo-A1, apo-B/apo-A2; TC/HDL, LDL/HDL) showed significant association with overall VTE after age- and sex-adjustment. However, by restriction to idiopathic VTE, apo-A1, apo-A2 and ratio of apo-B/apo-A2 showed a significant age- and sex-adjusted association with VTE (P<0.04). After additional adjustment for various cardiovascular risk factors, prior arterial thromboembolism, levels of C-reactive protein and use of lipid-lowering therapy, only apo-A1 (P=0.018) and apo-A2 (P=0.002) maintained significant inverse association with idiopathic VTE. Each 10% decrease in apo-A1 and apo-A2 levels were associated with 9% (HR, 1.09; 95%CI, 0.99-1.23) and 12% (HR, 1.12; 95%CI, 1.02-1.23) idiopathic VTE risk elevation, respectively.
Conclusions: Low apolipoprotein-A1 and -A2 levels are independent risk factors of idiopathic VTE.

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ANTHROPOMETRIC MEASURES OF OBESITY, BODY HEIGHT AND RISK OF VENOUS THROMBOEMBOLISM. THE TROMSØ STUDY

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Keywords: body height, weight, venous thromboembolism

Background/Aims: Obesity is a well established risk factor for venous thromboembolism (VTE). Recent studies have suggested that body height is a sex-specific risk factor for VTE in men. The aim of our study was to investigate the combined effect of obesity and body height on the risk of VTE in men and women.
Methods: Personal characteristics and anthropometric measures of obesity and body height were collected in 26 714 men and women, aged 25-97 years, who participated in the fourth survey of the Tromsø study in 1994-95. Incident VTE events were registered through the end of follow-up (September 1, 2007). Subjects were divided into subgroups based on body mass index (BMI) and quartiles of height. Cox regression models were used to calculate hazard ratios (HR) with 95% confidence interval (CI) for VTE.
Results: There were 457 incident VTE events during a median of 12.5 years of follow-up. A tall stature was associated with increased risk of VTE both within lean (BMI<25 kg/m2) and obese (BMI≥30 kg/m2) men, but not in women. Multivariable HRs for upper (≥182 cm) versus lower (<173 cm) quartile of height were 2.5 (95% CI: 1.3-4.8) in lean and 2.7 (95% CI: 1.1-7.0) in obese men. Tall, obese men had more than 5-fold (multivariable HR 5.4; 95% CI: 2.5-11.9) increased risk of VTE compared to lean men with a short stature. Tall (≥168 cm), obese women had a 3-fold (multivariable HR 3.1; 95% CI: 1.4-7.0) increased risk of VTE compared to lean, short (<160 cm) women.
Conclusions: In our study, body height was a risk factor for VTE in both lean and obese men, but not in women. The combination of obesity and a tall stature was associated with a substantially increased risk of VTE, especially in men, and suggest additive effects of obesity and height on risk of VTE.

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RISK OF RECURRENT VENOUS THROMBOEMBOLISM IS NOT INCREASED IN SUBJECTS WITH ELEVATED ALBUMINURIA: RESULTS OF A POPULATION BASED COHORT STUDY

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Keywords: albuminuria, recurrent VTE

Background: Recently we identified elevated albuminuria as a risk factor for first venous thromboembolism (VTE). The risk of recurrent VTE (rVTE) in patients with elevated albuminuria is unknown, yet an important issue for their clinical management.
Material and Methods: On account of the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, all inhabitants of the city of Groningen, aged 28-75 years (n=85,421), were sent a questionnaire regarding arterial risk factors and a vial to collect a first morning urine sample. Subjects with VTE were identified by national registries of hospital discharge diagnoses and death certificates and by the regional anticoagulation clinic. Events were objectively verified. First morning urine, collected in 1997 and 1998, was used to collect data on albuminuria. Albuminuria was classified as elevated at a concentration of more than 20 mg/L. Follow-up started after discontinuation of anticoagulant therapy and ended at time of rVTE, death or end of study (June, 2007).
Results: Out of 40,856 responding subjects, 537 developed VTE between 1997 and 2007. Because of ongoing anticoagulant therapy, 156 subjects were excluded. Of the 381 remaining subjects (46% men, median age at enrolment; 58 years), 48 subjects developed rVTE and 49 subjects were diagnosed with elevated albuminuria. Median Follow-up time was 3.51 years. Annual incidence of rVTE was 3.1% (95%CI, 1.0-7.3) in subjects with elevated albuminuria, compared to 3.4% (95%CI, 2.5-4.6) in subjects with normal albuminuria. Crude hazard ratio of rVTE was 0.9 (95%CI, 0.4-2.3) in subjects with elevated albuminuria, compared to those with normal albuminuria. After adjustment for age, sex and VTE before start of study, this hazard ratio was 0.6 (95%CI, 0.2-1.5).
Conclusions: The risk of rVTE is not increased in subjects known with elevated albuminuria. Therefore, prolonged anticoagulant treatment seems not necessary in such patients after VTE.

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☺ P351

INCIDENCE, RISK FACTORS AND OUTCOME IN CHILDREN WITH THROMBOSIS - A POPULATION BASED STUDY IN DENMARK 1994-2006

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Keywords: paediatric,thrombosis, incidence

Aim: To asses the incidence, risk factors, treatment modalities, and outcomes of venous and arterial thromboses among Danish children (0-18 years).
Methods: This population-based historical follow-up study comprises all children with arterial or venous thrombosis, in Denmark, between 1994 and 2006. Patients with thrombosis were identified in the nationwide registry of patients. Data on patients with symptomatic thrombosis were combined with information from registries of birth and death, and from medical records, and all diagnoses were validated.
Results: Incidence rates (95% confidence interval) are presented as number per 100.000 person years. In total, 623 cases of thrombosis have been identified corresponding to an incidence rate of 3.9 (3.6-4.3). Cerebral thrombosis (arterial ischemic stroke and sinus-venous thrombosis) occurred with an incidence rate of 1.6 (1.4-1.8). A linear trend of an increasing incidence rate of cerebral thrombosis was found during 1994-2006 with a slope of 3.4% on log scale (p=0.048). The incidence of deep venous thrombosis and pulmonary embolism was 2.1(1.9-2.3). Peak incidences of thrombosis were in neonates (11.0 (8.9-13.4) per 100 000 live births, males comprising 67.0%) and in adolescents aged 15-18 years (10.5 (9.1-11.7), females comprising 71.0%).
Underlying conditions or additional risk factors precipitating thrombosis were present in 72.3% of patients. Thrombophilia was diagnosed in 141 out of 436 cases investigated. Supportive care was given to 172 children, 442 patients received antithrombotic treatment. All cause mortality was 7.4%; death was directly attributable to thrombosis in 2.1 %. Morbidity associated with thrombosis was reported in 57.0% of survived patients with available follow-up details one year after thrombosis (192/337).
Conclusions: The study shows age and gender disparities in incidence rates of paediatric thrombosis; an increasing trend of cerebral thrombosis during the study period; and a significant morbidity of thrombosis in paediatric population in Denmark.

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LONG-TERM INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH ACUTE SPINE INJURY: A PROSPECTIVE STUDY

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Keywords: spine cord injury, venous thrombosis

Background: Venous thromboembolism (VTE) is a frequent complication in patients with acute spine injury. However, scarce data are available on the duration of the risk of VTE in this clinical setting.
Aims: To prospectively evaluate the long term risk of VTE in a cohort of patients with acute spine injury during their rehabilitation and post-rehabilitation follow-up.
Patients and methods: Consecutive adult patients with recent spine injury hospitalized for rehabilitation course in the Spinal Unit of Piacenza Hospital. Patients were followed-up every six months after discharge for symptomatic VTE or death. For the outcome VTE we performed survival analysis using Kaplan-Meier and Cox regression statistics.
Results: From January 2003 to November 2007, 94 patients (male 81, 86%; mean age 40.3 years) were recruited; 40 tetraplegic (42.5%) and 54 paraplegic (47.5%). All the patients undergone thromboprophylaxis during hospitalization (range 1-19 months; mean 5.8 months) with low-molecular-weight heparin and compressive stoking. Mean duration of follow-up was 34.3 months (range 1-80 months). The cumulative incidence of VTE was 22.3% (21/94). DVT was diagnosed in 20/21 of the cases (95%) and isolated pulmonary embolism (EP) in 1/21 (5%). The majority of VTE events were recorded during the first three months of follow-up (78.2 vs 11.2 VTE events/1000 patients/year); the major determinant of VTE was age over 45 years (HR 3.7; 95% CI 1.4-9.2). Sex, severity of disability and hypertone were not related to the development of VTE complications.
Conclusions: The long term risk of VTE in patients suffering from acute spinal injury is high. In addition, VTE complications are more common during the first three months after trauma and in patients over 45 years old.

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SEASONAL AND MONTHLY VARIABILITY IN THE INCIDENCE OF VENOUS THROMBOEMBOLISM: A SYSTEMATIC REVIEW AND A META-ANALYSIS OF THE LITERATURE

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Keywords: venous thromboembolism, seasonal

Background: Many studies showed that the occurrence of cardiovascular and cerebrovascular events exhibits a seasonal and monthly variation. On the other hand, evidences on the existence of a seasonal and monthly variation in the incidence of venous thromboembolism (VTE) are more conflicting. Therefore, we conducted a systematic review and a meta-analysis of the literature to assess the presence of an infradian rhythm of this disease.
Methods: MEDLINE and EMBASE databases were searched up to January 2010. Monthly and seasonal variation in the incidence of VTE were analyzed.
Results: Eighteen studies for a total of more than 35,000 patients were included in our systematic review. Thirteen studies (34,557 patients) analyzed the seasonal variation and 10 studies (22,825 patients) the monthly variation of VTE. Our data confirmed the existence of a seasonal variation in the incidence of VTE with a peak incidence in winter (p< 0.001). Furthermore, we found a monthly variation in the incidence of VTE with a peak incidence in January (p< 0.001). Subgroup analyses including only idiopathic venous thromboembolic events confirmed the results of principal analyses.
Conclusions: Our data support the presence of a infradian pattern in the incidence of venous thromboembolic events, with a significantly higher risk in winter and in January. Future studies are needed to better clarify the mechanisms behind this pattern.

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☺ P654

GENOTYPE-INDEPENDENT IN VIVO OXIDATIVE STRESS AND PLATELET ACTIVATION FOLLOWING A METHIONINE LOADING TEST. COMPARABLE ACTIVATION IN SUBJECTS WITH A HISTORY OF EARLY-ONSET ARTERIAL OR VENOUS THROMBOSIS

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Background: The methionine loading test identifies individuals in whom fasting total plasma homocysteine (tHcy) may be normal but the post-methionine load (PML) tHcy is abnormally high.
Methods: In 96 subjects [54 males, 42 females, mean age 40.4±12.3 yrs, 28 with the 68bp844 ins polymorphism of the Cystathionine-β-synthase (CBS) gene; 20 homozygotes for the C677T mutation of the methylene-tetrahydrofolate reductase gene (MTHFR++); 13 with the combination of the two, and 35 without any] we have evaluated tHcy, folate, vitamin B12, and vitamin B6 and correlated them with in vivo oxidative stress (as reflected by urinary excretion of 8-iso-PGF2α) and platelet activation (as reflected by the urinary excretion of 11-dehydro-TXB2), before and after the ingestion of 100 mg/kg methionine.
Results: Baseline vitamin B12 and tHcy significantly (p<0.05) predicted PML tHcy. Baseline and PML tHcy were maximal in subjects with the combination of MTHFR++ and the 68bp844ins polymorphism. PML 8-iso-PGF2a exhibited a 21% increase (p<0.005) and 11-dehydro-TXB2 a 8% increase (p<0.005) vs. baseline, their increases being similar in the genotypes evaluated. 8-iso-PGF2a correlated with 11-dehydro-TXB2 (r=0.26, p<0.05). Fifty-two subjects had a history of early-onset thrombosis (18 arterial, 32 venous, 2 both). Their PML platelet activation was comparable and higher than that of subjects without such history (p<0.05).
Conclusions: *In vivo* oxidative stress and platelet activation, independent of genotypes associated with moderate hyperhomocysteinemia, occur following a methionine loading test. *In vivo* platelet activation is maximal in subjects with a history of early-onset thrombosis and comparable in subjects with a history of arterial and venous events.

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