DLTH2009 Belgrade

ABSTRACT BOOK

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Danubian League against Thrombosis and Haemorrhagic Disorders

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Abbreviations:

PL - Plenary Lecture
SOA - State of the Art
OP - Oral Presentation
PP - Poster Presentation

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Venous Thromboembolism: Risk Factors, Diagnosis and Therapy

PL 1

THE OLD VERSUS THE NEW IN ANTICOAGULATION FOR VENOUS THROMBOEMBOLISM

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We have recently entered into a new era with the approval of two orally available, predictable and selective coagulation inhibitors – dabigatran etexilate and rivaroxaban. Other agents are soon to follow. This is undoubtedly a welcome change of paradigm after 60 years with vitamin K antagonists. However, this will by no means eliminate the old friends. Unfractionated heparin (UFH) with its short half life and complete reversibility remains as the anticoagulant of choice in open heart surgery, and with its non-renal elimination still the preferred drug in the acute treatment of venous thromboembolism (VTE). Another specific subgroup, patients with massive pulmonary embolism, should immediately be started on intravenous UFH until further therapeutic choices are made. Low-molecular-weight heparin (LMWH) has so far unmatched documentation in a wide range of indications for prophylaxis against VTE and during the hospitalization period subcutaneous injection is not considered a major disadvantage. In patients with established VTE the subgroup with cancer derives benefits regarding effect/harm ratio and possibly also survival from LMWH compared to warfarin. This may be due to pleiotropic effects of LMWH that are unlikely to be reproduced by the selective inhibitors. About one third of the patients on long-term anticoagulation do very well on warfarin without any changes to their maintenance dose over 6 months, requiring infrequent monitoring and the total cost of treatment will be difficult to beat.We still remain with large patient populations where current treatment is suboptimal. For the extended prophylaxis against VTE after major orthopedic surgery the oral drugs that don't require monitoring are clearly advantageous. In the long-term treatment with anticoagulants (stroke prophylaxis in atrial fibrillation, secondary prophylaxis after VTE) we will in a few months have information whether dabigatran meets the expectations and may replace warfarin. For a major proportion of the patients, who don't achieve complete stability in warfarin, this would be a relief. There are, however, unanswered questions regarding the new agents. Will absence of monitoring lead to reduced compliance and an increased rate of thromboembolism in patients who are less motivated than those consenting to participate in studies? Will elderly patients during long-term therapy slowly slip into unnoticed renal failure with increased risk of bleeding due to drug accumulation? How will patients with need for emergency invasive procedures be handled? Until these and many other questions have been answered, the transition from the old to the new should be cautious, stepwise and accompanied by clever studies to resolve this new issues.

SOA 2

OBSTACLES IN DIAGNOSTICS AND THERAPY OF HEPARIN-INDUCED THROMBOCYTOPENIA

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Considering the complexity of clinical status and pathogenesis of heparin-induced thrombocytopenia type II (HIT II) patients as well as introduction of novel anticoagulant drugs, clinicians have faced numerous obstacles in HIT II diagnostics and therapy, which demands great care, dexterity and cooperation of a multdisciplinary expert team of various profiles. Due to the administration of the low-molecular-weight heparins (LMWH), which takes ever conspicuous place in the VTE prophylaxis with unfractionated heparin (UFH), the occurrence of HIT induced by LMWH has been more frequently observed recently, i.e. in about 0,25-1%. The solution to the complex diagnostic problems arising in interpreting each laboratory HIT assays performed may be found in focusing on the following: 1. the corelation with HIT clinical probability test like 4T'score; 2. the interpretation of the laboratory findings dependent on the time of the thrombocytopenia onset as well as 3. the sensitivity and specificity of each test respectively. The HIT diagnostics in the presence of other comorbid states which may also induce thrombocytopenia, more precisely known as pseudo HIT, represents a specific clinical problem. The introduction of new anticoagulants by a certain number of clinicians raised suspicion whether some anticoagulats, such as foundaparinux, play the role only in the prevention of HIT onset, regarding the fact that they still lack the official registration for HIT therapy as anticoagulant drugs. Also, it is of high importance to apply adequate adjunctive therapeutic methods, such as plasmapheresis, antiplatelet drugs, intravenous immunoglobulin in order to achieve successful outcome in certain forms of HIT.

OP 3

AN OVERVIEW OF GENETIC RISK FACTORS IN THROMBOPHILIA

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Thrombophilia is a multifactorial disorder, involving both genetic and acquired risk factors that affect a balance between procoagulant and anticoagulant factors and lead to increased tendency to thrombosis. The concept that thrombophilia could be associated with genetic defects was first proposed in 1965 after discovery of familiar antihrombin III deficiency. Further family studies showed that deficiency of protein C or protein S also increased thrombotic risk. In order to identify the exact nature of these deficiencies, the advent in DNA technology, especially invention of PCR reaction, was employed and consequently enabled new insights in genetics of thrombophilia. In 1993, activated protein C resistance and Factor V Leiden mutation were discovered. Although originally identified within the context of family study, Factor V Leiden mutation was so prevalent in general population that the focus shifted from family to population-based case-control studies. Shortly afterwards, a mutation in the 3' untransleted region of Factor II gene (FII G20210A) associated with increased concentration of factor II in plasma, was described. Large epidemiologic studies have conformed that these two common mutations are significant risk factors for thrombophilia. In the last decade several prothrombotic genetic risk factors have been described, including genes variants associated with increased levels of coagulation factors, defects of natural coagulation inhibitors, altered platelets function, defects of the fibrinolytic system and hyperhomocysteinemia. These genetic defects and/or their combination have been extensively studied in an attempt to elucidate the possible association with increased thrombotic tendency. In the past years, large number of candidate genes variants has been investigated, but, so far, due to their minor impact on the thrombotic risk, they are not included in the diagnostic panel. The large-scale DNA analysis systems are now becoming available, opening a new era in genetic studies of thrombophilia. New technology will enable many genes to be studied in a single patient leading to personalized medicine.

OP 4

TAFI LEVELS AND THR325ILE POLYMORPHISM INFLUENCE CLINICAL EXPRESSION OF THROMBOPHILIA IN CARRIERS OF FV LEIDEN OR FII 20210A MUTATIONS

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Background: It is considered that TAFI levels may influence clinical expression of hereditary thrombophilia in carriers of thrombophilic mutations, but the results of previous studies regarding this issue are inconsistent. Influence of Thr325Ile polymorphism in TAFI gene on occurrence of thrombosis or spontaneous pregnancy loss in individuals with hereditary thrombophilia has not been investigated so far.
Aim and methods: In this study we measured TAFI levels, and distribution of Thr325Ile polymorphism in 100 symptomatic and 20 asymptomatic heterozygous carriers of FV Leiden or FII 20210A mutations.
Results: Mean values of TAFI were similar in symptomatic and asymptomatic carriers of FV Leiden or FII 20210A mutations, (672±104 vs. 662±88 IU/L, $p>0.05$), as well as in carriers with venous thrombosis (678±106) and carriers with fetal loss (650±96), ($p>0.05$). Higher TAFI levels were observed in patients with spontaneous than in patients with provoked venous thrombosis (693±118 vs. 655±87), but the difference was not significant. Between carriers with recurrent thrombosis and those with single episode of thrombosis there were no differences regarding mean TAFI levels. Among symptomatic patients prevalence of Thr325Ile variant was significantly higher than in asymptomatic group (47% vs. 25%, $p<0.05$).
Conclusions: Our results indicate that characteristics of TAFI system may significantly influence clinical expression of hereditary thrombophilia in carriers of FV Leiden or FII 20210A mutations.

SOA 5

THROMBOSIS IN ANTIPHOSPHOLIPID SYNDROME: RESULTS FROM THE SERBIAN NATIONAL APS REGISTRY

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Introduction: Antiphospholipid Syndrome (APS) patients suffer from repeated venous and arterial thrombosis with the presence of antiphospholipid antibodies (aPL). APS may manifest itself as a primary disease (PAPS), or as a secondary disease, most commonly in the context of Systemic Lupus Erythematosus (SLE).
Goals: Demonstration of the initial thrombosis-related results from the Serbian National APS Registry.
Patients and Method: The project started in January 2005. Data currently comprises total of 256 patients: 162 PAPS patients (94 female and 68 male; mean age 45.2 + 12.9 years) and 94 SLE patients with secondary APS (83 female and 11 male; mean age 43.1 + 15.4 years); aPL analysis includes analysis of aCL (IgG/IgM), b2GPI, and LA.
Results: Of the 162 PAPS patients 94 were female and 68 male; mean age was 45.2 + 12.9 years, and 94 SLE patients with secondary APS. Thrombosis was diagnosed in 83 (51.2%) PAPS patients and in 36 (38.3%) SLE patients, ($p = 0.045$). Of that number, 56 (34.6%) PAPS patients had arterial thrombosis, compared to 32 (34%) SLE patients, ($p = 0.932$). Venous thrombosis was diagnosed in 42 (25.9%) PAPS patients and in 8 (8.5%) SLE patients, ($p = 0.001$). The following thrombosis localizations were diagnosed in PAPS significantly more often than in SLE: superior extremity ($p=0.027$), superior extremity venous thrombosis ($p=0.004$), inferior extremity deep vein ($p=0.027$), and inferior extremity superficial thrombophlebitis ($p=0.004$). Comparative analysis of aPL did not show a statistically significant difference between the type and localization of thrombosis and the type of antiphospholipid antibodies ($p < 0.05$), except for the statistically significant correlation between aCL-IgM and cerebral venous sinus thrombosis ($p=0.040$), and with jugular venous thrombosis ($p=0.040$).
Conclusions: The Serbian National APS Registry allowed us to ascertain a significantly increased incidence of thrombosis in PAPS patients, as compared to SLE patients with secondary APS.

OP 6

DIFFERENT D-DIMER TESTS COMPARING TO ULTRASOUND EXAMINATION OF DEEP VEIN THROMBOSIS

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Background. The American Academy of Family Physicians and the American College of Physicians recommends the diagnosis guideline for deep vein thrombosis (prediction rule -modified Wells, D-dimer and compressive ulrasonography).
Aim. The aim of our study was to evaluate the sensitivity and specificity of three D-dimer tests (DDPlus Dade Behering, Hemos IL, Vidas) comparing to ultarsonography examination.
Methods. This study has been performed over the June 2007-October 2008 period at the Cardiovascular Disease Institute of the Clinical Center of Serbia. We have examined 110 outpatients. All of them have undergone through the same protocol which was composed from: first, primary care physician systematically obtained patients´ history and physical examination and subsequently referred patients to the laboratory to undergo D-dimer testing as second step. Finally, real-time compression ultrasonography of the symptomatic leg was used as reference test in all patients. Data analysis was assessed using statistical evaluation in addition to various descriptive and analytic statistical methods (T-test, chi-square test and McNemar test).
Results. From 110 patients 5 patients were excluded from study because they had thrombosis of greater saphenous vein (GSV). Using ultrasonography examination (CUS) we have found thrombosis in 43 (40,1%) of our patients (from which proximal localization had 26 (60,5%) patients). Without thrombosis were 62 (59,9%) patients. Comparing ultrasonography examination with D-dimer tests we have found following sensitivity (Sn) and specificity (Sp): for Behring D-dimer test Sn 93,0%, Sp 40,0%, for HemosIL test Sn 84%, Sp 66% and for Vidas test Sn 95%, Sp 59%.
Conclusions. The study results suggest that the addition of Vidas D-dimer tests to diagnostic algorithm could improve the management of the patients with suspected deep vein thrombosis in daily practice. The main reason why the Vidas is used as the D-dimer test of the first choice in many laboratories worldwide is that it has the greatest sensitivity value and it's time needed to be performed is significantly shorter comparing to the other tests.

OP 7

VALUE OF TRANSTHORACIC ECHOCARDIOGRAPHY IN THE DIAGNOSIS OF PULMONARY EMBOLISM

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Background: Pulmonary embolism (PE) is despite the development of new diagnostic procedures still a disease with high mortality. Transthoracic echocardiography (TTE) is easily available, rapid bedside diagnostic modality, and it can play a very important role in the management of patients with suspected PE.
Aim: The aim of this study is to determine the sensitivity and specificity of TTE in the diagnosis of PE.
Methods: Forty eight patients with suspected PE were enrolled in this study. The study protocol included assessment of clinical probability by Wells score, TTE, perfusion scan and computed tomography pulmonary angiography (CTPA). Data were collected retrospectively by reviewing the records of patients with suspected PE admitted to the Institute for pulmonary diseases between January 2007 and January 2008. The diagnosis of PE was confirmed or excluded by a CTPA.
Results: In 20 patients the signs of right ventricular dysfunction were observed on the TTE and the diagnosis was confirmed by CTPA in 32 patients. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TTE for diagnosis of PE were 41.18%, 57.14%, 47% and 51%, respectively.
Conclusions: From the results of our study we can confirm that the sensitivity and specificity of TTE in PE diagnosis is low. Diagnostic value of this technique is limited; therefore CTPA should follow in all inconclusive cases. TTE should be considered as orientational examination and, where available, it should be followed by CTPA.

PP 8

NATIONAL REGISTRY OF THROMBOPHILIC STATES IN SLOVAK REPUBLIC

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Background: Thrombophilic states (TS) are inherited or acquired hemostatic disorders associated with increased risk of thrombosis. Their most important clinical manifestation is venous thromboembolism (VTE).
Aim: Primary goal of our project was to find out prevalence of the most frequent inherited TS in families of patients with thrombosis in Slovakia using National Registry of Thrombophilia States (NRTS).
Methods: Patients from all regions of Slovakia with history of thrombosis and their relatives were examined for TS including FV Leiden and prothrombin mutation, protein C (PC), protein S (PS) and antithrombin (AT) defects, sticky platelets syndrome (SPS), hyperhomocysteinemia, etc.
Results: In 1770 subjects was confirmed presence of TS. The most frequent TS was FV Leiden mutation with prevalence of 42%, PS defect was found in 13%, prothrombin G20210A mutation in 10%, SPS in 9%, PC and AT defects in 5%. VTE was proved in 52% of TS (45% venous thrombosis and 7% combination of VT and pulmonary embolism), arterial thrombosis was present in 5% and other complication in 3% of patients. 600 patients with unexplained cause of thrombosis were examined for SPS, which was confirmed in 150 patients (25%).
Conclusions: Our results from NRTS suggest that TS are common causes of thrombosis in Slovak population. The most frequent TS in families of patients with thrombosis are FV Leiden mutation (42%) and PS defect (13%). Interesting finding seems high prevalence of SPS in patients with unexplained thrombosis (25%).

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PP 9

PREVALENCE OF THROMBOPHILIC DEFECTS IN WOMEN WITH VENOUS THROMBOEMBOLISM IN PREGNANCY AND PUERPERIUM AND ITS CONNECTION WITH THROMBOSIS LOCALIZATION

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Pregnancy and puerperium are independent risk factors for the development of venous thromboembolism and this risk is further increased by the presence of thrombophilia. In order to determine prevalence of thrombophilia in women with pregnancy related venous thromboembolism we have investigated 169 women with first episode of venous thromboembolism during pregnancy and puerperium and 128 healthy women with at least one uncomplicated pregnancy. We have determined the antithrombin, proten C and protein S activity, APC resistance, presence of lupus anticoagulant, as well as the presence of mutations FV G1691A, FII G20210A and MTHFR C677T. Inherited thrombophilia was diagnosed in 91 patients (53.8%) and 11 controls (8.59%). The prevalence of FV Leiden in the investigated group was 22,48% compared to 1,56% in the control group (Chi square= 25.6, p=0.000). The relative risk (RR) of venous thromboembolism was 18.27 (95%CI 4,3-77.3). The prevalence of the FII G20210A mutation among patients was 11.24% compared to 2.3% in controls, (Chi square =6.44, p=0.011, RR 4.97, 95%CI 1.43-17.25). Prevalence of antithrombn, PC and PS deficiencies taken together was 5.9% compared to 0, (Chi square =6,125, p=0.013). Prevalence of combined thrombophilia was 8.87% compared to 0% (Chi square =11.0, p=0.000) Prevalence of MTHFR C677T homozygous mutation was similar in both groups (4.14% in pts vs 4.69% in controls). Venous thrombosis have occurred more frequently in the iliofemoral segment in women with thrombophilia, comparing to women without thrombophilia (59 vs. 32, Chi square =7.66, p=0.0056). The difference of the other locations of thrombosis between the two investigated groups was not statistically significant.

PP 10

HOMOCYSTEINEMIA LEVELS AND METHYLENTETRAHYDROFOLATE REDUCTASE C677T GENOTYPES IN PATIENTS WITH PULMONARY EMBOLISM

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Introduction: Hyperhomocysteinemia is a well known risk factor for pulmonary thromboembolism (PTE) while methylentetrahydrofolate reductase (MTHFR) 677TT genotype as a major cause of hyperhomocysteinemia (HHcy) may be associated with a higher risk of venous thromboembolism.
Aim: To determine the differences in HHcy incidence, homocysteinemia levels and distribution of MTHFR 677 genotypes (C/C, C/T and T/T) between patients with PTE and healthy persons.
Methods: The study encompassed 64 patients with PTE and 50 healthy persons were investigated. Homocysteine was measured using HPLC method. HHcy was defined as homocysteinemia above 12 μmol/L. PCR based method was employed for MTHFR 677 genotypes determination. Statistical analyses included chi-square and Mann Whitney U tests.
Results: Median homocysteinemia value was significantly higher (p=0.017) in patients (12.10 μmol/L) than in controls (10.35 μmol/L). Incidence of HHcy in patients with PTE (51.5%) was significantly higher than in controls (30.0%) (p=0.021). There was no significant difference in distribution of MTHFR 677 genotypes (C/C, C/T and T/T) between patients and controls. In patients, homocysteinemia was significantly higher (p=0.002) in men (14.05μmol/L) than in women (10.08 μmol/L). HHcy was present in 67.6% of men with PTE, which was significantly higher (p=0.006) than the incidence in women with PTE (33.3%). Healthy males had significantly higher (p=0.001) homocysteinemia (12.54 μmol/L) than healthy females (9.39 μmol/L).
Conclusion: Since the finding of MTHFR 677 genotypes is only one of HHcy causes and the study enrolled a limited number of persons, it is clear that HHcy in PTE has a greater clinical importance than MTHFR 677 genotype.

PP 11

REVIEW OF PATIENT WITH THROMBOPHILIA ASSOCIATED WITH ESSENTIAL THROMBOCYTHAEMIA

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Background. Leiden mutation is a hereditary disorder producing mutated factor V that can not be normally inactivated by protein C, which leads to increased risk of venous thrombosis.
Aim and Methods. Paper represents a 50 yrs. Old male patient, with no previous family history of thrombosis. Due to the weakness of the right side of the body, he was checked by a neurologist, who diagnosed mild right hemiparesis, followed by CT scan which revealed ischemic lesions in the brain tissue. Blood count demonstrated elevated neutrophile count (19.1x109/l) and platelets (675x109/l), with normal coagulation parameters (PT, aPTT), and elevated D dimer (750 U/l). Neutrophilia and high platelet count were considered as a reactive phenomenon, and further diagnostic procedures were directed toward diagnosis of thrombophilia or suspected brain vasculitis. Kariotype analyses confirmed factor V Leiden mutation (heterozygot), and patient underwent periodic symptomatic therapy administered by both haematologist and neurologist. During the next 7 months, blood count analyses showed the increase of platelet count (770, 900, 1200 x 109/l), and therefore the patient was suspected of myeloproliferative disorder. After the bone marrow aspirate and kariotype analyses were done and spontaneous growth of megakaryocytes were confirmed, the diagnosis of essential thrombocythaemia was established. After the induction therapy with anagrelide and anti aggregation drugs, this patient is under control and without any of thrombogenic events.
Conclusion. Although association of thromophilia with essential thrombocytemia represents a complex disturbance of haemostasis, the fact that the first clinical manifestation was stroke (e.g. arterial occlusion), lead to the conclusion that high platelet count was the main trigger of thrombosis. Further therapeutic efforts were directed towards the reduction of thrombocytosis with simultaneous elimination of the risk factors for venous embolism, such as infection, trauma and preventive therapy with anticoagulants.

PP 12

MESO-ATRIAL SHUNT FOR MANAGEMENT OF FACTOR V LEIDEN RELATED BUDD-CHIARRI SYNDROME: CASE REPORT

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Introduction: Factor V Leiden (fVL) is the most common form of inherited thrombophilia, present in 5% of people. FVL is product of gene mutation that results in more active fV. Mild form of fVL in heterozygous carriers is more frequent than severe form in homozygous carrier. The Budd-Chiari syndrome is characterized by hepatic venous outflow obstruction at the level of the hepatic venules, the large hepatic veins, the inferior vena cava, or the right atrium. Hepatic veno-occlusive disease refers to obstruction of hepatic venous outflow at the level of the central or sublobular hepatic veins, or both.
Case report: A 25 years old male patient was admitted in our hospital with abdominal pain and swollen right leg. In medical history no comorbidites were present, except recent tooth extraction. Thrombosis of hepatic veins and inferior v. cava were noticed on computerized tomography. Further investigations revealed presence of fVL and heterozygous origin. Patient was scheduled for meso-atrial shunt construction with Dacron graft. Gradual regression in liver size and resolution of ascites were noticed following the procedure.In postoperative period, we managed coagulation disorders. Patient was discharged from hospital 43 days after surgical procedure, without ascites and instructed to remain on oral anticoagulants.
Conclusion: In patients with Budd-Chiari syndrome, fVL is common. This is a case of successful liver decongestion and ascites resolution after meso- atrial shunt in fVL related Budd-Chiari syndrome.

PP 13

RECURRENT DEEP VENOUS THROMBOSES IN A PATIENT WITH FACTOR V LEIDEN AND MTHFR C677T MUTATIONS

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Introduction: Mutations in Factor V gene - Factor V Leiden and MTHFR gene - C 677 T are assosciated with recurrent thrombotic disorder - thrombophilia.
Case report: Patient T.Z, 45 years old is presented. As of 35 he had frequent and recurrent phlebothromboses episodes. He had four episodes of deep venous thrombosis: thrombosis v. iliace externe, v. femoralis communis and v. femoralis sinistra. Then, on two occasions he had deep venous thrombosis of the right axillary vein and later on recurrent thromboflebitis of the right on three occasions. Patient experienced total of nine episodes of venous thromboembolisam in a ten years interval. As of the third thrombotic episode he was treated with oral anticoagulant therapy. Further thrombotic episodes occurred both during the interupption of therapy and sometimes even during oral anticoagulant therapy within therapeutic ranges. Blood count and hemostasis screening test were within reference values. ANA and lupus anticoagulant tests were negative. AT III, PC, PS activity were within reference values. APC ratio was slight decreased. Molecular investigation showed that he is heterozygot for FV Leiden mutation and homozygot for MTHFR C677T mutation.
Discussion: Patient has a serious risk of recurrent thrombotic disorder because of the association between Factor V Leiden (heterozygous form) and MTHFR C677T (homozygous form) mutations During the past six months, the patient was treated with oral anticoagulant therapy (INR 2,5-3,5) and folic acid and had no thrombotic episodes.

PP 14

CONGENITAL-CYANOTIC HEART DISEASE AND HYPERCOAGULABILITY OF BLOOD - CASE REPORT

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Introduction: Cyanotic heart disease associated with high values of haematocrit and high risk of coagulation abnormality. Most of the patients with cyanotic disease were thrombocytopenic. Polycythemia, associated with cyanosis caused hypercoagulability and thrombosis, but also with antiphospholipid syndrome (APS). A thrombophilia screening performed to detect thrombogenic complications.
Case report: We report the case of 49-year-old women was admitted to our hospital with thrombocytopenia, high values of haematocrit and cyanosis. She had previous repair of cyanotic congenital heart disease at age 6. She had a history of complete heart block and acute renal failure. The results of relevant laboratory investigation were as follows complete blood count (CBC) : WBC 13,4x109/l, Er 5,93x109/l, Hgb186g/l, Hct 57,6, PLT 45x1012/l. Screening coagulation tests PT 51%, Fibrinogen 3,57g/l, APTT 26,9, D-dimer 1210, lupus anticoagulant (LAC) 1,07 FVIII 170%. Thrombophilia test: PS no coagulation, AT III 98%, PC 138%, APCR 1,74, LAC 1,14. pH 7,3, pCO2 36mmHg, pO2 42,2 mmHg, SatO2 72,8%, FII 47%, FV 34%, FVII 13%, FIX 32%, FX 25%. Transtoracic echocardiography found arterial pulmonary pressure 155 mmHg and stenosis main pulmonary artery (PA), right ventricular hypertrophy and dilatation (RV), tricuspidal regurgitation TR1+ and dilatation right atrium. The patient was treated with glucocorticoids. A pectoral pacemaker was implanted.
Conclusion: This report draws attention to an interesting association of thrombogenic complications and highlights the possibility of a coexistent antiphospholipid syndrome.

PP 15

POSTOPERATIVE DEEP VEIN THROMBOSIS AFTER LAPAROSCOPIC CHOLECYSTECTOMY

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Background: Venous thromboembolism is a relevant problem in operated patients, which can results in pulmonary embolism, even in mortality.
Aim: The aim of this study is to examine presence of postoperative deep vein thrombosis (DVT) and to determine coagulation status in patients who are undergoing laparoscopic (LC) and open cholecystectomy (OC).
Methods: We have randomized 114 patients into 2 groups. The first group consisted of 58 patients with LC and the second group consisted of 56 patients with OC. The coagulation parameters (prothrombin time, partial thromboplastin time, D-dimer, antithrombin III and factor VII) were monitored preoperatively, during the operation and 24 and 72 h after the operation. Patients in both groups did not receive thrombosis prophylaxis.
Results: In LC group 4 patients (6,9%) developed postoperative DVT, and 9 patients (16,1%) in OC group. Coagulation parameters in both groups were without statistical difference. In patients in both groups who developed DVT only decrease of FVII had statistical significance (p 0,05).
Conclusion: The incidence of postoperative DVT in patients with OC is higher comparing with patients with LC (p 0,05). The decrease of F VII after the operation can be a parameter indicating the risk of DVT.

PP 16

THROMBOSIS OF THE INFERIOR VENA CAVA AND PULMONARY THROMBOEMBOLISM

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Diverse risk factors induce pulmonary embolism (PE). In 90% of the cases, the thrombus stems from the low extremity veins, and having been formed between the venous valves, by the circulation and via the inferior vena cava, it reaches the right heart and then the pulmonary artery, inducing PE, which is manifested by perfusion oscillations of diverse intensity. In case 50% of the pulmonary bed is excluded due to hemodynamic disorders, a fatal outcome may occur. Thrombosis of the inferior vena cava (VCI) is due to the ascending spread of the thrombus from deep veins of the upper leg in 4-15% of the cases, or to the compression-induced local spread, involvement of the inferior vena cava by a tumor, or to iatrogenic factors as in pregnancy. Due to the size of the thrombus emerging from the VCI, the resulting pulmonary embolism is usually massive and fatal.
This is a report of three cases with thrombosis of the inferior vena cava (VCI) and PE. The admission diagnosis of a female patient was PE, which was confirmed by respective diagnostic procedures. While searching for the departure point of the thrombus, the contrastive computerized tomography (CT) of the abdomen verified the right kidney tumor and a thrombus in the VCI. Having administered heparin therapy and stabilized the patient's general condition, the right nephrectomy and thrombectomy from the VCI were performed. Postoperatively, the patient was transferred to oral anticoagulant treatment (OACT). The second patient with a recurrent PE and a thrombus in the VCI under the renal veins, which was due to the ascending spread of DVT, had a vena cava filter (VCF) implantation and was transferred to OACT. The third patient was admitted in the state of sepsis with bilateral infarction pneumonia. The thrombus verified in the VCI was localized under the orifice of the renal veins so a VCF was impossible to be implanted, and the patient was treated with heparin and then transferred to OACT.
We have reported three patients with PE and VCI thrombosis due to different causes, who were effectively treated in our Institute, applying different methods to resolve thrombosis of the VCI as the dominant etiological factor of PE.

PP 17

PULMONARY EMBOLISM IN INTENSIVE CARE UNIT

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Background: Pulmonary embolism (PE) is common in Pulmonary Intensive Care Unit (ICU), connected with high mortality risk and often under diagnosed.
Aim: To evaluate diagnostic tools, associated medical conditions and outcome in PE patients (pts).
Material and Methods: Retrospective analyses of PE pts. medical data for: D-dimer, right ventricular dysfunction (RVD) assessed by echo-cardiogram, lover limb vein ultra sound (US), ventilation-perfusion lung scans, arterial gases, high resolution CT (HRCT), underlying diseases and outcome.
Results: Of 304 pts. treated in our ICU 36 were diagnosed of PE (11,8%) due to: 1.elevated D-dimer (100%), 2.RVD in 29 pts (80,5%), 3. positive lover limb vein US in 22 pts (61,1%), 4. conclusive ventilation-perfusion lung scans in 34pts (94,5%) (1pt. refused to perform examination), 5 hypoxia in 21pts (58,3%), 6. HRCT performed in 6pts, positive of PE in 5pts. Underlying diseases were: 1. malignancy in 5 pts (13,9%) 2. abdominal surgery in 10pts (27,8%), 3. osteosyntesis in 2 (5,6%), 4. systemic disease in pts (11,1%) 5. heart failure in 5pts (19,4), 6. endocarditis and sepsis in 2 pts., 7. COPD in 16 pts (44,4%), 8. respiratory failure in
5 pts (13,9%), 3 of them in obesity hypoventilaton syndrome (OHS), 9. lover limb thrombosis in 22 pts (61,1%) There were 6 (16,7%) lethal outcomes: 1 due to lung cancer, 1 with endocarditis, 1 with systemic disease and 3 with terminal OHS.
Conclusions: Growing number of PE (6% in previous year) is due to improved laboratory and imagining methods and clinicians guides. Prognosis depends of underlying disease and hemodynamic status. Upgrading diagnostic tools (BNP and HRCT) are helping both to patients and clinicians.

PP 18

CORRELATION BETWEEN PAI-1 AND Lp(a) IN DEEP VENOUS THROMBOSIS

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Aim: To establish the correlation between fibrinolytic parameter PAI-1 and lipoprotein fraction Lp(a) in patients with deep venous thrombosis (DVT).
Method: It was used ELISA-sandwich method, manufacturer Immunogenetics-Belgium.
Results: Control group assembled 100 blood donors, age 50-55, equal sex precipitate. The examine group constituted 100 patients with clinic possibility of DVT, proved by ultrasonography method and ventilation-perfusion scan, same qualified as the control group. The obtain results in examine group were for PAI-1 56.03±12.2 ng/mL versus 23.12±9.25 ng/mL in control group and for Lp(a) the obtain results were 33.96±12.23 mg/dL in examine group versus 21.03±9.16 mg/dL in control group. The values for PAI-1 and Lp(a) in patients with DVT are increased compared with the values of the same variables in control group ($p<0.001$). There was found positive significance correlation $r=0.490$ between fibrinolytic parameter PAI-1 and lipoprotein fraction Lp(a) in patients with DVT.
Conclusion: Our investigation established strong positive correlation between fibrinolytic parameter PAI-1 and lipoproteins fraction Lp(a) in patients with deep venous thrombosis.

PP 19

FIBRINOLYSIS DISTURBANCE IN DEEP VENOUS THROMBOSIS

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Aim: To determinate the efficiency of 3 different fibrinolysis system parameters namely t-PA, PAI-1 and DD in diagnosis and development of acute deep venous thrombosis (DVT).
Method: At the moment of clinical suspicion, a sample of venous blood was obtained to measure levels of t-PA, PAI-1 and DD using ELISA (enzyme-linked immunosorbent assay) method, manufacturer Immunogenetics, Belgium.
Results: Control group is assembled of 100 blood donors at the age of 50-55 years with equal participation. Examined group is constituted of 100 patients with clinic assessment of DVT, proved by ultrasonografy method and ventilation-perfusion scan, same qualified as the control group. The obtained results for t-PA values provide no difference compared with control group (4.6±0.5 ng/mL versus 4.8±0.7 ng/mL). The obtained results for PAI-1 and DD values provide high increasing compared with the control group. The values for PAI-1 in examined group were 56.03± 12.2 ng/mL versus 23.12±9.25 ng/mL ($p<0.001$) and for DD 721±125 ng/mL versus 122±38 ng/mL ($p<0.001$).
Conclusion: We have consolidated the association between fibrinolytic parameters (t-PA, PAI-1 and DD) with diagnosis and development DVT.

PP 20

THE USE OF D-DIMER WITH NEW THRESHOLD CAN BE USEFUL IN DIAGNOSIS OF VENOUS THROMBOEMBOLISM IN PREGNANCY

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Background. D-dimer testing has an important role in the exclusion of acute venous thromboembolism (VTE) in the nonpregnant population. Establishing D-dimers role in the diagnosis of VTE in pregnancy is hampered because of the substantial increase of D-dimer throughout gestational age.
Methods. In a prospective study we followed 107 healthy pregnant women to establish the reference range of D-dimer for each trimester. D-dimer testing was also performed in 12 women with clinical suspicion of VTE and their results were compared with the established new reference range of D-dimer, and with the recorded ultrasound findings.
Results. In the first trimester, 84% women from reference group had normal D-dimer, in the second 33%, and by the third trimester only 1%, which suggests that D-dimer has no practical diagnostic use in ruling out VTE if the threshold for abnormal 230 ng/ml is used. All pregnant women with thrombosis who had positive ultrasound findings had also statistically significant elevation of the D-dimer level, considering the established reference range of the corresponding trimester. We found 100% sensitivity of D-dimer test. A women developed thrombosis in the first trimester had 6.7 to 7.6 time higher level of D-dimer than the mean value in the reference group, and in the third trimester thrombotic women had 2.0 to 3.8 time higher level of D-dimer, $p< 0.0001$.
Conclusion. D-dimer test with the new threshold for: the first of 286, the second of 457 and the third trimester of 644 ng/ml can be useful in diagnosis of pregnancy related VTE.

PP 21

THE MANAGEMENT OF VENOUS THROMBOEMBOLISM

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Background: Deep vein thrombosis (DVT) and pulmonary embolism (PE) comprise different manifestations of the same clinical entity referred as venous thromboembolism (VTE). Post-thrombotic syndrome (PTS) can be a consequence of DVT.
Aim: To describe the management of patients with VTE in Ambulance for thrombosis and hemostasis (ATH), in ITM for three months, 2008.
Methods: Retrospective study with dates from our electronic information systems
Result: In this period, 8 661 patients came in our ambulance, from which 1 988 came for first time and 6673 for control. From this number, 4 531 (52.3%) were women. From total patients with VTE, 57.6% were women; from this number 71 % were with Thromboflebitis superficialis, 45.9 % with DVT, 38.5 % with PE and 62.8 % were with PTS.75 % of women had VTE (Thromboflebitis superficialis, DVT, PE and PTS) from women came in ATH.
Conclusion: From the total number of examined patients with haemostatic disorders were more women (52.3%); more came for control. 57.6% from all VTE were women; most frequently were with Tromboflebitis superficialis, on second place were with PTS, and then with DVT and less were with PE. For the initial treatment 1) low molecular weight heparin were used given subcutaneously, without monitoring, 2) intravenous or subcutaneous unfractionated heparin given with monitoring and 3) overlapping administration of an oral vitamin K antagonist, acenocumarol minimum three months or longer in patients with a high risk or recurrence (target INR 2.5: range 2.0 to 3.0). Our ITM provide excellent means of monitoring the oral anticoagulation.

PP 22

THE USE OF THROMBOPROPHYLAXIS IN THE PATIENS WITH GENERAL AND GYNECOLOGIC SURGERY

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Introduction: The deep venous thromboembolismus process is one of the most important medical problems, because it is very difficult to be diagnosed.
Aim: To show the positive effects of the prophylactic therapy with low molecular weight heparin (LMWH) or by standard heparin between the patients with high risk for deep vein thrombosis and possible pulmonary embolism after complicated surgery treatment.
Material and methods: 2500 blood samples were analyzed on automatic analyzer (coagulometar- Amax) for the period of 2 years. The blood samples were from the patients of the Obstetrics and Gynecology and Surgery Clinics, Medical University-Skopje. In this research was used screening haemostasis (PT, aPTT and TT) and D-dimer. Evaluation of the thromboprophylaxis is made according the patient's diagnosis, the gravity of the surgery and the thrombosis past of the patient. There were also used the data from the patient's medical records, the first diagnosis and the previous haemostasis screening. The results of the used preventive therapy between the high risk patients (possible presence of deep venous thrombosis or pulmonary embolism) were analyzed by the retrospective method.
Results: The thromboprophylactic therapy was used before and after the surgical intervention for the period of 15-21 days. It was found that after the preventive treatment, the complications of the pulmonary thrombosis were present in 2% of the treated patients and deep venous thrombosis in 3% of the treated patients.
Conclusion: Thromboprophylaxis is very important for the patients with complicated surgery treatments, long period of immobilization or with detected haemostatic disorders.

PP 23

ANTITHROMBOTIC THERAPY IN PATIENTS WITH MECHANICAL HEART VALVES

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Background. Patients with mechanical heart valves have been treated with a long term oral anticoagulant therapy, in order to prevent thrombosis. Maintaining a targeted level of INR and its adequate range is of extreme importance, along with minimization of possible bleeding and thromboembolic complications.
Aim. Random retrospective evaluation of bleeding events and thromboembolisms in patients with mechanical heart valves, treated with vitamin K antagonists.
Methods. Data have been gathered from 201 current patients. INR has been determined through a Thrombotest method using capillary blood. Bleeding events and thromboembolisms have been shown in percentages per patient year. Patients are divided according to type of mechanical valve and age.
Results. Total number of monitored patient years equals 1783. Patients observed have 1,46% bleeding events per patient year and 0,9% thromboembolisms ppy. In a group with mechanical valve in aortal position there are 1,30% ppy with bleeding events, and 0,74% thromboembolisms ppy. Group with a replaced mitral valve consists of 1,67% bleeding complications ppy and 1,07% thromboembolisms ppy. When it comes to age, a group of patients under 65 has 1,13% bleeding events and 1,63% thromboembolisms ppy. Group of patients older then 65 have 1,72% bleeding events ppy and 0,30% thromboembolisms ppy.
Conclusions. 1.Targeted INR as well as the range of INR should be maintained according to the position of the mechanical valve, while individualising it if needed. 2. Specialized teams should guide the anticoagulant therapy.3. National references / guides should be made, for leading a therapy of vitamin K antagonists.

PP 24

ANTITHROMBOTIC THERAPY IN ATRIAL FIBRILLATION IN MACEDONIA

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Background: Atrial fibrillation (AF) is the most common cardiac rhythm disorder; a major independent risk factor for ischemic strokes and due to cardiogenic embolism. Oral anticoagulant therapy (OAT) is highly effective for both primary and secondary prevention of stroke in patients with AF. Recommended targeted at an International Normalized Ratio (INR) of 2.5: range 2.0 to 3.0.
Aim: To describe the management of patients with AF anticoagulated with acenocumarol.
Methods: 89 patients, total number of analyses 1079, average per patient 12 analyses per year (monthly). The dosing parameter was INR, performed in ITM and was measured with TRINITY-Biopool, ISI-1,04.
Results: The follow up of the 89 patients with AF (62 man and 27 women), with a median age of 69 years (range 53 - 84) during the 2008 year showed that the patients were anticoagulated with acenocoumarol. The average INR value was 2.42 with STD 0.23, which means all the patients were excellently anticoagulated. But, the additional analysis in function of time showed that 36.33% of 12 INR controls were out of targeted range, 63.67 % of patients had recommended target INR.
Conclusion: Anticoagulation controls vary widely among patients taking acenocoumarol for AF. Monthly controls of INR are suggested, but if the results are not appropriate, the frequency of controls should be increased during the stabilization of the targeted INR. Slow careful dosage titration, regular laboratory monitoring and patient education can substantially reduce the risk of complications. Adequate software for OAT is very important to avoid subjective attitude of every doctor who dosages the acenocumarol.

PP 25

HEMORRHAGIC AND THROMBOEMBOLIC COMPLICATIONS DURING ANTITHROMBOTIC PREVENTIVE THERAPY IN ATRIAL FIBRILLATION

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Introduction: Atrial fibrillation is the most common long-term, heart rhythm disorder in elderly persons, with an increased risk of developing thromboembolic complications. Oral anticoagulant therapy is recommended for prevention of thromboembolism.
Objective: The aim of this study was to assess the frequency of hemorrhagic and thromboembolic complications during antithrombotic preventive therapy in patients with atrial fibrillation.
Material and Methods: The study included 133 patients, mean age 69±8.56 years, on the whole treated for 519 patient-years. The patients were followed-up at the Thrombosis Prevention Department of the Clinical Center of Vojvodina. Effects of oral anticoagulant therapy were evaluated using capillary thrombo-test. Hemorrhagic and thromboembolic complication rates are given in percentage per patient-year. Hemorrhagic complications were recorded in patients receiving oral anticoagulant therapy, as well as in patients receiving a combined treatment - oral anticoagulant therapy with antithrombotic therapy.
Results: On the whole, hemorrhagic and thromboembolic complications occurred in all 133 patients per patient-year: 2.8% and 0.5%, respectively. Hemorrhagic complications were recorded in 0.7% of patients receiving oral anticoagulant therapy, and in 2.1% of patients receiving combined antithrombotic therapy, per patient-year.
Conclusion: In comparison with single oral anticoagulant therapy, combined antithrombotic therapy increases the risk of hemorrhagic complications. In order to decrease the risk of hemorrhagic and thromboembolic complications, continuous individual drug maintenance of the Target International Normalized Ratio is necessary.

PP 26

FOLLOW UP OF ORAL ANTICOAGULANT THERAPY

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Introduction: Oral anticoagulant therapy (OAT) is used to prevent thrombus formation, its extension and the risk of embolisation. Hypocoagulation of blood can be achieved by application of oral anticoagulant drugs. Prothrombine time is the test of choice for laboratory follow up of oral coagulation therapy. The obtained results should be expressed in INR system. Underdosage may lead to thromboembolic complications, while overdosage may cause bleeding.
Aim: The aim of the paper was to determine the number of prothrombin time tests during the therapy in patients who were controlled at our laboratory from January 1st to December 31st 2008.
Material and Methods: Samples were taken using standard procedure for the determination of coagulation status. Prothrombine time was determined using PT-fibrinogen rabbit recombinant thromboplastin (HemoIL) with ISI 1.036-1.088. All analyses were done by ACL7000 coagulometar. Total of 3033 samples were processed.
Results: Out of the total, INR 2-3 was found in 1024 (33.76%) samples; INR 3-4 was found in 385 (12.69%), INR below 2 was in 1468 (48.40%), while INR over 4 was found in 155 (5.11%) samples. The results show that in 48.40% of samples there was no therapeutic effect, which may be due to genetic factors, inadequate diet, alcohol or antagonism with other drugs, whereas 5.11% patients were overdosed with the risk of bleeding. Our data are accordance with the published data.
Conclusion: The number of patients treated with OAT is ever increasing; therefore it is necessary to establish anticoagulant clinics for their follow up in order to avoid problems related with the beginning and efficiency of treatment and to treat the patients with the risk of bleeding.

PP 27

FOLLOW UP OF THE ORAL ANTICOAGULANT THERAPY – OUR EXPERIENCE

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Background: Problems in the follow up of the therapeutic effects of the oral anticoagulant drugs can occur due to their small therapeutic range and various interactions with other drugs and food.
Aim: Survey of nonhospitalized patients (pts) treated with OAT at the CC `Dr Dragiša Mišović`.
Material and methods: Detailed history data were taken from 202 pts, on reasons for the initiation of OAT therapy (hospital discharge lists, specialists` reports), dosage, other drugs and any possible complications. Based on those information and obtained results of PT time according to Quick, expressed as INR (using Sysmex CA1500 and BFT II, DADE BEHRING, reagent Thromborel S), we determined further therapy, made appointments for the follow up and instructed pts to perform additional tests (liver function, thyroid gland and kidnies, and in overdosed ones we excluded occult bleedings).
Results: Out of 202 registered pts, 110(54,5%) were men and 92 (45,5%) women., average age 68 yrs (32-82). 112 pts had cardiac rhythm disorder, 26 had heart attack associated with insufficiency and enlarged heart, 9 had thrombus in the heart cavity, 7 had coronary by pass, 17 DVT of the lower extremities, 5 lung embolisms, 24 artificial aorta and/or mitral valves and two had brain insults. Out of additional risk factors responsible for the occurrence of thromboembolism, 70 had arterial hypertension, 34 diabetes mellitus and 19 had hyperlipoproteinemia (separately or associated). Chronic renal insufficiency was the additional problem in 10 pts, malignancy in 6 and liver cirrhosis in 2 pts. At the first admission, in 30 (14,8%) pts INR ranged from 4-10. `Only` 4 pts had bleeding episode (hematuria, hematoma, eye bleeding ans epistaxis). Out of 2339 performed controls, INR was above the upper therapeutic limit in 49 controls: INR = 4-6 in 33, INR = 6-9 in 16 controls. Those pts were treated with antibiotics (8), diklofenak (6), for virus infections (5), etc. Two overdiagnosed patients developed bleeding episode. One had pneumonia treated with 3 kinds of antibiotics, INR=9 and he developed rectorrhagia. Another patient had epistaxis with INR= 4,28. Two pts with the INR=3,15 had hematoma/epistaxis. 56 pts were simultaneously treated with Cardiopirin and 3 pts with Clopidogrel in addition. 46 pts were treated with Amiodarone and OAT. During the so far conducted follow up of pts underdosed for a long time prior to admission to our center, we have not gathered data on the occurrence of any thrombotic complications.
Conclusion: Detailed knowledge of the effect of other drugs and associated diseases on each and every OAT patient could reduce the most severe complications in the sense of bleeding or thrombosis. Data that 15% of pts at the first admission were overdosed, imposes the need of a more detailed and more frequent observation of OAT pts. Out patient treatment should be more available as well as the staff adequately educated regarding the necessity of regular controls and respect of the recommended drug dosage.

PP 28

HOW TO USE GENETIC INFORMATION DURING THE TREATMENT OF PATIENTS WITH ANTIVITAMINS K

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The danger of the thrombosis today is impossible to underestimate. Only from atherothrombosis every year dyed almost 18 millions peoples and from venous thromboembolism - near 6,5 millions. As the death appeared mostly in the first half of an hour the most effective way to prevent the death is the prevention of it by different ways. The drugs prophylaxis is worldwide accepted. For the venous thromboembolism the most popular is the prophylaxis by the use of oral anticoagulants, the most correct-by the antivitamin K. At the developed countries treatment by OAK-AVK get every one from 200 people. Because of the valve prothesis-25%, because of the defect of the heart valves-13%, atrium fibrillation-16%, venous thromboemblism-26%. In spite of the use modern requirements and measurement of INR-international normalizing ration with ISI –international sensitivity of thromboplastin some times it is very difficult to chose the proper doses of the drug. It was a very big hope for the physicians when it was discovered the cause of different sensitivity of different peoples to these drugs. The measurement of the genes of VKORC1, which regulate the vitamin K turnover, and genes of CYP 450 2C9, which regulate the vitamin K elimination, gave to the doctors the possibility of patients reaction for the treatment. In our clinic there are many patients, who were investigated for these genetic markers. Unfortunately our proper experience sometimes gave us unpredicted results. We are planning to share it with the audience.

PP 29

EFFICACY AND SAFETY OF UNFRACTIONATED AND LOW MOLECULAR WEIGHT HEPARIN USED AS PERIOPERATIVE BRIDGING THERAPY IN PATIENTS WITH MECHANICAL HEART VALVES

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During perioperative period patients with mechanical hart valves, particularly at mitral position, are at a high risk of thromboembolism The aim of the study was to evaluate the incidence of thromboembolic and haemorrhagic complications in patients with mechanical heart valve prothesis during perioperative administration of unfractionated (UF) or low molecular weight (LMW) heparin. Data on 648 patients with mechanical heart valves who have visited our anticoagulant clinic from 1979 until 2008 have been analyzed. In 352 pts mechanical valve was in aortic position, in 227 in mitral, in 2 pts in tricuspid position and 67 pts had both aortic and mitral heart valve replacement. Total follow up was 5025 patient/ years, average 7.75. In 210 pts bridging therapy have been implemented, 61 of them had major surgery, 149 had minor surgery including dental procedures or invasive procedures. Oral anticoagulant was withheld and LMWH or UFH was given prior to surgery. Oral anticoagulant was restarted 1-5 days after surgery, and treatment with heparin was continued until INR reached target range. Thromboembolic or bleeding complications that occurred within one month period have been considered to be perioperative. The incidence of major bleeding for major surgery was 6.55% and 0% for minor surgery and invasive procedures. The incidence of minor bleeding for major surgery was 14.75% % and 3.35% for minor surgery or invasive procedures. There were 3 thromboembolic events (1.59%), all of them had happened in pts with mitral valve replacement. There was no difference between two heparins in terms of efficacy and safety. Bridging therapy provides satisfactory protection from thromboembolic events in patients with mechanical heart valve replacement.

PP 30

BLEEDING CONTROL AFTER TEETH EXTRACTIONS IN PATIENTS ON ORAL ANTICOAGULANT TREATMENT WITHOUT THERAPY INTERRUPTION

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Background: Patients on anticoagulant therapy who require oral surgery face a conflict of interests: to discontinue anticoagulant therapy with the risk of thromboembolism, or to continue anticoagulant medication with risk of bleeding. In recent years, continuation of anticoagulant therapy in minor oral surgical procedures has gained more attention in the literature, emphasizing the role of local haemostasis.
Aim: The purpose of this investigation was to evaluate and compare haemostatic effects of two local haemostatic agents, Gelatamp ® and FastAct ® following teeth extraction, in patients on anticoagulant therapy.
Methods: Sixty patients, with INR between 2 and 4, were underwent single or multiple tooth extractions, without altering anticoagulant therapy. The INR was measured on the day of the procedure. According to used local haemostatic agents, they were divided into two groups.
Results: Of the hundred teeth extracted during this investigation, bleeding was recorded in four cases, two in Gelatamp ®, and two in FastAct ® group. All bleedings were easily controlled with measures of local haemostasis.
Conclusion: Tooth extraction can be safely carried out without altering anticoagulant therapy when local haemostatic measures are used to control bleeding.

PP 31

HEPARIN-INDUCED THROMBOCYTOPENIA OCCURRED AFTER CARDIOSURGICAL TREATMENT OF ATRIAL MIXOMA: A CASE REPORT

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Background: Heparin-induced thrombocytopenia (HIT) is an acquired prothrombotic disorder, caused by antibodies to a complex of heparin and platelet factor 4 (PF4) that activate platelets, resulting in release procoagulant microparticles, thrombocytopenia, generation of thrombin, and frequently thrombosis.
Case report: We present a case of severe HIT at a 68-old female occurred after cardiosurgery of left atrial mixoma with aim to point a importance of differential diagnosis of thrombocytopenia at patients who have recent heparin exposure. Platelet count drop at eleventh postoperative day on 4x109/l and patient developed hemorrhagic syndrome. Diagnosis at first is missed, and when thrombocytopenia remained refractory on corticosteroid treatment and platelet transfusion, patient hospitalized 13th postoperative day in the Institute of Haematology. Diagnosis of HIT was confirmed with clinical score (4T's) and positive anti heparin-PF4 ELISA test, such as positive platelet aggregation test. Treatment was started with danaparoid at doses of 750 U in intravenous (iv) bolus followed by continuous infusion of 400 U per 4 h. Twelve hours after danaparoid therapy, intravenous gammaglobulins added at doses of 0.4 g/kg four days because severe thrombocytopenia persisted with complication of hemorrhagic syndrome. Platelet count started to arise third day and its completely normalized 5th day of danaparoid therapy.
Conclusion: We showed successful treatment of severe HIT with low doses of danaparoid with addition of intravenous gammaglobulins. Awareness of heparin-induced thrombocytopenia is still lacking, and we pointed to need for consideration of HIT in differential diagnosis of thrombocytopenia at patients who have been recently exposed to heparin.

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Current and Future Assays of Haemostasis

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HOW TO USE DIC DIAGNOSIS TODAY?

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The prevalence of DIC (disseminated intravascular coagulation) is different - from 0,1 % all patients, admitted to hospital (G.Muller-Berghause), to 1:867 (Zbilut J.P.1980). But, the DIC phenomenon was detected at 30 -50% patients with Gram-negative septicemia, at 50-70% patients with severe trauma, and at 15-20% of patients with acute leukemia (M.Levi et oth. 1999). DIC was in 10-22% the cause of the maternal death (R.Bick, 1988). Why there is such a big difference? We suppose that this may be connected with the different approach to this problem. The consensual recommendation of the ISTH Subcommittee on DIC is as follows: DIC is the URGENT clinical phenomena, which is characterized by the presence of microclots of blood inside the body vessels, which may be different in size, structure, and the intensity of their formation is so high, that produce obvious damage in the body tissues or organs , originate different clinical picture and may be even dangerous to the life ". What shall we get from the adoption of such definition? We explain to everybody, that this phenomenon is dangerous, that such microclots of blood may be different, but that they may be so numerous, that may produce some danger to the life of the organism. Very important for the physicians is to diagnose DIC at its origination, at the beginning. The most practical way is to note the changes in the level of such laboratory data as fibrinogen and platelets. We have made the analysis of the data from more than 1500 patients and healthy persons. It was measured soluble fibrin- monomers, D-dimers, platelet factor 4, fibrinogen, platelets number, and the clinical picture of the disease – CHD, arterial hypertension, diabetes mellitus, leukemia, hemophilia, rheumatoid arthritis. The data allowed us to conclude, that there is Constant Intravascular Coagulation-CIC, which has different expression, structure and clinical manifestation. We propose to use the term of DIC-only in the most serious variant of CIC that can be dangerous for the human live. We propose, that the Concept on Continuous Intravascular Coagulation attract attention to "The Phenomena, which characterized by the constant presence of microclots inside the body vessels, which may be different in size, structure, intensity of formation and can produce, or not produce different clinical pictures. It may be the solution of problems, which take place during search of difference between pre-DIC and DIC. Our data also allow us to see the difference in this phenomenon as of CIC so as DIC. Both of them characterized by intravascular microclots formation in the microvasculature, arising from different cause, which may have different morphology and clinical picture, or have no clinical manifestation at all, and may lead to acute organ dysfunction and even to death, or may have no harm to the people and came to the normal intensity by itself.

SOA 33

FACTORS INFLUENCING FIBRIN STRUCTURE AND FUNCTION. RELEVANCE TO CLINICAL DISEASE

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The fibrin gel (or clot), is a structural element of blood clot derived from fibrinogen at the sites of the lesions in the blood vessel wall. Information about the quality of fibrin in examined plasma samples can be obtained by studying fibrin gel porosity with a method previously developed by Blombäck B. and Blombäck M. with collaborators. Using the original liquid-permeation technique interesting findings were obtained, indicating the most important determinants of fibrin gel structure both in physiological and pathological conditions. The permeability coefficient (Ks) became an established parameter that provides information on the overall gel structure and reflects the size and shape of the pores in the gel. Low value of Ks indicates a tighter, less porous fibrin network which is more resistant to fibrinolysis, and individuals with this type of fibrin structure are at grater risk for developing atherothrombosis. This important correlation between final fibrin structure and predisposition to atherothrombosis has been repeatedly demonstrated. Both genetic and environmental factors contribute to the structure of the fibrin gel, which in turn influences an individual's risk of the disease. Genetic variants in clotting factors, specially fibrinogen and FXIII have a role in modulating fibrin structure. Recent observations by our group also indicate that platelet derived microparticles may influence the structure of the fibrin network. A large number of environmental factors including diabetes, low-grade inflammation, hyperlipidemia, smoking and different drugs also affect the final fibrin gel structure. As fibrin gel formation represents the final step in the atherothrombotic process, it is essential to fully understand the mechanisms involved in determining fibrin structure in order to develop new preventive and therapeutic strategies aimed to decrease morbidity and mortality of this disease.

OP 34

THE ROLE OF ROTEM IN REAL TIME ASSESSMENT OF HEMOSTASIS IN SURGICAL SETTINGS

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Background: In contrast to conventional tests, rotational thromboelastometry (ROTEM) provides an automated measurement of interactive dynamic processes starting with initial hemostasis and proceeding through humoral coagulation, clot crosslinking and fibrinolysis at a given time point.
Aim: To highlight the usefulness of ROTEM in making the correct diagnosis and adoption of therapeutic approaches in a timely manner in some different clinical situations in surgery.
Materials and Methods: We describe the value of ROTEM in three hemostatically compromised patients. In the first case, after operation multitraumatised patient developed uncontrolled massive bleeding unresponsive to conventional treatment and rFVIIa administration. Cessation of bleeding was achieved after guided therapy according to ROTEM results. In the second case, we present the ROTEM-based dynamic assessment and goal-directed treatment of acute hemorrhage in liver transplant recipient. The third patient with strong lupus anticoagulant, unusual prolonged APTT (>250s), decreased PT (33%) and undetectable activity of coagulation factors VIII, IX, XI and XII underwent surgery. ROTEM has shown the reliability in screening this patient who was suspected for concomitant increased risk of bleeding and thrombosis.
Conclusion: In severely injured trauma patients, orthotopic liver transplantation as well as in complex thrombophilic conditions, where events may proceed at a fast and dramatic pace in perioperative period, ROTEM enables rapid and accurate detection and differential diagnosis of multifactorial coagulopathies. Also, it provides the basis of rational approach to the use of blood component therapy and pharmacological interventions.

OP 35

ESTIMATION OF THROMBIN PRODUCTION IN DIFFERENT SUBJECTS

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Background: During the decade of 90s new methods, of global impact, for the evaluation of the activation of blood coagulation appeared and mainly after the presentation of Hemker and Beguin method. We thought of a newer and simpler method (global thrombin test, GTT) using the already existing aggregometer (Chrono-log, P.I.C.A). Our method is based in thrombin production using platelet rich plasma (PRP) in which CaCl2 is added.
Aim: The aim of our study is the presentation of the new test and checks it in normal subjects, and patients in different pathologic situations.
Materials and Methods: We applied our method in 114 subjects (30 normal subjects (NP) with mean age 37,9±9,4 to estimate the mean values and SD, 16 haemophiliacs, 36 patients under LMWH, 56 patients under antiplatelet drugs - 48 under Aspirin and 8 under Plavix, and 6 patients under anti-vitamin K treatment). Principe of method: The addition of CaCl2 in PRP activates it and produces coagulum. In 450 μl of PRP we add 250μl CaCl2 (0,025mm) in a plastic tube of aggregometer (Chrono-log P.I.C.A). We also tested the reproducibility of the method by repeating the test 12 times in the same normal subjects CV for the area under curve 5,9%, and for the lag time was 13%.
Results: The results presented in table I. For normal subjects for example the mean lag time of GTT was 161,3±63,4sec while in haemophiliacs was 573,2±218,7sec (with a t-test statistically very significant, p<0,001).
Comments: The advantages of our method besides the fact that it is without cost is: The mean values of lag time in these categories differ accordingly; which is statistically significant and proves their diagnostic value. Categories mean Volume ± SD p NP vs. hemophiliacs 161,3±63,4 vs. 573,2±218,7 <0,000168 NP vs. thrombophiliacs 161,3±63,4 vs. 298,2±122,7 <0,0235 NP vs. antiplatelets 161,3±63,4 vs. 320,9±55,8 <0,020 NP vs. LMHW 161,3±63,4 vs. 349,6±144,3 <0,01.

SOA 36

HOW TO MEASURE PLATELET MICROPARTICLES USING FLOW-CYTOMETRY?

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Microparticles (MPs) are small membrane vesicles (0.1 - 1 µm) released from various cell types after activation and/or apoptosis. MPs have a potent pro-inflammatory and pro-coagulatory effect and may affect vascular function. Platelet MPs (PMPs) constitute 70-90% of all circulating MPs. The method most widely used for determination of PMPs is flow cytometry. Abundant data indicate that PMPs are sensitive to preanalytical conditions while the lack of standardization makes comparisons of results between different studies difficult. Therefore we have tried to determine preanalytical settings and a method for quantitative measurements of PMP using flow-cytometry. Platelet free plasma (PFP) was obtained by double centrifugation at 2000 x g for 30 min at 15°C and frozen at -80°C. Prior to analysis, the frozen PFP was thawed and double centrifuged at 20800 x g for 45 min at 10°C to obtain a pellet. The PMPs were identified as CD42+/CD62P+ or as CD42+/CD142+ particles sized < 1.0 µm. The mean fluorescence intensity (MFI) was translated into molecules of equivalent soluble fluorochrome molecules (MESF) using a calibration standard. Intra- and inter assay CV were 6.2 % and 7.2%. CV between different flow-cytometers was 2.8% if results were expressed as MESF values compared to 29.6 % when data were quantified as numbers of PMPs per volume. The MESF values were approximately twice as high in patients with type 1 diabetes compared to healthy controls. It seems that the use of proposed preanalytical settings enables flow-cytometric determination of PMPs in frozen samples. Quantification of the fluorescence intensity of PMPs and its expression as MESF-value gives a higher reproducibility and may be used for differentiation between patients and healthy individuals.

OP 37

FLOW CYTOMETRIC AND FUNCTIONAL INVESTIGATIONS OF CRYOPRESERVED AND LIQUID STORED PLATELETS

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Background: Factors responsible for cryoinjury as well as optimization of platelet freezing, are still a question of large interest.
Aim: To determine of the correlations between cryopreserved and liquid stored buffy-coat derived platelet concentrates (PCs), investigating of cell quantity, functional recovery, surface antigen expression on biggest, middle and smallest platelets.
Methods: PCs (27) cryopreserved by our original controlled-rate technique, using 6% DMSO, were compared with liquid-stored units (24). The quantitative and functional recovery was compared before/after cryopreservation. The platelet count, hypotonic shock response (HSR), aggregation with ADP, colagen, epinephrine and ristocetin were investigated. Percent of expression and MFI of surface glycoproteins were investigated by monoclonal antibodies anti-CD41, anti-CD42a, anti-CD42b, anti-CD62p, anti-CD63, anti-CD36, and Annexin V-FITC, using Epics XL.
Results: The recovery of cryopreserved platelets was (91.0%) significantly (p 0.01) better than in 48 hours liquid-stored vs. control group (79.9%). Similar HSR-answer (68% vs. 72%) aggregation with ADP (33% vs.33%), and epinephrin (35% vs. 30%) were obtained. Aggregation with collagen (31% vs. 27%) and ristocetin (63% vs. 76%) were obtained in controlled-rate group and liquid-stored platelets for five days. There were no differences in CD41 and CD42a expression between cryopreserved vs. liquid-stored or control group, but for CD42b (76% vs. 95%), CD62p (28% vs. 15%), CD63 (3% vs. 1.4%), CD36 (73% vs. 94%) and PS (28% vs. 7%) were significant ($p<0.05$).
Conclusion: PCs cryopreserved by controlled-rate freezing (with compensation of released fusion heat) allows recovery that is no inferior to 48 hours liquid-stored platelet.

SOA 38

PLATELET FUNCTION TESTS AND RESISTANCE TO ANTIPLATELET THERAPY

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The clinical efficacy of the antiplatelet therapy (aspirin, P2Y12 and glycoprotein IIb/IIIa receptor antagonists) to prevent recurrent vascular events in coronary and peripheral atherothrombotic disease is well established. Despite the proven benefits of antiplatelet therapy, many patients continue to experience thrombotic events. Many factors may influence the response of platelets to antiplatelet therapy and some patients with adequate compliance to the treatment may exhibit failure of platelet inhibition determined by ex vivo laboratory tests, a phenomenon termed »resistance« to antiplatelet therapy. Light transmittance aggregometry is considered as a gold standard for platelet function testing. The assay is widely used, but requires a relatively large amount of blood, is highly dependent on sample preparation, on technical procedure, is time-consuming and the results between different laboratories are poorly comparable. Due to its limitations, point-of-care devices such as platelet function analyzer (PFA-100®) have been recently introduced. The device is easy to use, has a short turn-around-time and the use of commercially available cartridges facilitates that results coming from different laboratories are comparable. Other laboratory tests, namely bleeding time, impendance aggregometry, whole blood platelet aggregation measured by platelet counting, thrombestastography, flow cytometry, measurements of serum and urinary levels of thromboxane B2 and its metabolite, or point-of-care devices such as VerifyNow® have been investigated for detection of resistance to antiplatelet drugs. The laboratory methods used and the clinical accuracy for individual monitoring during antiplatelet therapy are still a matter of debate.

OP 39

STUDY OF THE RESISTANCE TO ASPIRIN BY DETERMINATION OF GLYCOPROTEIN ON THE PLATELET MEMBRANE

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Introduction: Several studies have been made in order to determine why some individuals that are under anti-platelet treatment have relapses of thromboembolic episodes. The platelets function in individuals under aspirin or clopidogrel was checked using aggregometer, PFA-100, thromboxane derivative 11-dehydrothromboxane B2 in the urine. In order to follow up the changes of platelet membrane receptors in patients under anti-platelet treatment, we decided to examine the glycoproteins: Gp-Ib, Gp-Ib , Gp-IIb, Gp-IIIa and P-selectins. The reason for this was to see if these receptors were inhibited by this treatment.
Material: We studied 83 people (36M and 47F with mean age 45,2±23,9 and 52,3±19,1 correspondingly). From these; 43 (12Ms and 31Fs) without treatment and 29 (15Ms and 14Fs) under aspirin, 7 under clopidogrel (5M and 2F) and 4Ms were under both.
Methods: We applied platelet aggregation with AA and ADP. Also PFA-100 with Colla/EPI and Colla/ADP cartridges. In the same time we determined the CD41a, 42a, 42b, 61 and 62P antibodies with flow cytometry. The determination of glycoproteins was made in PRP. In order to evaluate the accuracy of this method we determined the glycoproteins in one person 10 times. As shown by CV our method has good reproducibility. Our results are shown a significant decrease of the number of all glycoproteins receptors in the patients under antiplatelet treatment except for the P-Selectins.
Comment: It is a method of confirmation of the other methods as it presents the decrease in the number of the receptors.

PP 40

COMPARISON OF THREE METHODS FOR D-DIMER DETERMINATION

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Background: The clinical utility of D-dimer assays is in diagnosis of DVT, PE, DIC. Exactly the usefulness of this test, therefore lies in its ability to safely exclude that clinical situations.
Aim: The aim of this study is to evaluate the comparability of results from three different routinely used immunoassay methods.
Methods: We compared results from three immunoassay automatic assays: Biomerieux (Vidas) and Dade-Behring (Sysmex 1500), il (ACL-6000/7000) for 108 (54 normal patients; 54 pathological persons). We callculated statistical parameters: # x, sd, cv, sensitivity, specificity. We used linear-regression corellation analisys and roc–curve analisys to compare those results.
Results: The performance of different D-dimer assays is summarized : Dade-Behring/ IL: regression line y= 231,9 + 1,261x, r= 0,577, s y,x= 896,01; Dade-Behring/ Biomerieux : y= 489,4 +4,125x ,r= 0,801,sy,x = 1544,26; IL/ Biomerieux y= 1073,4 +1,477x , r= 0,628, sy,x= 0110,8. Sensitivity (sens) and specificity (spec) for each method: IL: sens=83,3 %, spec= 39,5% ,Biomerieux: sens= 90,7 %, spec = 82,8 %; Dade-Behring: sens= 94,4 , spec =47,2 % for manufacturer #s recommended cut-off point.
Conclusion: coefficients of corelation between diferent metods is not satisfactory (from the higher Dade-Behring/Biomerieux r = 0,801 to the lowest Dade-Behring/IL r = 0,57). Good laboratory practice need emergency standardisation and harmonization methods to improve usefulness results for D- dimer.

PP 41

D-DIMER TEST APPLICATION IN THE HEALTH CARE CENTRE OF SOMBOR

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Aim: The aim of this research is to get an insight into the results of D-dimer analysis undertaken during a three years period and the importance of the assessment of this analysis in thromboembolism diagnosis.
Material and Method: A number of D-dimer analyses illustrated in this work were carried out in a period 2005-2008: 12 analyses in 2005, 110 analyses in 2006 and 216 analyses in 2007. Out of 338 analyses there were 220 positive (65%), 112 negative (33%) and 6 were immeasurable (2%). In the first quarter of 2008, the research comprised of 73 patients in eight hospital departments and 8 non-hospitalized patients. Total of 115 analyses were done, two or more analysis were done to 34 patients. The method of descriptive statistics in the review and data analysis was used. The method of single linear correlation was used to assess correlation between positive D-dimer test and diagnosed thromboembolism.
Results: Out of 73 patients 63 had positive D-dimer (86.4%) and 10 patients (13.6%) have negative findings, whereas 29 (40%) patients had confirmed diagnosis of thromboembolism while 44 patients (60%) did not. Out of 63 patients, 35 (55,6%) with positive findings were treated at the internal medicine department and 28 (44,4%) at surgical departments, According to D-dimer test margins the largest number of examined subjects (29 or 46%) had over 1000 ng/ml.
Conclusion: The examination of association of the positive D-dimer test and diagnosed thromboembolism points out the presence of statistically relevant positive correlation (r=0.92).

PP 42

HAEMOSTASIS SCREENING TESTS IN POTENTIAL PLATELET APHERESIS DONORS

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Introduction: In accordance with the regulations regarding criteria for the selection of platelet apheresis donors (PAD) in use by the end of 2007, apart from the platelet count above 150x109/L, favourable haemostasis screening test result (HST) was another relevant criterion used at the Blood Transfusion Institute of Serbia (BTIS). Questionnaire for the selection of PADs was introduced, with a particular stress on questions regarding personal and family history of possible bleeding and thrombosis tendancies. According to the Council of Europe and AABB recommendations, haemostasis testing is not included in the criteria for the PADs selection, thus as of 2008, these tests are no longer performed in PADs at the BTIS.
Objective: Analysis of pathological haemostasis screening test results which were the reason of PADs deferral in 2006 and 2007 and to determine if the exclusion of HST affected the safety of PADs.
Method: Retrospective 2006 and 2007 data analysis considered the reasons for the deferral of PADs due to pathological HST results. Besides, unfavourable PAD reactions were surveyed during 2006, 2007 and 2008.
Results: Out of 3953 tested PADs, 103 (2,59%) were rejected due to pathological HST results. Not one severe haemostasis disorder that might have geopardized PADs was detected. In 63 (1,59%) APTT test was slightly prolonged. Besides prolonged APTT, in 4 cases (0,10%) there was FXII deficiency, in 3 ceses (0,08%) prolonged PT , in one case there was (0,02%) lupus anticoagulant, in 3 cases (0,08%) mild FVIII deficiency, in one case (0,02%) slightly decreased VWF:Ag and in one case (0,02%) associated FVIII and FXII deficiency. In 9 cases (0,23%) PT was decreased, while in 18 cases (0,45%) along with decreased PT there was a mild FV and FVII deficiency.
Conclusion: Exclusion of HST had no effect on the safety of PADs, since no unfavourable reactions that might have been related with haemostasis disorders were recorded during 2008, and the number of PADs deferrals was reduced.

PP 43

MULTIPLATE® - BASED POINT-OF-CARE MANAGEMENT FOR DIAGNOSIS AND TREATMENT OF BLEEDING DURING HEART SURGERY

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Background: Bleeding disorders during heart surgery are currently diagnosed with classical coagulation tests and treated on empirical rules. New electrical impedance method, Multiplate®, as a point-of-care (POC) method enables detection of platelet dysfunction.
Aim: Establishing an algorithm for targeted treatment of bleeding disorders with specific blood products (platelets), desmopressin and antifibrinolytic drugs using Multiplate during heart surgery.
Material and Methods: During 2008, Multiplate measurements of 142 heart procedures were analyzed in pre-, peri- and post-operative phase, and 1 hour after administration of platelets and/or desmopressin (DDAVP). Two tests (COL and TRAP) were routinely used. In cases of suspected anti-platelet drugs (aspirin and/or clopidogrel) effects an additional test (ASPI and ADP) was performed.
Results: According to our experience in Multiplate-based haemostasis management during 142 cardiac procedures we developed our Institution algorithm. In contrast to Multiplate-analysis, classical coagulation tests do not detect platelet function. Prophylactic administration of tranexamic acid and/or DDAVP is indicated in case of preoperative drugs therapy. Therapeutic administration of antifibrinolytic drugs is indicated as prophylaxis of bleeding complication of hyperfibrinolysis, during and post extracorporeal circulation phase. According to the dynamics of the bleeding situation the algorithm may have to be restarted at any point when necessary. Administration of colloids and crystalloids for volume substitution may also lead to additional dilutional coagulopathy. Multiplate - as a point-of-care (POC) method - enables a rapid diagnosis of haemostasis disorders and a more goal-directed use of blood products and coagulation factor concentrates (rFVIIa) during and after heart surgery.
Conclusion: During cardiac surgery differential diagnosis of bleeding disorders and indications for administration of cryoprecipitate (fibrinogen), platelets, fresh frozen plasma (FFP) and rFVIIa are more precise new method - Multiplate analysis. Multicenter studies are necessary to validate the presented algorithm and results.

PP 44

QUALITY CONTROL - DETERMINATION OF LOCALLY OWN REFERENCE RANGES USING MULTIPLATE ®

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Background: The roles of platelets in hemostasis and arterial thrombosis include their adherence, aggregation and acceleration of the coagulation. There is a new device developed for measuring platelet aggregation in whole blood. Multiple electrode platelet aggregometry is sensitive and reproducible method for measuring spontaneous and stimulated platelet aggregation. It is also very useful in evaluating antiplatelet drugs who affect platelet plug formation by different mechanisms, in clinical every day work.
Aims: The goal of the study was to determine our own reference ranges by testing healthy blood donors without the ingestion of platelet function inhibitors. At the same time with this testing we perform regularly quality controls. We also want to compare these reference values with those obtained from the manufacturer.
Methods: Blood was collected from healthy blood donors who fulfilled in questionnaire that they refrained from taking medication affecting platelet function for at least 14 days before the collection of specimens. We used vacuum tubes containing LH according to NACCLS H21-A4, 2003. Whole blood aggregation was determined using impedance aggregometer (Multiplate ®). We tested 38 blood specimens. For TRAP test 38, ASPItest 33, for ADPtest 30 and COLtest 30.
Results: In our series of tested healthy blood donors average were: for TRAPtest 1041 AUmin, for ASPItest 790 AUmin, for ADPtest 737 AUmin, for COLtest 811 AUmin.
Conclusion: Our results revealed that our locally own reference ranges are correlating with reference ranges for European population given by manufacturer.

PP 45

STICKY PLATELET SYNDROME: ROLE OF FLOW CYTOMETRY

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Background: Sticky platelet syndrome (SPS) is a hereditary, autosomal dominant thrombophilia associated with an increased incidence of arterial and venous thrombosis. Typically, there is 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 an abnormality of platelet surface glycoprotein (GP) receptors that leads to their hyperfunction.
Aim: Aim of the study was to detect any abnormality in the expression of platelet membrane GP receptors in patients with SPS.
Methods: 75 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: The significant differences between the patient and control groups were 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.

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PP 46

PLATELET FUNCTION AND COAGULATION ABNORMALITIES IN TYPE 1 GAUCHER PATIENT

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Background: Gaucher patients (GP), may suffer from bleeding, usually attrubuted to thrombocytopenia. Deficiencies of coagulation factors and platelet disfunction in GP were reported in the literature.
Aim: The aim of the study was to investigate platelet function and coagulation factors in Serbian GP.
Methodes: 31 treatment-naive, type 1 Serbian GP (M/F: 17/14, median age: 40yrs, 11/31 splenectomized) were assessed. Complete blood count, protrombin time (PT), activated partial tromboplastin time (aPTT) and coagulation factor activity were measured according to the standard methodes using commercial kits. Platelet aggregation was asessed by a whole-blood aggregometer.
Results: Bleeding was registered in 10/31GP. Mean platelet count was 150x109/L (range: 46-428); 22/31 patients had platelet count <150x109/L; 3/22 <50x109/L. Platelet count inversely correlated with spleen volume ($p < 0.05$). PT and PTT were prolonged in 16/31 (52%) and in 13/31 (42%) of patients, respectively. The most frequent clotting factor deficiencies were of: FV (9/31), vWF (5/31), FVIII (3/31), FX (3/31), FXI (4/31), FXII (5/31) either isolated or combined. Platelet aggregation abnormality was registered in 19/31 (61%) GP. GP with bleeding episodes had significantly lower platelet count comparing to the nonbleeding patients ($p<0.01$). There were no significant difference in clottong factor concentrations and platelet function between bleeding and nonbleeding phenotype. Splenectomized patients had significantly higher platelet count (286x109/L vs 94x109) ($p<0.05$) and no bleeding ($p<0.01$).
Conclusions: Thrombocytopenia was the main cause of bleeding in GP. Although not proven by our study clotting factors deficiencies and platelet disfunction might contribute to the bleeding tendency in certain GP.

PP 47

THE USEFULNESS OF PFA-100 CLOSURE TIME AS SCREENING TEST FOR VON WILLEBRAND DISEASE

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder. Laboratory diagnosis of mild type 1 VWD remains a problem due to temporal variability in the level of von Willebrand factor. Many of these patients remain undiagnosed due to mild bleeding symptoms and borderline or occasionally normal laboratory values. Therefore good bleeding history is of major importance. In the Slovenian National Registry of patients with inherited bleeding disorders 129 patients with VWD are registered, among them 10 pts. with type 3.
Aims and Method: Since year 2000, PFA-100 testing was performed in 72 patients (24 pts. <18 years) with confirmed - probable or possible VWD. The closure time (CT) (PFA-100® system, Siemens) was measured with both cartridges (collagen/epinephrine (EPI) and collagen/ADP (ADP). Reference ranges were previously established.
Results: All 5 pts. with type 3 VWD had prolonged CT with both cartridges. Among 67 pts. with type 1 or 2 VWD, CT with both cartridges was prolonged in 35 pts. (52,2%) and with one cartridge in 15 pts. (EPI 13 pts, ADP 2 pts) (22,4 %). CT was in normal range with both cartridges in 17 pts (25,4 %).
Conclusion: CT may be useful in clinical practice as a screening test for detection of VWD. Sensitivity of PFA-100 system in our patients with type 1 or 2 VWD was around 75%, similar to other studies. In the case of normal laboratory level, measuring of CT, VWF antigen and its activity should be repeated when clinical suspicion is strong.

PP 48

IMPLEMENTATION OF THE SEMIQUANTITATIVE ANALYSIS OF VON WILLEBRAND FACTOR MULTIMERS USING SDS AGAROSE ELECTROPHORESIS METHOD

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Von Willebrand factor (VWF) is a glicoprotein necessary for the establishment of normal haemostasis, taking part in the platelet adhesion at the site of the vascular damage, binding specific platelet glicoproteins and subendothelium structures. VWF is found in circulation in the form of multimers of a rather large molecular weight, containing numerous platelet and blood vessel structures binding sites having platelet forming potential. Objective of this study is to define the implementation of the semiquantitative analysis of VWD F multimers using SDS-agarose electrophoresis in routine practice. Voluntary blood donors` samples were analyzed, platelet concentrates during storage, as well as the samples of 22 patients with previously diagnosed VWD. This method basically implies fast agarose gel electrophoresis using TRIS-SDS urea buffer. Visualisation of over 15 protein straps is considered a normal finding. We also designed our own model of scoring the intensity of multimer `bands` in the following way: value of 0-2 was added to each band`s intensity and values were summed up (0-50). Obtained results of the detection of VWF multimers in platelet concentrate plasma demonstrated the variations within the average score values between the first (44.75) and the fifth (22.5) storage day. Intensity score was calculated for each tested patient. Type 1 patients had the average score value of 24-42, type 2A 10-20, type 2B 18-22. When results of all diseased were scored, significant differences among variouis types of VWD were detected. However, despite the score value, it is necessary to additionaly describe electrophoresis.

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Periodontal Disease and Haemostasis

PL 49

IS LIPOPROTEIN (a) REGULATING THE PROSTAGLANDIN I_2-SYNTHESIS STIMULATING PLASMA FACTOR?

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Background: Prostaglandin I_2 (PGI_2) is known as one of the key mediators regulating blood vessel wall interaction. The plasma factor (PF) stimulating its synthesis, however, is widely unknown, its chemical structure not yet identified. However, there are numerous papers indicating that in certain bleeding disorders an increase in formation and availability might coregulate hemostasis.
Methods: We examined 185 females and 200 males. Patients were divided as to the presence of PF activity. Plasma level of lipoprotein (a) (Lp(a)), lipids, lipoproteins, risk factors as well as clinical data were assessed.
Results: The median level of Lp(a) was significantly ($p < 0.001$) increased in men (94 mg/dl) vs. 19.3 mg/dl) and women ($p < 0.001$; 94 mg/dl vs. 18 mg/dl) with a deficiency of PF activity. In males lacking PF activity the median fibrinogen levels were lower (319.5 vs. 348 mg/dl; $p < 0.05$) and diabetes was less frequent (12.3 vs. 23.6 %; $p < 0.05$). These connections, however, could not be discovered in females. Furthermore, the influence of exogenously added Lp(a) was tested for its ability to stimulate PGI_2-synthesis. Up to a concentration of 500 mg/dl no significant influence was found.
Conclusions: These findings indicate that the PGI2-synthesis stimulating PF might be a so far undervalued property being coregulated by plasma Lp(a) and thus a relevant coregulator of hemostatic balance.

OP 50

EFFECTS OF ENAMEL MATRIX DERIVATIVE (EMD) ON PROLIFERATION, MIGRATION AND ANGIOGENIC FACTOR EXPRESSION OF ENDOTHELIAL CELLS IN VITRO

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Objectives: In the present study we tested the effects of enamel matrix derivative (EMD) on proliferation, migration and expression of a ngiogenic factor and adhesion molecules in human umbilical vein endothelial cells (HUVECs).
Materials and Methods: The influence of EMD on the proliferation of HUVECs in time intervals of 24 and 48 h was measured using MTT-assay. Cell migration was observed in an especially adapted in vitro monolayer wound healing model. The expression of angiogenic factor angiopoetin-2 (ang-2) and adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular endothelium-selectin (E-selectin), was quantified with real-time polymerase chain reaction.
Results: After 24 and 48 h incubation, proliferation of HUVECs was promoted by low EMD doses (0.1-10 µg/ml) and inhibited by higher doses (50-200 µg/ml). Cell migration in the wound healing was stimulated by EMD at doses of 0.1-50 µg/ml and inhibited at 100 µg/ml. The expression levels of ang-2, ICAM-1 and E-selectin in HUVECs were stimulated by higher EMD concentrations (50-200 µg/ml).
Conclusions: EMD has demonstrated to have a concentration-dependent effect on the proliferation and migration of HUVECs: stimulation in low doses and inhibition in high doses. EMD elevated expression level of angiogenic factor and adhesion molecules in HUVECs. These in vitro results showed the potential effects of EMD on the angiogenic activity of HUVECs, which play an important role in periodontal tissue regeneration and wound healing in vivo.

OP 51

INFLUENCE OF PERIODONTAL DISEASE ON CARDIOVASCULAR DISEASE PARAMETERS

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Background: In recent years the relationship between coronary heart disease (CHD) and periodontal disease has attracted considerable attention. Cardiovascular disease ranks as one of the most common systemic conditions affecting periodontitis patients.
Aim: To assess the influence of cardiovascular risk factors on the periodontal tissue in 68 adults (47 with CHD, 21 controls).
Method: For this purpose, coronary risk factors (age, gender, cigarette smoking, overweight, increased serum lipids, increased plasma glucose level, hypertension, reduced physical activity, alcohol abuse, stress) were correlated to the periodontal condition of the patients through two periodontal indices (Plaque Index by Silness and Löe, Periodontal Disease Index).
Results: The statistical evaluation showed a significant relationship between:

- age, waist circumference, high-density lipoprotein cholesterol, plasma glucose level and plaque accumulation (Plaque Index (PI)).
- age, waist circumference, body mass index, plasma glucose level and gingivitis (GingivaPI)
- age, high-density lipoprotein cholesterol, plasma glucose level and attachment loss (AttachmentPI).

In addition, a relationship between hypertension and plaque accumulation as well as gingivitis was observed, possibly caused direct by hypertension or by the associated life style behaviour.
Conclusions: It can be concluded that a consistent prevention of cardiovascular disease in combination with well-known oral hygienic measures also contributes to an efficient prevention of periodontal disease.

OP 52

(ISO-)EICOSANOID PRODUCTION PROFILE IN PERIAPICAL GRANULOMAS AND THEIR ROLE IN BONE DESTRUCTION

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Background. Periapical inflammation stimulates the formation of granulomas and cysts, with the concomitant resorption of bone surrounding the roots of affected teeth. To date, a paucity of epidemiological research exists regarding systemic health consequences of endodontic disease; thus patients and practitioners lack potentially important knowledge about health risks to patients with apical periodontitis. F2-IP, a family of prostaglandin (PG) isomers, are produced in vivo by free radical-catalyzed peroxidation of arachidonic acid (AA) independent of the cyclooxygenase (COX) enzyme.
Aim. The present investigation sets out to establish whether:
(1) the (Iso-)eicosanoids 8-epi-$PGF_{2\alpha}$, PGI2 and its stable biologically inactive metabolite 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE2 and TBX2 would be suitable as a marker for the level of bone destruction and inflammation.
(2) the conversion rate of ^{14}AA to all IP
(3) the influence of tobacco smoking on IP-synthesis.
Methods. Periapical specimens from 46 patients (29 male, 17 female) with radiographic and/or clinical signs and symptoms of chronic and acute apical periodontitis were obtained and immediately frozen. They were between 18 and 81 years of age (20 smokers, 14 non-smokers and 12 former smokers). The samples were subjected to immunochemical analysis (RIA) and to radiothinlayer chromatography (RTLC), too. PGI2 was examined by Bioassay. At the same time they were evaluated by image analysis of their radiographs and their clinical dimension of the granuloma after tooth extraction.
Results. On RTLC, 14C-labelled AA was found to be converted into PGE2 (0,35%±0,21) and 6-oxo-$PGF_{1\alpha}$ (0,53±0,37) in all the 46 samples studied. TXB2 (0,04%±0,05) and $PGF_{2\alpha}$ (0,13%±0,10) could not be detected in every sample. RTLC also showed a LO (2,84%±0,72) pathway in all the samples. Quantitatively this was found to be more significant than the COX pathway in almost all the cases.
Conclusions. Because inflammation is associated with oxidation injury, which in turn stimulates release of IP, eicosanoid metabolites may modulate the osteolytic process going on in the diseased periapical area. Consistent data suggest that IP- formation is increased in a variety of clinical settings associated with inflammation and oxidant stress such as in smokers, for example. Measurement of F2-isoprostanes might provide a useful diagnostic indicator of the extent of pulpal inflammation and infection.

OP 53

(ISO-)PROSTANES - A MARKER FOR PARODONTOPATHY ?

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Background: Iso-prostanes are important indicators of in-vivo oxidative stress. They are used as markers for different inflammatory processes.
Aim: To assess the value of iso-prostane 8-epi-prostaglandin (PG)$F_{2\alpha}$ and/or prostaglandins in saliva as marker for diseases of the periodontal ligament.
Methods: 121 adults, (aged 21-73 years, 90 non-smokers, 31 smokers) had saliva samples taken and the saliva concentration of 8-epi-$PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$ (a stable metabolite of PGI2), thromboxane (TX)B2 and $PGF_{2\alpha}$ was measured. On the basis of periodontal indices a periodontal status of each test person was assessed.
Results: 8-epi-$PGF_{2\alpha}$ increased with the plaque index. 8-epi-$PGF_{2\alpha}$ in the saliva of smokers (115.5±23.5 pg/ml) was significantly higher than that of non-smokers (70.2±20.4 pg/ml), while 6-oxo-$PGF_{1\alpha}$ was significantly ($p < 0.001$) in smokers. More than 80% of the smokers showed slight gingivitis.
Conclusions: Salivary 8-epi-$PGF_{2\alpha}$ seems to be a promising diagnostic in-vivo marker in saliva of oxidative stress reflecting cigarette smoking and extent of parodontopathy.

OP 54

PLASMA BUT NOT SERUM THROMBOXANE B2 IS CORRELATED TO PERIODONTAL DISEASE INDICES

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Background: Recently, the association between periodontitis and atherosclerosis becomes more and more established. We examined the role of platelets which also act as an inflammatory cell in periodontitis, a disease caused by a small number of gram-negative bacteria, some of them (e.g. streptococcus sanguis, P. gingivalis) having been shown in-vitro to induce platelet aggregation. So far no other studies on platelet function in patients with periodontitis are available.
Method: In normolipemic non-smoking patients (37 – 55 years) suffering from periodontitis (n = 71) and an age- and sex-matched control group (n = 71) blood was drawn for the determination of serum thromboxane B_2 (TXB_2) (standardized coagulation at 37°C for 60 minutes) as well as plasma-TXB_2 and 11-dehydro-TXB_2. Parodontal disease was assessed by the community periodontal index of treatment needs (CPTIN), plaque index (PI) and papillal-bleeding index (PBI). Serum TXB_2 was comparable to the values found in the control group, while plasma and 11-dehydro-TXB_2 in plasma were significantly ($p < 0.01$) elevated. In the individual patients there was a strong correlation ($p < 0.01 – 0.001$) to the disease indices (PI > PBI > CPITN), in line with elevated TXB_2-formation at the local gingival site.
Results: These findings indicate that periodontitis induces an increase in circulating TXB_2, a prothrombotic state which might favour initiation and progression of vascular disease.
Conclusions: There are plenty of explanations for the association between periodontal disease and atherosclerosis. One of them is that periodontal inflammation is clearly sufficient to induce systemic inflammatory responses, reflected by an increased CRP, paralleled by increased plasmatic thromboxane, as shown in this study.

OP 55

EFFECTS OF PORPHYROMONAS GINGIVALIS LIPOPOLYSACCHARIDE ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS WITH REGARD TO EXPRESSION OF CELL ADHESION MOLECULES

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Several studies suggest an association between periodontal disease and cardiovascular diseases, particularly atherosclerosis. One of the bases for such a link seems to be the involvement of various periodontal pathogens, particularly Porphyromonas gingivalis. In the present study, we investigated the effect of P. gingivalis lipopolysaccharide (LPS) on the expression of cell adhesion molecules (CAMs) in endothelial cells, which is a hallmark for early inflammatory stages of atherogenesis. The effect of P. gingivalis LPS was compared with that of E. coli LPS and, in addition, the contemporaneous stimulating effect of these two pathogens was studied. Human umbilical vein endothelial cells (HUVECs) were stimulated with P. gingivalis LPS (concentrations of 0.1, 1.0, 10.0 µg/ml) and/or E. coli LPS (concentrations 0.01, 0.1, 1.0 µg/ml) for 24 hours and the change in the mRNA expression levels of ICAM-1, VCAM-1, and E-selectin was measured using real-time PCR. We found that both P. gingivalis LPS and E. coli LPS induced a significant dose-dependent increase of mRNA expression levels of all CAMs. The level of E-selectin mRNA was increased up to 300 times compared to control, whereas the mRNA level of ICAM-1 and VCAM-1 was increased maximally by factors of 10 and 20, respectively. Surprisingly, contemporaneous stimulation of HUVECs with two pathogens substantially attenuated the stimulatory effect. Our data show that P. gingivalis can enhance progression of early atherosclerotic lesions by up-regulation of CAMs expression by endothelial cells, which may facilitate recruitment and adhesion of macrophages.

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OP 56

TREATMENT OF INTRACRANIAL HAEMORRHAGE WITH RECOMBINANT FACTOR VIIa IN A PATIENT WITH APLASTIC ANEMIA

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Background: Haemorrhagic episodes in patients with aplastic anaemia occur usually secondary to thrombocytopenia and require frequent support with platelet concentrates and other blood products. Repeated transfusion often results in alloimmunization and lack of increments to further platelet transfusion. Activated recombinant factor VII (rFVIIa, NovoSeven®) has been used to control bleeding episodes in a wide spectrum of congenital and acquired bleeding disorders.
Aim: We describe our experience in the management of intracranial haemorrhage in an adolescent patient with aplastic anemia.
Case report: In a 17-year-old boy diagnosed with very severe acquired aplastic anemia, platelet count remained under 10x109/L so the patient developed intermittent haematuria and an episode of subcutaneous haemorrhage requiring supplementation with platelet transfusions. One month after the treatment started, the patient complained about headache, hypoaesthesia and slurred speech. The urgent CT showed intracranial haematoma (27x23x20 mm) of interventricular localization. The patient was treated with rFVIIa 70 µg/kg q2h and platelet transfusions q6h, for 24 hours. Control CT after six hours of treatment was unchanged with moderate amelioration of clinical symptoms. Three days later, CT showed reduced volume of haematoma and the patient was rendered symptom-free.
Conclusion: our case report provides supportive evidence of the efficacy and safety of rFVIIa along with platelet transfusions in a patient with severe thrombocytopenia. Based on this case, we suggest considering the use of rFVIIa in patients with severe bleeding due to thrombocytopenia.

OP 57

KASABACH-MERRITT SYNDROME - ASSOCIATED KAPOSIFORM HEMANGIOENDOTHELIOMA: SUCCESSFULLY TREATED VASCULAR NEOPLASMA WITH CONSUMPTIVE COAGULOPATHY

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Background: Kaposiform hemangioendothelioma (KHE) is an extremely rare and locally aggressive vascular neoplasm often associated with consumptive coagulopathy or Kassabach-Merritt Syndrome (KMS). The vascular lesion causes platelet trapping and activation, with consumption of coagulation factors.
Aim: The authors introduced a successful trial of a chemotherapy regimen to treat hemangioma, thrombocytopenia and coagulopathy.
Methods: We report a case of a 3-month-old boy suffering from a KHE associated with KMS. Physical examination revealed a large mass in his left anterior chest wall and neck. Laboratory analysis verified severe consumption coagulopathy with thrombocytopenia (20x10(9)/L), hypofibrinogenemia (1,0 g/l) and elevated D-dimer level (21825 ng/ml). Hemolytic anemia was also present (Hb 82,4 g/l). Computerized tomography (CT) of the chest wall and neck showed soft tissue mass occupying most of the left hemithorax, mediastinum and neck. Histopathologic findings after biopsy described features of KHE. We treated the patient with corticosteroids, vincristine and a chemotherapy regimen consist of vincristine, actinomycin D and cyclophosphamide (VAC).
Results: Corticosteroid and vincristine treatment initially induced minimal reduction in tumor size and partial reversal of the consumption coagulopathy. After 6 week course, it was decided to use a chemotherapy regimen of vincristine, actinomycin D and cyclophosphamide (VAC). The patient responded to the second cycle of chemotherapy with complete reversal of the thrombocytopenia and consumptive coagulopathy. After nine cycles of the applied three-drug regimen he was in complete remission, which has been maintained for 5 months since stopping therapy.
Conclusion: Severe consumption coagulopathy associated with KMS-KHA can be successfully treated with chemotherapy.

OP 58

KASABACH-MERITT SYNDROME: A STATE OF ALARMING CONSUMPTIVE COAGULOPATHY

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Introduction: Kasabach-Merritt syndrome (KMS) is an association of a vascular lesion and consumptive coagulopathy, due to localized intravascular coagulation (LIC). KMS is a diagnostic and therapeutic challenge to the clinicians, especially when it is accompanied with severe bleeding episodes.
Purpose: We analyzed retrospectively our patients with KMS, in order to assess the postulates of such a serious condition and its treatment modalities.
Method: For the last 20 years, we have treated 9 cases of large hemangiomas of different localization, accompanied with thrombocytopenia and the signs of severe consumptive coagulopathy. Most patients suffered from episodes of massive bleeding into the tumor and surrounding tissue, some of them into the visceral organs and central nervous system (CNS). Due to unpredictable clinical course and potentially fatal outcome we applied combined modalities of treatment in order to stop the hemorrhage, stabilize and cease the consumption of coagulation factors.
Results: Initial treatment with Prednisone in large doses of 5 mg/kg was successfully applied in 3 cases. As there was no benefit with steroid therapy, for the most two weeks, Alpha-2a Interferon was administered in 6 patients, in a dose of 1-3 million iu/m^2, as well as Ciclokapron and Dipiridamol in adequate doses. The intensive substitute treatment with blood derivates was applied in every case of hemorrhage. Complete remission of disease was achieved in all patients, combining medicaments and adequate surgical treatment. There was no need for chemotherapy or mutilating surgery.
Conclusion: Due to our experience, we consider that Prednisone and especially Alpha-2a Interferon are exceptionally useful in treatment of large hemangiomas complicated with KMS. Disadvantage of such treatment is its long duration and expensiveness of Interferon.

OP 59

COAGULATION DISTURBANCES IN PATIENTS WITH VENO-OCCLUSIVE DISEASE AFTER BONE MARROW TRANSPLANTATION

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Hepatic veno-occlusive disease (VOD) is a life threatening complication after bone marrow transplantation (BMT). Its prediction, precise diagnosis and treatment remain unclear. To determine the incidence, outcome and changes in haemostatic parameters in patients with VOD we prospectively evaluated all consecutive patients receiving BMT in the period February 2004 to July 2008. Our goals were to determine coagulation disturbances in the patients with VOD and their practical significance in early diagnosis. All of them were diagnosed according to Siettle criteria. During the study period 74 BMT were performed in Mother and Child Health Care Institute of Serbia. VOD was diagnosed in 11 patients. 10 of 46 antilogous and 1 of 28 allergenic BMT developed this complication. VOD was classified as mild in 7, moderate in 1 and severe in 3 patients. PT, aPTT, fibrinogen, FVIII, AT and vWF were measured on the day prior to starting conditioning regiment and on the days 1,7 and 14 from the moment of VOD diagnosis. At the moment of establishing the diagnosis all patients had significantly increased activity of vWF and FVIII and decreased AT and fibrinogen. All of them were dependent on platelet transfusions. In our group of patients the incidence of VOD was 14,8%. Platelet transfusion dependence suggests coagulation activation with great significance and indicates possible development of VOD. Our results also suggest that monitoring of coagulation parameters levels in the first five days from diagnosis establishment may have significance predictive value for the VOD outcome.

OP 60

IMPORTANCE OF LIFELONG ORAL ANTICOAGULANT THERAPY IN ADOLESCENT WITH CONGENITAL THROMBOPHILLIA

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We are presenting a 17 years old male adolescent who had been admitted in our hospital in September 2007 due to fever, pain and swelling of the left lower limb. Family history was positive for thrombophillia. Patient s father died because of thrombotic event in mean forties. Screening for thrombophillia showed resistance of activating protein C. Color Doppler revealed thrombus in vena saphena magna. Therapy started with low molecular weight heparin (LMWH) and parenteral antibiotic. After 10 days we discharged LMWH and started therapy with oral anticoagulant agent. Control ultrasound performed 14 days latter showed completely resolution of the thrombus so we preceded oral anticoagulant therapy for three months. In the meantime we obtained molecular and genetic analyses which shoved that the patient is heterozygote for FV Leiden and MTHFR C677T mutation. From January to November 2008 he received prophylaxis with low doses of acetylsalicylic acid. Second episode of the thromboplebitis in left popliteal vein occurred in April 2008. In August 2008 we decided to start with continuous oral anticoagulant therapy for prevention thromboembolic complication, bur patient redrew therapy by himself. In January 2009 he developed third episode of thrombophlebitis in left lower limb. This case shows the importance of lifelong per oral anticoagulant therapy even in adolescence, in patients who have documented congenital thrombophillia and at least one episode of thromboembolic event. This therapy reduces the risk of novel, potentially much more severe thrombotic complications.

OP 61

INFLUENCE OF OBSTETRIC FACTORS ON QUALITY OF UMBILICAL CORD BLOOD UNITS

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Objectives: The umbilical cord blood (UCB) units volume as well as haematopoietic stem cell (HSC) count are affected by the collection method and obstetric factors. Based on contemporary study results, it was unambiguously established that birth weight (BW) and placental weight (PW) affect the volume as well as HSC count in UCB samples, while the contribution of other obstetric factors is less clear.
Aim: The aim of this study was to investigate the influence of obstetric factors to the volume and HSCs count in CB units.
Methods: Retrospective analysis of obstetric factors was performed in 99 UCB units. Volume of UCB units, total nucleated cell (TNC), CD34+ cell and colony forming cell (CFC) counts were used as product quality indicators.
Results: Total of 99 units was collected. The study results showed that BW > 3500 g and PW > 700 g (72 units), significantly increases the volume in collected samples, TNC, CD34+ and CFC counts (mean volume = 96 ml, TNC = 12,5 x 10^8, CD34+ = 3,9 x 10^6, CFC = 104,1 x 10^4) comparing to BW from 2500 g – 3500 g and PW from 600 g – 700 g (27 units), (mean volume = 74 ml, $p<0,05$; TNC = 9,2 x 10^8, $p<0,05$; CD34+ = 2,9 x 10^6, $p<0,05$; CFC counts = 64,3 x 10^4, $p<0,001$). In addition, length of umbilical cord (≥ 30 cm) also increases significantly the volume and TNC count comparing to cord length of ≤ 30 cm ($p<0,05$). Other obstetric factors do not affect significantly the quality of UCB units.
Conclusions: Our study confirmed that birth and placental weight and length of umbilical cord independently influenced volume, TNC, CD34+ cells and CFU content in collected units. These obstetrical parameters could be added to standard cord blood donor criteria in order to improve the bank efficiency.

PP 62

LUPUS ANTICOAGULANT ANTIBODIES IN CHILDREN

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Background: Lupus anticoagulant antibodies (LA ab) are increasingly being recognized in children. One LA ab type is pathogenic, causing thrombosis and trombocytopenia, second is non-thrombotic, almost transient and diagnosed incidentally. We differentiate two groups of children: first with LA ab and thrombotic events, and second with childhood infection or autoimmune diseases or asymptomatic and LA ab.
Aim: To find frequency both types of antibodies, what is the difference between them in laboratory diagnostic tests, as well as to determine presence of clinical manifestations in patients with pathological LA ab, their connection with inherited thrombophilia.
Methods: Two groups of 74 children were tested. The LA was detected by coagulation assays: aPTT - LA sensitive reagent, DRVVT test (LA Screen, LA Confirm) and KCT.
Results: In thrombophilia group 23% of patients had LA ab, and their median age was 6.5 years, the frequent diagnoses were infarctus cerebri, deep vein thrombosis of the lower limbs and thrombosis v. portae et v. lienalis. The patients mostly had other risk factors for thrombosis: high FVIII, oncological or metabolic diseases, MTHFR or FV Leiden mutation. In group without thrombosis 50% of patients had LA ab, median age was 8.5 years, with tonsillitis chr, SLE, Hepatitis and AIHA as common diagnoses. The difference between the groups (thrombosis/asymptomatic) was statistically significant for aPTT (31.19 ±9.7/41.4±19.5), Lac Screen (39.15±10.5/44.69±13) and KCT (80.7±34.4/110.8±59.1) $p<0.01$.
Conclusions: Prolongation coagulation assays (mildly or markedly) were not associated with severity of clinical symptoms in children. Lupus anticoagulant ab may serve as a "second hit" in children with hereditary thrombophilic risk. Mainly associated with another risk factors LA ab can to cause thrombosis in children.

PP 63

HYPERCOAGULABILITY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Hypercoagulability is not a frequent occurrence in children with acute lymphoblastic leukemia (ALL), but once it appears, it represents a serious condition. AIM: The case study of patients that have developed a hypercoagulability disorder during ALL treatment at the Children's Clinic in Niš, in the period from 2003 to 2008.
Patients and Methods: Of 28 children with ALL, treated according to protocol BFM2002, hypercoagulable disorder was present in two: a seven year old boy in the induction phase and a four year old girl in the reinduction phase. With both children a clinical picture of sepsis developed, and locally the progression of changes from thrombophlebitis and phlegmone to abscess and deep ulcers.
Results: Laboratory results show that in the boy there are higher values of FVIII, FI, FDP and lupus anticoagulans, and in the girl along with the lowered Protein S, high values of FV, FVIII and FDP. Only after the application of anticoagulant therapy is there normalization in laboratory tests and the improvement of local changes.
Conclusion: The prevalence and pathogenesis of hypercoagulability associated to ALL are obscure. The risk factors with these children are numerous: the primary disease itself, chemotherapy, infection, trauma, vein paths and genetic predisposition. Although most of the events occurred during the induction phase of therapy, a continuous observation of children during treatment is necessary for early recognition and due time therapy application.

PP 64

THROMBOTIC MICROANGIOPATHY INDUCED BY CMV AND HHV6 INFECTION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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We report a girl with MDS, RAEB - T who developed thrombotic thrombocytopenic purpura (TTP) after allogeneic bone marrow transplantation (BMT). Girl was admitted at the age of 4 due to fever, fatigue, craving for water and polyuria. At the admission the girl had hepatomegalia and anemia with neutropenia and lymphopenia. According to history of illness and laboratory findings the diagnosis of neurohormonal diabetes insipidus had been established. Due to hepatomegalia and maintaining of anaemia bone marrow biopsy had been performed. It showed mylodisplastic syndrome, RAEB - T with monosomia 7. The girl had identical sibling donor, thus the allogeneic BMT had been performed. After conditioning regiment (Busulphan, Fludarabin, Thiothepa and Cyclophosphamid) she received 300 ml of allogeneic bone marrow with 6,86 x 106/kg BW CD 34 + cells. Immunosuppression started with Cyclosporine A. On the day +4 she developed acute GVHD of the skin and the gut, which was treated with methilprednisolone. Neutrophil and thrombocyte engraftment was achieved on the days +23 and +37. 53 days after BMT the girl developed acute headache and right hemiparesis. CT scan showed massive intracranial hemorrhage. According to clinical picture and increased values of the F VIII, vWF, LDH, findings of the fragmented erythrocytes in peripheral blood smear and positive PCR on CMV and HHV6 the diagnosis of TTP was established. The girl was treated with Defibrotide and therapeutic plasmapheresis. Despite all therapeutic procedures she passed away on the day +102.

PP 65

COAGULATION TESTING OF NEWBORN WITH CEPHALHEMATOMA

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Background: There is a significant deficiency of the K vitamin dependent coagulation factors in the normal newborn. The plasma levels of these factors normally fall even further during the first two to five days of life. This normally does not produce bleeding, but in hemorrhagic disease of the newborn the initial fall is accentuated and restoration is delayed and incomplete.
Aims: Discovering the cause of bleeding in six days old newborn with cephalhematoma, which sample of blood was sent to our blood bank department. Also our aim was to help the pediatricians in treatment of this patient.
Methods: Coagulation screening test (PT, aPTT, TT, D-dimmer test) and specific coagulation tests were performed on ACL 9000. Whole blood aggregation was determined using impedance aggregometer (Multiplate ®). ROTA thromboelastometry was performed on Rotem ® .
Results: Six day old newborn was admitted at pediatric department because of infection of umbilical stump and jaundice. After two days he developed bilateral parietal cephalhematoma. There was no record about K vitamin administration after delivery. Coagulation screening test PT 22 sec., aPTT 43 sec., TT 15.4 sec. Special test FII 29.3%, FV 70.5%, F VII 56.7%, FVIII 99.1%, FIX 43.1%, FXI 6.86%, FXII 34.4%, D dimmer 1428 ng/ml. In order of these results we suspected on hemorrhagic disease of newborn and sepsis. There was no pathologic results in platelet aggregation and thromboelastometry. Intravenous application of 3 mg vitamin K corrected pathological results in screening tests. Coagulation screening test after therapy: PT 13.2 sec., aPTT 31.8 sec., TT 19.5 sec. Special test FII 56.8%, FV 111%, FVII 91.1%.
Conclusion: Coagulation testing of newborns with hemorrhagic syndrome are necessary for establishment diagnosis and differential diagnosis of this patients. Prompt recognition is critical, since early therapy directed to ward achieving haemostatic, can be life saving.

PP 66

VITAMIN K DEFICIENCY BLEEDING IN A CHILD WITH CHOLESTASIS

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Background: Late vitamin K deficiency bleeding (VKDB) peaking at 3-8 weeks, typically presents with intracranial haemorrhage often due to undiagnosed cholestasis with resultant malabsorption of vitamin K. Other types of bleeding are less frequent. Infantile choledochal cyst usually presents as jaundice but almost never as a bleeding disorder. We report a case of a two-month-old infant with choledochal cyst presenting as suspected late VKDB.
Case report: A two months old female infant presented to the emergency room due to prolonged bleeding after capillary blood sampling for routine blood counting. Otherwise the patient was in a good general condition with unremarkable physical findings. Haemostasis screening tests were markedly abnormal: PT prolonged >1000 s and PTT 788,2 s. Detailed coagulation examination showed low values of vitamin K dependent coagulation factors. Administration of one dose of 1 mg vitamin K and one dose of fresh frozen plasma enabled normalization of coagulation tests after 12 hours. Biochemistry analyses revealed conjugated hyperbilirubinemia and subsequent abdominal ultrasound showed choledochal cyst. Urgent surgical treatment with liver biopsy was performed with no bleeding or other complications. Choledochal fusiform type I cyst with normal liver tissue was confirmed by histology. Normalization of liver tests was confirmed during the follow up of six months.
Conclusion: Early recognition of diseases predisposing to VKDB and immediate investigation and treatment of warning bleeds help prevent the worst consequences. Late VKDB is often the presenting feature of a serious underlying disease which may be recognized earlier.

PP 67

CHARACTERISTICS AND MANAGEMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD

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Background: Idiopathic thrombocytopenic purpura (ITP) often presents as an acute self-limiting disease in the pediatric patient. Because of this prognosis physicians debate the need for treatment of ITP in this patient population, especially in the presence of clinical symptoms. Nonetheless, a few children present with severe symptoms and require treatment.
Aims and Methods: The objective of this study was to review presenting features, response to therapy, and natural history of ITP treated at a single pediatric medical center.
Results: A retrospective chart review was made for all children diagnosed with ITP and treated at the University Children's Hospital in Skopje which is the only institution that provides tertiary care and copes with this problem in our country. Over the 10 years period 223 children aged 2 months to 15 years have been diagnosed with ITP and followed up. Acute ITP remitting within 6 months occurred in 202 and 21 had a chronic course. Sex incidence in the acute ITP was equal (male: female = 100:102) and the most cases in this group occur in children aged 2-10 years - 113 (55,9%). The mean platelet count on admission was 21x109L, lowest count 0x109L. Bone marrow aspiration was performed almost in all cases. Serious bleeding symptoms were registered in just 17 (8,4%). No patient suffered an ICH, and no death was reported. Initial management consisted of no drug treatment in 12 patients (5,9 %), intravenous immunoglobulin (IVIG) in 19 (9,4%) and glucocorticosteroids (GS) in 171 (84,7%). IVIG were used just in infants as they were expensive form of treatment. The majority of children with acute ITP recovered without recurrence. In 13 (5,8%) of patients resolution of the initial disease was followed by recurrent episodes of thrombocytopenia. Chronic ITP occurred in 21 (9,4 %) of the children with ITP, was more common in older children >10 years (33,3%) and in females (66,7%). Most received GS and/or IVIG, 3 were splenectomised. Although the course of chronic ITP was generally mild, 15 (71,4 %) had some serious bleeding episodes, and 1 child died from ICH.
Conclusions: ITP is a common pediatric disease presenting at any age with low morbidity and mortality. Most cases can be managed by pediatricians without hematology referral. Several equally successful therapeutic options exist. Chronic cases present at an older age with higher platelet counts. Annual incidence of acute ITP in our country was 4,0/100000 children under 15 years (for chronic ITP it was 0,4/100000). Serious bleeding in acute ITP was uncommon. Fatal ICH in our study (in a patient with chronic ITP) was low (0,4%).

PP 68

SAFETY OF LUMBAR PUNCTURE IN THROMBOCYTOPENIA - PROPHYLACTIC PLATELET TRANSFUSIONS BEFORE LUMBAR PUNCTURE: YES OR NO

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Background: Prophylactic platelet concentrate (PC) transfusions may result in complications and increase costs of treatment. It has not been shown that thrombocytopenia increase the possibility of lumbar puncture (LP) related bleedings and that platelet transfusions reduce the risk of these complications.
Aim: We wanted to find out how often the LP was performed at platelet number < 50 x 109/L, how often the PC was used and which were the complications.
Methods: 51 patients were included into the retrospective study. The patients were divided into 3 groups according to the platelet number (group 1: < 10 x 109/L; group 2: 11-20 x 109/L; group 3: 21-49 x 109/L).
Results: 61 (6/19/36) and 74 (19/23/32) LP were performed in the individual groups of the patients considering results of the platelet number measured with analyzer and Neubauer counting chamber respectively. The PC transfusions were used before LP at 5/6 (analyzer) and 9/19 (chamber) patients in group 1, at 7/19 (analyzer) and 3/23 (chamber) patients in group 2, and at 2/36 (analyzer) and 1/32 (chamber) patients at group 3. Complications were not detected in any of groups.
Conclusions: Our results confirm findings of previous studies in which the safety of LP at platelet number higher than 10 x 109/L was confirmed.

DLTH2009 Belgrade

Principles of Haemophilia Care

PL 69

HAEMOPHILIA CARE IN EUROPE – HOW CAN WE IMPROVE IT?

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Those with haemophilia can lead almost normal and fulfilling lives if their bleeding disorder is well managed. Increasingly this requires that individuals are responsible for their own treatment. This autonomy has, however, to be within a therapeutic arrangement, which is able to provide the appropriate specialist care for the different manifestations of haemophilia and its complications. For children to grown up relatively free of the ravages of repeated haemarthroses and muscle haematomas requires more than just clotting factor concentrates infusions. Whilst this therapy is essential to prevent and treat bleeds it can only be effective if it is in the context of a Comprehensive Care Haemophilia Service. This requires a partnership between those who fund the service, the professional Haemophilia Centre staff and those with haemophilia and their families. The Comprehensive Care environment requires a wide range of appropriate specialist services, apart from haemostasis experts, to be readily available including dentistry, orthopaedics and physiotherapy along with paediatrics and obstetrics and experts in the diagnosis of treatment and transfusion transmitted infections. The arrangements for Comprehensive Care must dovetail with other local medical and surgical services. Local, geography and the distribution of the population will dictate the optimal arrangements between larger Comprehensive Care Centres and Haemophilia Centres, which provide a more limited range of specialist services, particularly directly at the provision of immediate care of acute bleeds. There is, therefore, no single plan, which can be applied to all countries, but rather a series of principles, which can be used to help design appropriate arrangements. The European Association of Haemophilia and Allied Disorders (EAHAD) has set out in the European Principles of Haemophilia Care (Haemophilia, 2008, 14, 361-374, or www.eahad.org) guidance on the essential components of a high quality service. Furthermore because haemophilia is a relatively uncommon disorder, collaboration between Haemophilia Centres is becoming increasingly necessary particularly to undertake clinical audit and research. One such project is the establishment of the European Haemophilia Safety Surveillance System (EUHASS). Since the 1st of October 2008 this has gathered data on adverse events from 45 Haemophilia Centres in 25 European countries. This EUHASS enterprise in collaboration with the patients' European Haemophilia Consortium (EHC) and EAHAD is likely to expand to include further Haemophilia Centres. These and other developing EAHAD initiatives will hopefully lead to improved haemophilia care throughout Europe.

OP 70

BUILDING THE REGISTRIES AND NETWORKS FOR HEMOPHILIA CARE. A NATIONAL APPROACH: SLOVAKIA

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The National Registry of hemophilia and related bleeding disorders in Slovakia was established in the late 1960´. The system, operational at the National Hemophilia Centre in Bratislava was computerized in 1992. The data are collected regularly using questionnaires distributed among all hemophilia treatment centers in the country. The registry is a name based system, however the confidentiality is ensured at all times. The registry comprises the demographic data, severity of bleeding disorder, blood group, inhibitor, treatment modality, yearly factor consumption, virology status, surgical procedures, education, employment, disability and mortality, including the reasons of the death. At present the National Registry database accounts 1980 individuals with bleeding disorders (476 hemophilia A, 71 hemophilia B, 424 von Willebrand disease, 504 congenital FVII deficiency and 505 persons with other rare bleeding disorders), comprising a total of more than 100,000 records. The Registry provides important information about the hemophilia population at large. In 1991 the data derived from the Registry represented an essential prerequisite for allocation of adequate resources for the introduction of safe factor concentrates nationwide. Regular yearly survey of the Registry serves as the basis for the formulation of the real requests submitted to the National tender for the purchase of clotting factors concentrates. In 2008 a supply of FVIII of 4.0 IU/capita/year has been achieved. The institution of the National Registry helps to generate the pressure on the Health Authorities aiming at progressive increase of factors supply as well as the preservation and further promotion of the overall quality of hemophilia care.

OP 71

QUALITY OF HAEMOPHILIA TREATMENT IN SERBIA: NATIONAL HAEMOPHILIA REGISTRY REPORT

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Background. National Registry of patients with inherited bleeding disorders was established in 1963 and ever since it has been in charge of the Haemophilia Center, Blood Transfusion Institue of Serbia, Belgrade.
Aim and Methods. Purpose of the study was to assess the quality of haemophilia treatment in Serbia from 2000 to 2008 based on the National Registry data related with the organization of care and quantities and choice of products.
Results. A total of 392 haemophilia A, 64 haemophilia B, 217 vWD and 19 RBD patients are in the National Registry. Treatment can be obtained in seven Haemophilia Treatment Centers - haematological and pediatric institutes and clinics in Belgrade, Nis and Novi Sad, as well as in another twenty local hospitals. From 2000 to 2003 around three million units of FVIII concentrate were administered annually, e.g. 0,25 IU/capita/year. Besides, national cryoprecipitate was also available for the treatment. In 2003, National Hemophilia Committee was founded which, among other things, resulted in the introduction of centralized products supply. During 2004 and 2005, considerable quantities of concentrate were provided: around five million units annually, i.e. 0,65 IU/capita/year. The choice of products was also improved. Namely, until 2004 availability of DDAVP, antifibrinolytic drugs and rFVIIa concentrate was limited, while from 2004 these products became available for the treatment of haemophilia patients in Serbia . In order to further improve haemophilia care we also established international cooperation: education, training, consulting and participation in clinical and research projects. As a result of our activities, FVIII concentrate consumption in 2008 was 10,5 million units e.g. 1,35 IU/capita/year.
Conclusions. Considerable improvement of the treatment is the result of efforts made by the health care and regulatory institutions in Serbia. Significant support has been provided through the cooperation within highly useful twinning programs between Stockholm and Belgrade Haemophilia Centers in 2003-2004 and Hamilton and Belgrade Haemophilia Centers in 2005-2008.

OP 72

CONTINUOUS FVIII REPLACEMENT FOR OPEN HEART SURGERY IN THE NEWBORN - A CASE REPORT

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We present a case of successful open heart surgery in the newborn with transposition of great arteries and hemophilia A. Term male newborn developed a respiratory distress four hours after delivery and was referred to our hospital. Heart ultrasound revealed transposition of great arteries and immediate treatment with alprostadil was stared. Since the child has the positive family history for hemophilia A, immediately after birth a blood specimen for coagulation studies was taken and the diagnosis of hemophilia A was established. The next day baloonatrioseptostomy was performed with FVIII substitution of 50 U/kg every eight hours. Procedure and the course after intervention were uneventful and the child had satisfying rise in oxygen saturation. Substitution of FVIII every eight hours continued until open heart surgery, which was performed in the 12th day of life. Before procedure a bolus of FVIII 100 U/kg was given and then continuous infusion of FVIII started in the dosage 10 U/kg/h. With this regimen no unusual bleeding was detected during procedure as well as in the postoperative period. Levels of FVIII were in the range of 46-164%. Continuous FVIII infusion in the dosage of 10 U/kg/h took place for eight days and in the following six days FVIII was given every eight hours 50 U/kg. After fourteen days child was discharged and put on secondary prophylaxis with weekly bolus of FVIII 50 U/kg. This regimen of continuous FVIII was safe and effective in the setting of neonatal open heart surgery in newborns with hemophilia A.

OP 73

MOLECULAR GENETIC DIAGNOSTICS OF HEMOPHILIA A IN SLOVENE PATIENTS

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Hemophilia A is the most common X-linked coagulation disorder, with an incidence of about one in 5000 males. The degree of disease in FVIII activity correlates with increased severity and frequency of bleeding in patients. The F8 gene maps to the long arm of the X chromosome. Gene has 186 kb and is composed of 26 exons. Unusually, the intron 22 contains additional transcript, termed F8A. Two additional copies of F8A have been found 400 kb telemetric to the FVIII gene. The function of the F8A gene is still unknown, but it is implicated in almost half of severe hemophilia A via a partial inversion mechanism. Other patients have gene mutations specific for the family. There are 166 hemophilia A patients from 114 families in Slovene registry for Hemophilia, of which 76 have severe hemophilia A, 20 patients have moderate and the rest have mild form of the disease. In the Slovene population we determined genetic mutation in 133/166 patients, 38/133 have inversion of intron 22 and another 95/133 have 47 different mutations in F8 gene which cause hemophilia A – 21 of them are so far found only among Slovene patients. Between 1989 and 2008 14 prenatal diagnostics of hemophilia were performed. The determination of hemophilia mutations is of great importance for the discovery of the hemophilia carriers and the prenatal diagnostics of hemophilia A.

OP 74

PROPHYLAXIS IN ADULTS WITH HAEMOPHILIA A: BENEFIT OR RISK?

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Prophylaxis is regular infusion of coagulation factor concentrates to severe haemophiliacs that started in Sweden in the 1960s. The aim of the prophylactic treatment is to start early in life and to reduce overall and joint bleed frequency. Haemophilia patients who have been on prophylaxis from an early age have less physical impairment, reduced time lost from school and work and better socialization and quality of life compared with patients who were treated on-demand. There are different forms of prophylaxis, like primary, a continuous therapy, starting after the first joint bleed or before the age of 2 years. The goal of secondary prophylaxis is usually to avoid progression of joint disease. It can be continuous long-term treatment started after two or more joint bleeds or after the age of 2 years. However, intermittent periodic prophylactic treatment that is initiated after a period of frequent bleeds is regarded as secondary prophylaxis. The experiences with prophylaxis are mostly based on retrospective studies. Two large, prospective, randomized controlled clinical studies compare prophylaxis with on-demand therapy for prevention of arthropathy and haemarthroses in young children with haemophilia A. Results of these studies of prophylaxis showed improved outcomes by 6 years of age in children started at a young age on alternative day prophylaxis in comparison with on-demand treatment. Prophylactic treatment in adults remains controversial. It is known that patients who begin secondary prophylaxis in adulthood may drive some benefit despite haemophiliac arthropathy being already established at that age. It would reduce bleeding frequency and may slow the rate of arthropathy progression. The survey by Richards confirms the lack of consensus on the management of prophylaxis in severe haemophilia in adulthood. Further clinical studies have and continue to be conducted in this field in an attempt to determine the ideal regimens of prophylaxis, its benefits its risk and costs in adult haemophilia A patients.

SOA 75

HOW SAFE IS RADIOCTIVE SYNOVIORTHESIS IN HAEMOPHILIA

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From 1976 to 2005 we have performed 134 synoviorthesis (SHORTER Follow up 4 years) : 3 patients, 3 joints, 3 sessions, 4 patients, same joint, 2 sessions JOINTS. Right knee 42, left knee 34, right elbow 18, left elbow 19, right ankle 9, left ankle 19 and right shoulder 1. RADIOCOLLOID USED , GOLD - 198Au : 36 joints, RENIUM - 186 Re : 19 joints and YTRIUM - 90 Y : 79 joints RESULTS • HAEMARTHROSIS, N O N E 70% , L E S S 21% , FAILURES 6 (9 %) in 5 Knee that sustained Surgical synovectomies GLOBAL GOOD RESULTS 91 % IN ORDER TO ASSESS THE SAFETY OF INTRARTICULAR RADIOACTIVE MATERIAL INJECTION WE PERFORMED CHROMOSOMAL STUDIES by CULTURE, FLUORESCENSE AND BANDING TECHNIQUES CHROMOSOMAL STUDIES AFTER SYNOVIORTHESIS WITH 189 AU STUDY I 1978 : 354 Metaphases , BREAKAGES 61 (17.23%), FRAGMENTED 13 , NON PREMALIGN 6 STRUCTURAL CHANGES (1.69%), Dicentric 2,Marker2, Triradius 1,Segregated 1 PREMALIGN Any number below 2% is considered NOT DANGEROUS CHROMOSOMAL STUDIES AFTER SYNOVIORTHESIS WITH 189 AU STUDY II 1982 (4 years after) : 649 METAPHASES ON SAME PATIENTS-BREAKAGE 21 (3.24 %), SEGREGATED 2 FRAGMENTED 1, NON PREMALIGN TWELVE (12) Haemophilic Patients(Group A) BEFORE SYNOVIORTHESIS with 186 RH, SEVEN (7) Haemophilic Patients and (Group B) FIVE TO SEVEN MONTHS AFTER RADIOACTIVE INJECTION with 186 RH GROUP A 9 / 12 patients presented numerical abnormalities GROUP B 7 / 7 patients presented numerical abnormalities 2 / 7 patients presented breakages in low proportion CYTOGENETIC ABNORMALITIES Chromosome Patients abnormality with abnormalities ANEUPLOIDY BREAKAGE Nº % Nº % Nº % GROUP A - 11 - 13 - 9 81.81% GROUP B - 7 - 19 2 6 85.71% TABLE 3 CHROMOSOMAL ABNORMALITIES in 431 METHAPHASES of 11 patient treated with 186 Re Total Nº Total abnormal Aneuploidy Breakages Frequency metaphases cells nº % Nº % Nº % abnormal cells GROUP A (11) * 272 13 4.77 13 4.77 - - 4.7 *** GROUP B (7) ** 159 19 11.94 17 10.69 2 1.25 11.94 *** * Pre 186 Re **Post 186 Re *** NO PREMALIGNANT CHROMOSOMAL ABNORMALITIES CHROMOSOMAL STUDIES ON NON IRRADIATED HAEMOPHILIACS STUDY III : 281 METAPHASES BREAKAGE 1 (1.26%) ACROCENTRIC SEGREGATION 2 (2.32%) Chromosomal Structural Changes NON PREMALIGN NO PREMALIGN CHROMOSOMAL ABNORMALITIES (markers, segregations, triradials, dicentric, others) WERE FOUND IN ANY OF THE TWO GROUPS Difference with previous study with 198 Au. PRE MALIGN LESIONS (*) STUDY I 1978 (1-2 years post), Non specific structural changes 61 (17. 23 %) *6 Chromosomes (1. 69 %) and STUDY II 1982 (5-6 years post), Non specific structural changes 21 (3. 24 %)*0 Chromosomes (0 %) STUDY III (Non irradiated), Non specific structural changes 2 (2. 32 %) *0 Chromosomes (0 %) RESULTS CHROMOSOMAL INESPECIFIC CHANGES (ANEUPLOID and BREAKAGES) have been previously reported as BEING PRODUCED BY CHEMICAL, VIRUSES and RADIONUCLEOIDS. IN OUR STUDY, Breakages were found in VERY LOW PROPORTION CHROMOSOMAL CHANGES (MARKERS, TRIRADIALS and DICENTRICS considered PREMALIGN in irradiated patients or premalignant disorders WERE NOT OBSERVED IN THESE STUDIES CONCLUSION RADIOACTIVE SYNOVIORTHESIS as prevention of haemophilic haemarthrosis is a SAFE PROCEDURE WITH EXCELLENT CLINICAL RESULTS and NO LONG STANDING PREMALIGNANT CHROMOSOMAL ABNORMALITIES PRODUCED Ten of the patients of our first session with 198 AU, married and had absolutely normal children.

OP 76

ORTHOPAEDIC MANAGEMENT OF MUSCULOSCELETAL COMPLICATIONS OF HAEMOPHILIA PATIENTS IN HUNGARY

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Patients and methods: 45 % of haemophilia patients of Hungary are treated in the National Haemohilia Center (NHC), Budapest. In the past 15 years the musculosceletal complications of the disease were treated in close cooperation between the Orthopaedic Department of the Semmelweis Medical University and the NHC. The treatment courses of 65 patients with severe congenital bleeding disorder are presented by the authors. The diagnostic distribution of the patients: haemophilia A – 55; haemophilia B – 4; von Willebrand's disease – 6, with the mean age of 30 at the time of the surgical intervention.
Results: 43 patients went through on invasive orthopaedic procedure. 22 patients were managed exclusively by conservative methods. The indications for arthroscopy and open surgery included severe bleeding episodes unresponsive to factor replacement, and articular blockage. For the total prosthesis implantations the main indications were resistant bleeding and resting pain. The distribution of the different orthopaedic procedures was the following: total knee replacement – 12; total hip replacement – 7; arthroscopic synovectomy – 2; open synovectomy –1. The average FVIII consumption was 116 500 IU during the prosthesis operations and 49 190 IU during the arthroscopic procedures.
Conclusion: As a result of the operations the number of the recurrent bleeds has significantly decreased, the quality of life improved with the cessation of the resting pain. The range of motion of the joints did not decrease significantly.

OP 77

BENEFITS OF EARLY TREATMENT OF BLEEDING IN HAEMOPHILIA PATIENTS WITH INHIBITORS

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Identifying haemophilia patients with inhibitors for clinical trials is difficult due to the limited number of patients available. Registries are therefore being established as an additional means of data collection. HemoRec is an international registry for patients with haemophilia. The aim of presented analyses of bleeding episodes of the adult inhibitor patients from Czech Republic was to investigate the effect of different recombinant activated factor VIIa dose ranges and time schedules on the incidence of re-bleeding. Patients treated within 2 h of bleeding onset experienced less re-bleedings than patients treated after 2 and more hours of bleeding onset (5.2% vs. 13.7%, respectively). In addition, patients who were treated after 2 h of bleeding onset experienced fewer re-bleedings when high single dose rFVIIa was used (15.8% and 0%; <120 μg kg^{-1} and >250 μg kg^{-1}, respectively). Initial high single dose rFVIIa was also associated with a decline in total rFVIIa consumption needed to stop bleeding. We conclude that the early initiation of treatment (up to 2 h) is the most important factor for the effective management of bleeding episodes allowing significant reduction of the number of injections and the related cost of treatment. If the treatment has to be initiated with a time delay (> 2 h) the use of higher initial single dose rVIIa (>250μg/kg) was more effective than multiple low dose injections. The use of higher single dose rFVIIa resulted in significantly less re-bleeding episodes and significantly decrease the overall consumption when compared to treatment with initial low doses (<120μg/kg) requiring multiple injections. The HemoRec registry and the performed analyses provide unique insight into the bleeding patterns of inhibitor patients, highlighting the importance of early treatment initiation and appropriate dose.

PP 78

IS THERE AN IMPROVEMENT IN THE HEMOPHILIA CARE IN MACEDONIA?

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Macedonia is one of the former Yugoslav republics with a population of 2.1 million inhabitants. There are 313 registrated persons with hemophilia: 209 with hemophilia A and 104 with hemophilia B. The diagnosis is established by coagulation tests and determination of circulating procoagulant (percentage of f-r VIII and f-r IX). Genetic diagnosis has been performed in several cases where the hemophilia carrier state was determined. Fetal testing is still not regularly performed. The treatment of the patients is carried out with concentrates mainly derived from plasma and, very rarely, with recombinant concentrates. The treatment is founded on on-demand basis. Some patients (children) use home therapy for prevention and treatment of minor bleeding episodes. Very few patients use concentrates under prophylactic basis. Transmissible diseases (hepatitis B, hepatitis C and AIDS) have been detected but still are not completed for all patients. About 38% of patients have antibodies to antigens of hepatitis B, 35% for hepatitis C and none has antibodies to HIV. Three patients were detected with inhibitors (two of them were marked as high responders). The number of patients with affected joints is growing but complex rehabilitation therapy is insufficient. We can say “Yes” the hemophilia care in Macedonia is improving referring to the diagnosis, the regularly check up of patients, the usage of save products for treatment. The implementation of software for registration and follow-up of patients is ongoing. Regional educational workshops are periodically carried out.

PP 79

SOME CHARACTERISTICS OF PATIENTS WITH HEMOPHILIA IN REPUBLIC OF SRPSKA - BOSNIA AND HERZEGOVINA

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We performed a survey in 2007/08 on patients with hemophilia regarding their quality of life, which includes bleeding frequency, musculoskeletal problems, hepatitis infection, school life or social activities and social discrimination. In this survey we investigated the influence of bleeding frequency of total bleeding episodes and joint bleeding episodes on school life, which includes days of school absence and participation in school activities. Twenty four patients among fifty six registered hemophiliacs in Republic Srpska were analyzed for study in age group from six to twenty years old. There was correlation between the frequency of total bleeding episodes and school life. There was correlation between frequency of joint bleeding and school activities too. The result showed a relation between the articulate and physical state or situation of children and adolescent and perception that they have their quality of life. We should try to stop joint bleeding in children and young adolescent by introduction regular replacement therapy such as secondary prophylaxis for their increased participation in school activities.

PP 80

MULTICENTRIC EVALUATION OF HEALTH-RELATED QUALITY OF LIFE IN ROMANIAN HAEMOPHILIACS

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Introduction. In our country, most of the patients have limited access to substitution, with important impact on patients' health status, as well as on their health-related quality of life (HRQOL). Assessing and monitoring these parameters is essential for evaluating treatment strategies.
Aim. Aim of the study was to perform a multicentric cross-sectional study on young patients' health status and QOL in our specific therapeutic conditions.
Material and Method. Our study group included young haemphiliacs with age>7 and<40 years. It consisted of 120 patients, most of them (92.4%) with haemophilia A and severe form (74.2%). 46.2% were younger than 18 years. 26.5% were handicapped with social support, 24.2%-employees, and 49.2%-students. 43.2% lived in rural areas. Only 26.5% were treated in a Haemophilia Center with Comprehensive activity (HCC). We assessed QOL using EQ-5D questionnaire.
Results. Haemophilia severity influenced self-care domain (p=0.0338) while age impacted mobility (p<0.0001) and usual activities (p=0.0003). Significant differences were observed in mobility (p=0.0401), usual activities (p=0.0270), pain (p=0.0007) domains, in VAS (p=0.0033) and descriptive (p=0.0314) utilities, related to socio-professional status. Patients from urban areas had better descriptive utilities (p=0.0205). Comparing patients from different regions, we noticed significant differences (p=0.0274) only in self-care domain. Patients treated exclusively in HCC had better scores in mobility (p=0.0465) and usual activities (p=0.0493) domains, and better VAS utilities (p=0.0059).
Conclusions. Although we considered only young patients, our study revealed an equally affected HRQOL in the whole country, with some better results only in younger patients, urban areas and in haemophiliacs treated in HCC.

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PP 81

GENETIC DIAGNOSIS OF HAEMOPHILIA AND PRENATAL DIAGNOSIS. SLOVENIAN EXPIERENCES.

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Background. Haemophilia A/B results from a lack or decrease of FVIII/IX activity and is X-linked inherited. The locus for FVIII has been assigned to band Xq28 and for FIX to band Xq27. After known mutation in a family and detecting of a carrier it is possible to do prenatal diagnosis.
Aim. To study the prenatal genetic diagnostic methods for haemophilia A/B fetus.
Methods. We have analyzed DNA from 58 unrelated among 81 severe HA patients and 8 among 10 with severe HB. FVIII/IX gene defects and their usefulness in prenatal diagnosis were analysed.
Results. From 1989 to 2009 prenatal diagnosis by chorionic biopsy was done in 16 pregnancies of 14 carriers of HA/HB. The results were: 9 females, 2 healthy males, 2 haemophiliacs, 3 abortions (one of them due to mucopolysacharidosis Hurler). Twice we performed amniocentesis due to late counseling in HA carrier with unknown mutation in two pregnancies. In one of the pregnancies the fetus was male, therefore direct foetal blood sampling was performed later but was not successful. There was no foetal-loss caused by the procedures.
Conclusions. Prenatal diagnosis is safe and useful procedure. However in the last 5 years 6 pregnancies in carriers of severe HA with known mutation terminated without chorionic biopsy. The parents decided, due to positive experiences with haemophilia patients, that they are prepared to have a child with haemophilia.

PP 82

HEMOREC - A CLINICAL REGISTRY FOR HAEMOPHILIA PATIENTS

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Background: Besides the spectrum of designed and well controlled clinical trials, data registration is nowadays mainly related to hospital information systems. However, their role is rather archival and they are not adapted for collecting clinical data in a sense of statistical evaluation and interpretation. When considering non-interventional population studies, so called clinical registries can be used to carry out such an evaluation of treatment results and safety.
Methods: HemoRec is a web based application including a comprehensive master data set dedicated to collecting data on care for haemophilia patients. HemoRec system is designed for easily capturing of a broad array of data, including medical history, laboratory data, detailed information on the primary diagnosis, symptoms and manifestations, treatment, and potential complications. HemoRec provides unique customised forms feature. Any number of centre-specific data entry forms can be created by selecting the fields from the local master data set that best support the treatment practice of the individual centre. The application is developed and hosted in compliance with patient data privacy regulations in the EU (e-Privacy), US (21 CFR part 11, HIPAA), and Canada (PIPEDA).
Results and Conclusions: As an example of HemoRec system possibilities in data management and knowledge extraction, we provide an analysis of 121 primary bleeding episodes of 5 adult haemophiliacs with high-responding factor VIII inhibitors treated with rFVIIa. The analysis emphasizes the role of early initiation of treatment and the effect of the number of rFVIIa infusions according to the occurrence of rebleeding, overall consumption of rFVIIa per bleeding

PP 83

SUCCESFULL TREATMENT OF HUGE ILIOPSOAS HEMATHOMA IN PATIENT WITH MILD HEMOPHILIA A

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Background: Deep muscular bleeding is one of the most severe complications in patients with hemophilia A. It has a spontaneous onset, without data of previous trauma, and it is characterized by painful and limited movements in the affected limb. Due to a high mass of iliopsoas, a hemorrhagic shock may occur. In addition, hematoma may compress the surrounding tissues, leading to complication of the femoral artery or nerve. The main therapy is support of coagulation F VIII, leg immobilization and early rehabilitation. Rarely, surgical decompression is necessary. Recovery may require weeks, sometimes with residua such as sensomotoric deficiency or contracture.
Aim and Results: Paper presents a case study of a patient with a mild form of hemophilia A (F VIII 6%, aPTT 38 sec.) who spontaneously developed a severe pain in the left thigh, with limited movements and parestesia in the internal area of the left leg. At the admission, F VIII value was 1%, aPTT 56 sec, HCT 35%. Ultrasonography of the left femoral region showed spindly hematoma in the iliopsoas muscle, 25cm x 6cm in diameter. Neurological findings revealed anterolateral senso-motoric insufficiency with deep patellar reflex of the left leg. Patient was immobilized and treated with F VIII concentrate (25 units/kg in12 hours intervals). Three days after that episode, ultrasound confirmed a mild regression of the hematoma with better neurological findings. During the next seven days, a complete regression of hematoma occurred with the cessation of neurological complications. Due to a weakness of the left quadriceps, the patient started additional rehabilitation therapy. Four weeks after the hospitalization, the patient was in complete recovery and able to perform normal activities.

PP 84

ILIOPSOAS BLEEDING IN CHILDHOOD HAEMOPHILIA - SINGLE CENTER EXPERIENCE

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Background: Muscle bleeding episodes in patients with haemophilia are second only to haemarthrosis. Usually they occur after trauma, but they can also occur spontaneously. An important site of haematoma formation is the iliopsoas muscle, a well recognized complication of haemophilia, and is considered as potentially life threatening and significantly associated with morbidity.
Aim: In this retrospective study, we present the experience of our centre with iliopsoas bleeding.
Methods: A total of 30 patients with haemophilia (25 haemophilia A, 5 haemophilia B; 11.0 ± 5.0 years) followed between 2003 and 2008 at University Children's Hospital, Belgrade were involved in this study. In all patients presenting with thigh, hip and/or groin pain, abdominal tenderness and hip flexion contracture, ultrasonography was performed. Iliopsoas haemorrhage re-appearing more than 3 months from the initial episode was defined as repeat bleeding.
Results: Ten iliopsoas bleeding episodes were identified in four haemophiliacs. One patient had three episodes, one patient with inhibitor had two episodes and two had one episode. Three episodes were classified as repeat bleedings. The mean duration of therapy with clotting factor concentrate ranged 6-15 days. Ultrasonographic findings related to iliopsoas haematoma disappeared in all patients within 3 months from the initial episodes
Conclusion: Ultrasonography is the fastest and reliable diagnostic tool in patients with iliopsoas bleeding. Early and effective factor replacement therapy of optimal duration is essential in the prevention of the complications and rebleeding. Prophylactic therapy may also play an important role in reducing the bleeding frequency.

PP 85

ORTHOPEDIC STATUS OF HEMOPHILIACS IN ROMANIA – A MULTICENTRIC STUDY

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Introduction. Despite great advances in the treatment of hemophilia, joint lesions have remained hallmark of this disease.
Objectives. Therefore we decided to conduct a cross-sectional multicentric study on the orthopedic situation of young hemophiliacs.
Patients and methods. 6 centers participated, including 171 patients aged between 2-40 years, 90,64% with hemophilia A and 82,45% with severe form. Mean age was 22,70 years; 35,08% belonged to the age <16 years, 23,39% to the group of 17-23years, and 41,52% were older than 24 years. Socio-demographic data concerning number of joint bleeds, presence of chronic pain and treatment were collected. Joint evaluation was performed using Hemophilia Joint Health Score in children and Orthopedic Joint Score (WFH modified) in adults.
Results. Hemophilic arthropathy was present in 88,30%, 87,41% of them presenting target joints. The most frequent affected joint was the knee followed by elbow, ankle, shoulder, and hip. 64,32% presented in the last 6 months up to 6 hemarthrosis, 32,16% between 7-18 and 3,50% more than 18 hemarthrosis. Chronic pain was present in 44,44% of cases. Total joint score was 18,052±20,013 in children and 37,094±21,988 in adults. It was correlated in children and in adults with age (r = 0,567 and 0,527, respectively), but not correlated with residual FVIII/IX activity (r= 0,206 and 0,192, respectively) and onset of therapy (r= 0,232 and 0,074, respectively). That fact can be due to the scarce dosage of a late `on demand` substitution.
Conclusions. Frequency and severity of hemophilic arthropathy is overwhelming. It impacts in 90, 90% of teenagers the locomotor functionality, proportion increasing to 97,40% in young adults. It is an accurate reflection of inadequate quality of hemophilia care in our country.

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PP 86

HIP SURGERY IN A HEMOPHILIC PATIENT

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Background: It's well known that the risk of venous thromboembolism in patients undergoing total hip replacement is very high. Before thromboprophylaxis was used routinely, deep vein thrombosis (DVT) occurred in 40-60% of these patients. Although changes in surgical and anesthetic techniques and early mobilization may have reduced this risk, routine thromboprophylaxis remains extremely important and is standard of care.
Aim: We report a case of a patient with mild hemophilia A where total hip prosthesis was successfully implanted.
Methods: Case report.
Results: A 48-year old patient with mild hemophilia A got a traumatic injury of the right hip (f-ra prethrochanterica femoris dex.). He underwent surgery with implantation of total hip prosthesis. Purified freeze-dried human f-r VIII (Immunate-Baxter) was used for supportive treatment starting with a dose of 50IU kg kg-¹body weight twice daily. High level of f-r VIII was achieved between 120 and 160%. Supportive treatment was administrated along with low-dose LMWH (20mg enoxaparin s.c. once a day). This treatment was continued the first 7 postoperative days until the patient was completely immobilized. The operation and the postoperative period passed without any complication.
Conclusion: This is the first case of a successfully implanted total hip prosthesis in a hemophilic patient in our country. We used a low dose thromboprophylaxis and had no complications. There are no data of such treatment in literature. So, one question is propounded: As we made hip surgery in a hemophilic patient with supportive treatment that provide conditions approximately the same as in a healthy individual, is antithrombotic prophylaxis necessary?

PP 87

INHIBITORS IN HEMOPHILIA PATIENTS IN UKRAINE

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Background. It is well recognized that inhibitors currently represent the most serious complication of hemophilia. However, there was great difference in the reported incidence of the inhibitors. Our aim was to review the hemophilia inhibitor patients in our center.
Methods. Screening coagulation tests and one-stage APTT-based factor assay were performed. The inhibitor assessment was by Bethesda assay, and the results were expressed as Bethesda units (BU)/ml.
Results. 478 previously treated patients (417- hemophilia A, 61 - hemophilia B) have been recruited from 2000 to 2008. Frequency of FVIII inhibitors was 14,9 % (62/417), frequency of FIX inhibitors was 4,9 % (3/61). The presence of FVIII inhibitors was demonstrated in 42 out of 185 (22,7%) patients with severe severity (FVIII:C <1%) 35 patients (56,5%) were low responders (<5 BU/ ml) and 27 (43,5%) were high responders. In hemophilia B patients we revealed FIX inhibitors in 2 out 30 (6,7%) patients with severe severity (FIX <1%) and in 1 out 19 (5,3 %) patient with moderate severity (FIX 1-5%). All 3 patients (100%) were low responders. Among 47 patients with age <10 years, 5 child (10,6%) had inhibitors. The most frequencies of the inhibitors were 28,6% (22/77) in the patients between 20 and 30 years.
Conclusion: Our results have showed a relatively moderate frequency of FVIII inhibitors in previously treated patient with hemophilia in our center.

PP 88

SUCCESSFUL TREATMENT OF INTRACRANIAL HAEMORRHAGE AND COMPARTMENT SYNDROME OF THE NECK IN PATIENT WITH HAEMOPHILIA A AND INHIBITORS

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Intracranial haemorrhages (ICH) are affecting 3-10% of the haemophilia population who are mainly treated on demand. The risk is higher in inhibitor patients. Spontaneous haemorrhage is reported more frequently than trauma-induced haemorrhage in most studies. This complication is usually life-threatening in haemophilic patients with high mortality rate particulary at haemophilia A with inhibitors (20-35%). Nineteen years old man with haemophilia A and inhibitors (11 BU) admitted in hospital due to headache and suspicion for intracranial bleeding. Cranial CT scan confirmed multiple intracranial haemorrhages and he was treated with rFVIIa in dosage of 90 µg/kg/2h. He had a very bed vein access and central vein catheter /CVC/ was done. Few hours later, compartment syndrome of the neck was developed. After angiography patient was prepared for surgical intervention with rFVIIa in dosage of 120 µg/kg/2h. Exploration of the neck shown that jugular CVC was in correct position but it has been placed through the muscle. Haemorrhage in the muscle produced severe compartment syndrome. During the treatment with rFVIIa control aPTT was 42.7s and treatment with concentrate of hFVIII in dosage of 4000 U/8h was continued. During the treatment with concentrate of hFVIII monitoring of aPTT and FVIII were in normal level without inhibitors. Ten days later inhibitor to FVIII developed and treatment with rFVIIa continued to complete healing. This case report underscores the importance of individual decision making and continuous monitoring of response to therapy in patients with high-titer inhibitors and hemophilia.

PP 89

RECOMBINANT FACTOR VIIa IN TREATMENT OF NONTRAUMATIC SPONTANEOUS INTRAMURAL SMALL-BOWEL HAEMATHOMA IN HAEMOPHILIA A PATIENT - CASE REPORT

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Nontraumatic spontaneous intramural small-bowel haemathoma, once considered a rare complication of haemophilia, oral anticoagulant therapy, idiopathic thrombocytopenic purpura, lymphoma, pancreatitis and others, is being reported with increasing frequency. A 55 year old haemophilia A patient was admitted to the Clinic for hematology, Clinical Centre Vojvodina with abdominal pain, nausea and vomiting which began without an obvious cause. Tenderness in epigastrium and left hemiabdomen was present in physical exam. Factor VIII (FVIII) was 3%, inhibitors of FVIII 38 BU/ml, aPTT 74.9s, Rbc 5.41x1012/l, Hgb 174 g/l, Hct 0.506 l/l, Plt 221x109/l test for occult gastrointestinal bleeding repeated three times was positive. CT scan of abdomen showed distended jejunal loops with fluid and gas, bowel wall thickening suspicious of intussusception and hyperdensity of jejunal mesenterium. Patient was treated with concentrated FVIII 2000 units/day for three days, proton pump inhibitors and analgetics. Enteroclysis revealed an altered jejunal loop from the Traitz to 25-30cm distally, with nonocclusive luminal narrowing indicative of intramural jejunal haemathoma. Bowel morphology and elasticity distal to the lesion was normal. The patient then received treatment with recombinant factor VIIa in a dose of 90 g/kg every four hours for two days. Followthrough of small bowel performed after this treatment was normal, test for occult gastrointestinal bleeding repeated three times was negative, FVIII 2%, inhibitors of FVIII 48 BU/ml, aPTT 85.7s, Rbc 3.96x1012/l, Hgb 126 g/l, Hct 0.368l/l, Plt 276x109/l. We presented a haemophilia A patient with inhibitors successfully treated with recombinant factor VIIa for a rare type of intestinal bleeding.

PP 90

RECOMBINANT FACTOR VIIa FOR TREATMENT OF ACQUIRED HAEMOPHILIA - CASE REPORT

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Background: Acquired haemophilia is a rare coagulation disorder caused by factor VIII autoantibodies. The recombinant FVIIa is a drug of choice for treatment of acquired haemophilia.
Case report: A 22 year old woman was admitted to the hospital because of intensive bleeding from gums caused by pulling out of a tooth, and spontaneous appearance of haematoma in extremities. The diagnosis of acquired haemophilia was made on the basis haemostasis tests: aPTT 52,7 s, F VIII- 7%, F VIII antibodies 8 BU. The patient received recombinant FVIIa 90 mikrograms/ kg BW every 2h 2 doses, then every 4h 2 doses first day of therapy, and the bleeding was stopped. Treatment was continued for 3 days with reduced intervals of a treatment. The patient was on immunosuppressive therapy with prednisone and local received antifibinolytic. The patient was discharged after 4 days of treatment without signs of Syndrome of Haemorrhagia, with improvement in haemostasis tests, with recommendations for immunosuppressive treatment. The patient is presently healthy, without any treatment.
Conclusion: The recombinant factor VIIa treatment was successful to stop the bleeding in the cause of acquired haemophilia with no adverse effects.

PP 91

THE EFFECTIVENESS OF DDAVP IN PREVENTING OF BLEEDING IN PATIENTS WITH VON WILLEBRAND DISEASE UNDERWENT ORAL SURGERY PROCEDURES

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Objective. Oral surgery procedures in vWD patients are associated with a risk of intraoperative and postoperative bleeding.
Study design. This study was performed to asses the effectiveness of DDAVP to prevent bleeding in patients with vWD underwent oral surgery procedures. DDAVP was administered to DDAVP responsive patients in combination with antifibrinolytic agent and local hemostasis in 24 consecutive patients. The level of FVIII, vWF and vWF-Ag was measured before and after DDAVP application to assess optimal time for surgical procedure.
Results: 22 patients respond to DDAVP testing and administration and there were no intra- and postoperative bleeding. Two patients did not respond to DDAVP testing and replacement therapy was used for surgical procedures.
Conclusion: DDAVP should be given to all vWD patients to establish individual response and thus patients divided into two groups for oral surgery procedures: DDAVP responsive and DDVAP nonresponsive.

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Women and Haemostasis Disorders

PL 92

OBSTETRIC AND GYNECOLOGICAL PROBLEMS IN WOMEN WITH INHERITED BLEEDING DISORDERS

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The recent increased interest in coagulation disorders led researchers to improve the knowledge on laboratory testing, diagnosis and treatment of women affected with these disorders. However, this is not yet sufficient to diagnose or exclude an underlying coagulation defect in all women who present with gynaecological bleedings. These problems include excessive menstrual bleeding or menorrhagia, bleeding complications during pregnancy and after delivery and their related complications such as chronic anaemia. The prevalence of undiagnosed bleeding disorders among these women is high, with estimates ranging from 5 to 20%. Menorrhagia and chronic anaemia reduce significantly the quality of life and cause limitations in activities and work. These women could also have psychological problems due to alteration of their reproductive life. In the last years there has been a considerable progress in the identification of obstetric and gynaecological problems in these women raising clinical awareness amongst their care providers. However information regarding the management of menorrhagia and other gynaecological problems in these women remain still limited. With the exception of one small randomised trial, showing that heavy menses is seen in females with von Willebrand disease (69%), factor II deficiency (75%) and factor V, VII, X, afibrinogenemia, and combined V+VIII deficiency (50%), due the rarity of RBDs, studies are limited to case reports and small case series. Therefore clinical studies aimed to the enlargement of available data are deemed in order to draw up guidelines based on clinical evidence. The scarce information, in particular the lack of recognition of the coagulation cause of the symptoms may lead to inappropriate treatment (unnecessary surgical interventions or uncontrolled bleeding) that could be avoided with appropriate diagnosis. Prompt diagnosis, prophylaxis or treatment may significantly improve the quality of life, usually poor and debilitating. This is even more true in developing countries, with limited facilities and low economic resources, where it is necessary to re-allocate resources and to conduct appropriately designed studies. In conclusion, the ideal management of women with coagulation disorders who suffer from menorrhagia should be discussed and planned through multidisciplinary clinics. With such a team it would be possible to approach this problem in an accurate way. We propose an International study (more information on www.wrbd.org) to analyse the severity and prevalence of bleeding symptoms and gynaecological problems in women affected with coagulation disorders and to evaluate the type and efficacy of treatments used. The final aim of this study is to draw a specific guideline for diagnosis and treatment of women affected by coagulation disorders, based on experiences obtained from International treatment centres on women affected by coagulation disorders.

OP 93

MENORRHAGIA AND COAGULATION ABNORMALITIES

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Background: It has been described that mild hemostatic defects could be a cause of menorrhagia in substantial number of cases (etiology of menorrhagia is unclear in more than half of causes).
Method: We have investigated coagulation in 81 women (35.77 ± 9.40 years) with symptoms of menorrhagia, which has been verified using semiquantitive method - Pictorial Bleeding Assessment Chart (PBAC) with score >100 (equivalent > 80 ml blood). Bleeding time (BT), PT, aPTT, VWF:Ag and VWF:Ac, FII, FV, FVII, FVIII, FIX, FX and FXI activity, have been analyzed, as well as complete blood count including hemoglobin (Hb), serum iron and ABO blood group typing
Results: 47 women (58.0%) have had objectively verified menorrhagia (PBAC ≥ 100). Coagulation defects have been found in 8 (9.9%) women (17.0% of women with objectively verified menorrhaghia) - decreased FVII:Ac in 1 (12.5%), decreased FX:Ac in 1 (12.5%), decreased FXI:Ac in 1 (12.5%), while 5 women (62.5%) matched criteria for mild VWD type 1. Women with coagulation disorders have had longer duration of menstrual bleeding (8.2±3.8 vs. 10.2±7.0 days; $p<0.05$), and lower levels of hemoglobin (118.4±21.5 vs. 109.9±21.0 g/L; $p<0.05$) and serum iron (10.7±6.4 vs. 7.1±3.6 μmol/L; $p<0.05$) in comparison to those without such disorders. aPTT tended to be prolonged in women with coagulation disorders although with borderline statistical significance (27.9±3.8s vs 30.6±7.2s, $p=0.052$), while BT and PT showed no difference.
Conclusion: One sixth of women with verified menorrhagia have mild hemostatic defects. This condition is associated with longer period duration and more severe anemia in affected women. It seems that coagulation screening may be of some benefits in diagnostic algorithm of women with menorrhagia, at least in those with more severe clinical conditions.

OP 94

EFFICACY AND SAFETY OF THE LOW MOLECULAR WEIGHT HEPARIN NADROPARIN AND UNFRACTIONATED HEPARIN FOR TREATMENT OF VENOUS THROMBOEMBOLISM DURING PREGNANCY AND PUERPERIUM

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Introduction: The optimal treatment of pregnancy associated VTE has not been established yet.
Objective: The assessment of the the efficacy and safety of LMWH nadroparin and unfractionated heparin (UFH) used for treatment of pregnancy and puerperium related VTE. Primary outcomes were rate of recurrent VTE (proximal extension or PTE), thrombocytopenia, major and minor haemorrhages and skin allergic reactions. We have also studied the relationship between the presence of thrombophilia and the occurrence of complications during VTE treatment.
Materials and Methods: The data on 72 antepartal VTE treated with s.c.LMWH during entire pregnancy and 88 postpartal VTE initially treated with either s.c.LMWH or i.v.UFH were analyzed. Thrombophilia testing included antithrombin, PC and PS activity level, APC resistance, LA, aCLA, FV Leiden, FII G20210A and MTHFR C677T mutations.
Results: The twice daily weight based therapeutic regimen had been applied for LMWH and aPTT was applied for UFH dosing. After 2-6 weeks of antepartal DVT treatment the dose of nadroparin was reduced to intermediate level. Duration of LMWH therapy during pregnancy was 1-35 weeks, average 16 weeks. Among 88 women with postpartal DVT 51 were treated with i.v. UFH and 37 with LMWH. One case of DVT propagation into the vena cava and opposite iliac vein had occurred in woman with antithrombin deficiency treated with LMWH. Two women (0.0125%) had non major clinically relevant bleeding and 5 (3.125%) had minor bleeding episodes. Three cases of skin allergic reactions were detected. Thrombophila was found in 86 women (53.7%). No statistically significant correlation between the presence of thrombophilia and treatment complication occurrence was found.
Conclusion: Nadroparin is both safe and effective for the treatment of DVT during pregnancy and puerperium, and UFH is safe and effective for the initial therapy of immediate postpartum DVT and PTE.

OP 95

RECURRENT FETAL LOSSES: ETIOLOGICAL AND THERAPEUTIC APPROACH USING MAINLY THE INNOHEP

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Background. In the last three years we examined 191 women with recurrent fetal losses to determine the causes of these losses. In order to make an estimate on the success of the treatment we applied.
Material and Mehod. We studied 191 women with mean age 33,9±4,8 years that had about 2,9±1,3 fetal losses. We studied all the possible factors of haemostasis that can be implicated for disturbances and also full genetic control.
Results: We found 22 women heterozygote and 3 were homozygote for FV-Leiden, 9 women were carriers of the Gp-IIIa mutation and also 3more women had the FV -HR2 with the Gp-IIIa homozygote mutation,8 woman with FII 20210 G>A mutation (including 1 homozygote), 5 more had FII mutation and Gp-IIIa homozygote mutation, and 4 more had PAI-4G/4G and Gp-IIIa homozygote mutation, 2 women with the 455G>A mutation of fibrinogen, 25women with PAI-4G/4G polymorphism. Also 2 more women with protein S, 1 woman with FXII, 1 woman showed plasminogen deficiency, 3 women with antiphospholipid syndrome and 7 women had haemorrhagic diathesis (mainly mild Willebrand disease). Overall in 95 women we found risk factors connected with recurrent fetal losses. From the 191 women we examined 95 were able to have one effective pregnancy with the help of antithrombotic treatment (μMBH mainly innohep, or antiplatelets mainly aspirin) or with DDAVP in order to increase the levels of factor Willebrand. In the other women an effective therapeutic approach was not possible.
Comment: The determination of the causes of recurrent fetal losses can help in an effective pregnancy.

OP 96

CORRELATION BETWEEN THE LOW MOLECULAR WEIGHT HEPARIN DOSE AND THE PLASMA LEVELS OF ANTI Xa ACTIVITY IN PREGNANT WOMEN

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Low molecular weight heparin (LMWH) is the drug of choice for treatment and prevention of venous tromboembolism (VTE) during pregnancy. Whether or not the monitoring of anti Xa activity is necessary is a matter of debate. The objective of this study was to investigate the correlation between the dose of LMWH Nadroparin used for VTE treatment and prophylaxis during pregnancy and the plasma levels of anti Xa activity in 33 pregnant women, 25 of them received prophylactic and 8 therapeutic dose of LMWH. Anti Xa activity was determined using the Instrumentation Laboratory (IL) HemosIL Heparin. Blood samples have been taken 4h after LMWH application. Anti Xa activity was determined in 198 samples, 22 at first trimester, 71 at the second and 106 during the third trimester. The obtained value of Pearson`s correlation coefficient $r>0.7$, $p<0.05$ indicates that the drug dose per kg and the anti Xa activity found in plasma are highly correlated during second and third trimester. We have also used the artificial neural network (ANN) and Support Vector Machines (SVM) for prediction modeling of heparinemia level in pregnant women receiving LMWH. Four variables associated with heparinemia were considered in both the ANN and SVM analyses: body weight, body mass index, heparin dose per kg and trimester of pregnancy. In both ANN and SVM models there were no qualitative differences between prediction results and real data. The difference between the predicted and obtained value of anti Xa activity was less than 8%. This technology may become increasingly useful for real-time prediction of heparinemia level.

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Arterial Thrombosis: Risk Factors and Therapy

SOA 97

LIPOPROTEINS AND ATHEROGENESIS: AN UPDATE

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Cardiovascular diseases, one of main cause of mortality worldwide, recognize elevated lipoprotein levels as a risk factor. High blood concentrations of low-density lipoproteins (LDL) and low blood concentrations of high-density lipoproteins (HDL) are the primary factors for the development of atherosclerotic disease. The association between LDL cholesterol and atherogenesis has been initially established on animal and autopsy studies and later on the evidence that cholesterol-lowering therapy greatly reduced the clinical manifestations of atherosclerosis, i.e. morbidity and mortality due to coronary heart disease. Therefore LDL cholesterol is the primary target of hypolipemic therapy, with its levels modulated on the basis of individual cardiovascular risk. Low density lipoproteins are not a homogeneous class of lipoproteins: they include “small dense” and “large buoyant” LDL with a different pathogenetical role on atherosclerotic plaque progression; small dense LDL have a higher content of apoprotein B, are higher oxidable and more prone to enter the vessel wall. LDL cholesterol levels are influenced by several genetic and environmental factors, these capable to influence either cholesterol synthesis or dietary cholesterol absorption. The efficiency of intestinal cholesterol absorption ranges from 29% to 80% among healthy individuals with majority of individuals absorbing about 55% of dietary sterols. In the last years particular attention has been paid to the role of dietary cholesterol since we can use “functional foods” or drugs able to inhibit cholesterol absorption; in this contest the knowledge of the mechanisms underlying the maintenance of cholesterol levels allows to individualize hypocholesterolemic therapy.

Low HDL cholesterol is an independent risk factor for cardiovascular disease (CVD), with a strong inverse association between plasma HDL-cholesterol and the incidence of coronary artery disease. High density lipoproteins exert their protective activity through the “reverse cholesterol transport”, the modulation of cell adhesion molecules expression and the reduction of LDL oxidative changes.

Many epidemiological studies have reported also associations between serum triglyceride concentrations and the risk of coronary heart disease. In the early stages of atherogenesis, triglyceride-rich lipoproteins are directly involved in the delivery of lipids into the arterial tissue. Hypertriglyceridemia is a characteristic feature of highly atherogenic hyperlipemias such as familial combined hyperlipemia, familial hypertriglyceridemia, metabolic syndrome and diabetic dyslipidemia. It is often associated with small dense LDL and low HDL levels (the so called “atherogenic lipoprotein phenotype”), and with postprandial hyperlipemia, characterized by plasma accumulation of chylomicron remnants, very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), a highly atherogenic and thrombogenic condition.

When considering the lipid pattern we must also focus on lipoprotein(a), [Lp(a)], a lipoprotein particle consisting of an LDL linked by a disulfide bridge to an apolipoprotein (a), one of the most polymorphic proteins. A metanalysis of 27 prospective studies showed that Lp(a) is an independent risk factor for CAD and case control studies showed also an association with cerebrovascular disease. Lp(a) plasmatic levels are correlated with apo(a) isoforms: smaller size isoforms are associated with higher plasmatic levels and are more consistently associated with CVD.

Among the cause of lipid disorders, secondary factors that may affect lipid levels include obesity, diet, alcohol use, endocrine disorders such as diabetes mellitus and hypothyroidism, liver and renal diseases and many pharmacological agents. Primary dyslipidemias recognize a genetic basis, either resulting from a combination of polygenic predisposition with lifestyle habits or from monogenic disorders.

The improved knowledge of lipid risk factors and lipid metabolism can help to develop a cardiovascular prevention strategy. This will be useful to identify those at higher risk in order to target specific behavioural or drug interventions and reduce cardiovascular morbidity and mortality.

OP 98

FIBRINOLYTIC DISORDERS AND Lp(a) LIPOPROTEIN IN ISCHEMIC STROKE PATIENTS

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Background: Ischemis stroke is the third leading cause of mortality and morbidity in most countries in the world. Impaired fibrinolysis, as well as disordered lipid metabolism has been recognized as risk factors for this disease.
Aim: To study some of fibrinolytic parameters and Lp(a) lipoprotein in patients with ischemic stroke and to examine association between Lp(a) lipoprotein and studied fibrinolytic parameters.
Methods: Sixty patients with ischemic stroke (case group, 65% male and 35% female, mean age 63.48±9.62 years) and thirty age- and sex-matched healthy controls with no history or clinical evidence of ischemic brain disease (control group, 60% male and 40% female, mean age 60.2±7.96 years) were studied.
Results: Significantly longer euglobulin clot lysis time (219,7±78,8 min. vs 183,5±58,22 min; p=0,005) and higher levels of plasminogen activator inhibitor-1 (PAI-1) (48,5±17,1 ng/ml vs 27,1±10,1 ng/ml; $p=6,2x10^{-11}$) and tissue-type plasminogen activator antigen (t-PA) (11,1±7,14 ng/ml vs 6,20±3,66 ng/ml; $p=5,2x10^{-5}$) were found in cases compared to controls. There were no significant differences in fibrinogen levels or plasminogen activity between cases and controls (92,7±11,4% vs 96,9±9,5%; p=0,085). There was no significant difference in serum Lp(a) lipoprotein levels between cases and controls (0,15±0,11 g/l vs 0,12±0,11 g/l; p=0,261). However, in cases, but not in controls, multivariate analysis of association between fibrinolytic parameters and Lp(a) lipoprotein showed the highest correlation between t-PA and PAI-1, and a latent effect of Lp(a) lipoprotein on t-PA and PAI-1.
Conclusions: Our results show that there are significant differences in the characteristics of the fibrinolytic mechanism in ischemic stroke patients compared to healthy population. Major differences are prolonged euglobulin clot lysis time and elevated PAI-1 and t-PA antigen levels in ischemic stroke patients. In addition, Lp(a) lipoprotein appears to be involved in inhibition of fibrinolysis in ischemic disease through a mechanism unrelated to its serum concentrations.

SOA 99

THROMBOGENIC PROFILE OF LEUKOCYTES: CLINICAL AND EXPERIMENTAL EVIDENCES

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Epidemiologic studies have shown that the neutrophil count correlates with the risk of myocardial infarction and stroke and identify patients more susceptible to reinfarction and in-hospital death. In particular, neutrophils action was initially associated to the blood rheological changes, or to the effect of neutrophil-derived eicosanoids or proteases. Animal models indicate that platelet-leukocyte P-selectin dependent cross-talk contributes to fibrin deposition during in vivo thrombus formation. In fact, platelet P-selectin, through its leukocyte counter-receptor PSGL-1, determines the activation of leukocyte ß2 integrins, the binding of fibrinogen and the expression of tissue factor on leukocyte surface. Monocytes stimulated in vitro with LPS, PMA and P-selectin synthetise and express tissue factor. fMLP, P-selectin, TNF and C5a are effective stimuli that trigger the synthesis and expression of biologically active tissue factor in neutrophils. The experimental evidence well agrees with clinical observations: patients with acute coronary syndromes, acute respiratory distress syndrome, antiphospholipid syndromes, giant cell arteritis and myeloproliferative syndromes have increased expression of tissue factor on leukocyte surface. Moreover circulating neutrophils express mRNA codifying for full-length and/or alternatively spliced tissue factor, suggesting a new important link between thrombosis and inflammation. All together, clinical and experimental evidence suggest that the leukocyte thrombogenic profile is a relevant player in patients with high risk of thromboembolic events and possibly represents a suitable target for molecular intervention.

OP 100

EFFECT OF UMBILICAL CORD BLOOD CELLS IN THE "EXPRESSION" OF PLATELET GLYCOPROTEIN RECEPTORS

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Background. Recent proofs have been found that there is a heterotypic reaction between platelets and lymph cells. This reaction appears to play an important role in thrombosis, inflammation and atherogenesis (Wagner 2005, Hansson 2005). In order to study the effect of umbilical cord cells (UCC) in the "expression" of the glycoprotein of platelet membrane, we made an incubation protocol for these cellular elements.
Material: Platelet rich plasma (PRP) from 5 different individuals was incubated with 5 umbilical cord cells from different individuals and one with placenta cells.
Methods: The Gp-Iba, Gp-Ibβ, Gp-IIb, Gp-IIIa glycoprotein and P-selectin were measured with flow cytometry using the CD 41a, 42a, 42b, 61 and 62p.
Research protocol: All the glycoproteins from the PRP were measured before the incubation with the UCC. Then we incubated in 37°C for 1h, 24h and 10 days (in sterile environment). Our findings are a significant increase in Gp-IIb mainly in the 24h but also in the 1h and 10 days. For the Gp-Ibβ only in the 1h and for Gp-IIIa the significant increase appears only in the 10th day. On the contrary P-selectins decrease during the course of the incubation.
Comments: these observations appear much better in the analytical presentation since it is possible to follow the progress the phenomenon in each PRP. This could open new ways for the use of umbilical cord cells.

OP 101

EXTREMELY HIGH LEVELS OF PLATELET ACTIVATION MARKERS ASSOCIATED WITH CEREBROVASCULAR ACCIDENT

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Background. Few reports of increased platelet activation and formation of leukocyte-platelet complexes (LPCs) in end-stage heart failure patients on left ventricular assist device (LVAD) support are available.
Aim. We sought to identify relationship between cellular activation and cerebrovascular accidents (CVA) in these patients.
Methods. We used flow cytometry to measure platelet activation markers and LPCs in 20 patients during 6 months on LVAD support. P selectin (CD62P), CD63, thrombospondin (TSP), as well as circulating levels of LPCs, were analyzed.
Results. Platelet activation was increased before LVAD implantation and throughout the study. Average levels were: CD62P 24±15 %; CD63 8.1±5.7 %; TSP 8.2±5.7 %. LPCs increased after implantation and remained significantly elevated up to 90 days ($p<0.03$). We noted 1 episode of CVA (confirmed by computerized tomography) in patient admitted with acute onset of right hemiparesis and blurred vision. Although he was on warfarin and aspirin his PT was subtherapeutic at 11.6 seconds and INR was 1.1. Platelet count was 277 x 10^9/L. His percentages of activated platelets were highly increased when compared with the average of other patients: CD62P, 73.8 vs. 20.3± 9.0 %; TSP, 30.2 vs. 7.1±4.9 %; CD63, 53.8 vs. 6.7±3.2 %. Percentages of LPCs were similar to those of other patients. He was discharged home 3 weeks later.
Conclusions. Increased cell activation was observed in patients during long-term LVAD support. However, only in 1 case extremely high levels of platelet activation markers were associated with CVA episode. The clinical significance of our findings requires further study.

OP 102

HAEMOSTASIS PARAMETERS FOR THE PREDICTION OF ISCHEMIA DURING ADENOSINE-EXERCISE-SPECT STRESS TEST IN ASYMPTOMATIC CORONARY PATIENTS AFTER CORONARY STENTING

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Background. Activation of haemostasis during physical stress or during myocardial ischemia may be an important mechanism of stent thrombosis. We examine changes of haemostasis parameters and its association with myocardial ischemia during adenosine-exercise-SPECT (adeno-EX) stress test in asymptomatic coronary patients at least 4 months after coronary stenting.
Materials and methods. Twenty-eight patients on dual antiplatelet therapy (APT) and 11 patients on aspirin, 4-8 months after successful intracoronary stent implantation were enrolled in the study. We determined levels of platelet aggregability (PA) on ADP (PA-ADP) and epinephrine (PA-EPI), beta-thromboglobulin, platelet factor-4, von Willebrand factor, FVIII, protein C (PC) and antithrombin (AT) before and 15 minutes after intravenous injection of adenosine 140 μ/kg/min with supine ergo-bicycle exercise 50W for 5 minutes. Size of ischemia was measured by ^{99m}Tc-tetrofosmin SPECT within 17 myocardial segments in rest and after adeno-EX stress.
Results. In stepwise multivariable linear regression analysis with all haemostasis parameters included and adjusted for age and body-mass, the independent predictors for rest myocardial perfusion defect in patients on dual APT were antithrombin and protein C before stress (constant=52.10, p=0.039; p=0.002, beta=-0.47 for AT and beta=0.50 for PC). Size of stress ischemia also correlates with AT and PC (constant=22.75, p=0.11; beta=0.57, p=0.001 for PC and beta=-0.50, p=0.003). Only significant predictor for rest and stress ischemia was activity of PC after stress.
Conclusion. Levels of AT and PC activity before adeno-EX SPECT correlate with rest and stress myocardial perfusion in patients after coronary stenting on dual APT.

OP 103

THE EFFECT OF THE ANTIAGGREGATIONAL THERAPY ON THE BYPASS LONG-TERM PATENCY

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Background. Antiaggregational therapy can reduce thrombosis development following the arterial reconstruction surgery. In most cases acetylsalicylic acid and ticlopidine are used as antiaggregational agents.
Aim. The aim of this research was to examine the influence of different antiaggregational agents on a long-term femoropopliteal/crural bypass patency.
Methods. The research has been performed over the April 2007 - December 2008 period at the Cardiovascular Disease Institute of the Clinical Center of Serbia. Femoropopliteal/crural bypass reconstructions were performed on 142 patients. Depending on the antiaggregational agents that were used, three groups of the patients were formed: 1) acetylsalicylic acid, 2) ticlopidine and 3) acetylsalicylic acid combined with ticlopidine.
Results. There was no statistically notable difference in femoropopliteal/crural bypass long-term patency comparing to the usage of different antiaggregational agents.
Conclusions. The first-choice antiaggregative agent after surgical infrainguinal arterial reconstruction procedures is acetylsalicylic acid. The main reasons for that are the low cost and absence of side effect-neuthropenia.

PP 104

RELATIONSHIP OF ALCOHOL CONSUMPTION AND THROMBOGENIC FACTORS IN HYPERTENSIVE MALES

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Background: High alcohol intake is related to increased mortality, hypertension, alcoholic cardiomyopathy, cancer, and cerebrovascular events.
Aim: The aim of the present study was to evaluate the association of thrombogenic factors according to alcohol intake in a large number of hypertensive males.
Methods: We studied 10000 consecutive males with a mean age of 55 years with uncomplicated essential hypertension. After a fortnight wash-out period (46.5%), patients were evaluated with medical history in four groups according to alcohol intake (rare, light, moderate, and heavy) and full laboratory examinations including plasma levels of fibrinogen and serum levels of plasminogen activator inhibitor-1 (PAI-1). Plasma fibrinogen and plasminogen activator inhibitor-1 were defined as high when >400mg/dl and >3.5UI/ml respectively.
Results: Alcohol excess intake is related to elevated both thrombotic factors (high-fibrinogen, high-PAI-1) ($p<0.001$).

Alcohol Consumption	RARE n=3602	LIGHT n=5248	MODERATE n=858	HEAVY n=292
high fibrinogen (%)	26.2	26.2	40.1	45.5
high PAI-1 (%)	25.9	24.0	23.1	37.0
serum fibrinogen (mg/dl)	306±68	310±68	337±73	345±70
PAI-1 (UI/ml)	2.57±1.36	2.81±1.08	2.96±0.93	3.25±0.99

Conclusions: Heavy alcohol consumption in hypertensive males is accompanied with higher values and incidence of elevated thrombotic factors compared to rare, light and moderate use.

PP 105

FIBRINOGEN OF LOW MOLECULAR WEIGHT AND ITS ROLE IN ATHEROGENESIS AT PATIENTS WITH DIABETES MELLITUS 2ND TYPE

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Background: Fibrinogen of low molecular weight (FLMW) is one of components of fibrinogen pool. It appears from the normal molecule fibrinogen under influence of leukocytes protease. FLMW has no the A-alpha chain which contain a domains for linkage with plasminogen, and consists of only B-beta and G-gamma chains. The action of fibrinolysis on the fibrins net, made from FLMW, is complicated because of its structural features in the absence of the A- alpha chain.
Aim: The aim of our study was to investigate the role of FLMW in the development of a clinical picture of atherothrombosis at patients with diabetes mellitus 2nd type.
Materials and methods: We investigated 83 patients. They were divided into three groups. In the 1-st group there were 49 patients (30 women and 19 men in the age of from 40 till 80 years, middle age made up 64.7 + 10,9 years). All of them suffered from the widespread atherothrombosis of different localization (heart, brain, legs) and had diabetes 2-nd types, with the subcompensated and compensated carbohydrate metabolism. Duration of diabetes was from 2 until 35 years, average duration 13.8+8.7. The second group consisted of 34 patients - 19 women and 15 men, middle age - 58.4+8.9 years. They have got a diabetes mellitus 2nd type middle and heavy current with subcompensated and decompensated carbohydrate metabolism. These patients had no episodes of arterial thromboses at the moment of investigation and in the past history (anamnesis and ECG data). Control group consisted of practically healthy 27 persons - 15 women and 12 men, middle age 51,6 + 11,4. They had no disturbances of a carbohydrate metabolism and no clinics of atherothrombosis. Fibrinogen of low molecular weight was determined by method of B.Lipinski .
Results: The patients of both groups had average value of fibrinogen of low molecular weight almost twice as much as healthy persons ($p<0,05$) The highest level of FLMW was at patients of the first group, who had the combination of a diabetes with arterial thromboses (0,9+0,19 g/l). The level of FLMW at patients of the second group, who a diabetes of 2nd type, who had not episodes of a thrombosis of arteries, was 0,81±0,22 g/l.

Difference of the level of FLMW at the investigated groups of patients were statistically doubtful (p = 0,059). At the patients of both groups the increase of contents of the FLMW in comparison to the general fibrinogen was high. Its level in the first, and in the second groups was 21,6 % and 21.3 %, accordingly. At the both groups of patients the level of FLMW was authentically above in comparison with parameters of the persons of control group, where FLMW was 14,9 % of the whole plasmas fibrinogen (P < 0,001).
Conclusions: At the persons, who suffered with diabetes mellitus 2nd type, fibrinogen of low molecular weight is increased. This high level of FLMW is more increased at the patients where there were the combination of atherothrombosis and a diabetes mellitus. It is possible to think that this fact may influence on the earlier origin and heavy current of atherothrombosis at the patients with diabetes mellitus 2nd type.

PP 106

THE COMPLEX'S ACTIVITY "VON WILLEBRAND FACTOR - GLYCOPROTEIN IIB/IIIA" AT THE PATIENTS WITH ATHEROTHROMBOSIS OF THE HEART (CORONARY ARTERY DISEASE) AND PERIPHERAL ARTERIES (PERIPHERAL ARTERY DISEASE) PROGRESSING ON BACKGROUND OF DIABETES MELLITUS TYPE 2

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Background. In 1990 J. R. O'Brien created device which exactly the process imitating in blood stream of patient with atherosclerosis. It permit to measure in vitro the level of aggregation which depend on the complex's activity "von Willebrand Factor - glycoprotein IIb/IIIa". The complex "Factor of Willebrand - glycoprotein IIb/IIIa" form the thrombotic mass. After J. R. O'Brien was create the method permiting to determine the complex's activity "Factor of Villebrand - glycoprotein IIb/IIIa", the supposition arisen about the opportunity of the exact valuation of the intensity of thrombosis in the development of the ischemic event result of atherothrombosis.
Aim. The aim of this work was to study the complex's activity "Factor of Willebrand - glycoprotein IIb/IIIa" in patient with atherothrombosis of cardiac arteries (angina pectoris, myocardial infarction in anamnesis) and peripheral arteries progressing on background of diabetes mellitus type 2. We compared the index of the complex's activity "von Willebrand Factor - glycoprotein IIb/IIIa" received in practically healthy persons and patient with atherothrombosis of cardiac (Coronary Artery Disease) and peripheral arteries (Peripheral Artery Disease) progressing on background of diabetes mellitus type 2 and in patient diabetes mellitus type 2 without clinical display of atherothrombosis.
Materials and methods. 105 patients aged 39 - 75 (25 male and 80 female) were examined. All patients and practically healthy persons were determined the complex's activity "Factor of Willebrand - glycoprotein IIb/IIIa" by method J. O'Brien. We used the filters by J. R. O'Brien and Homburg – (RT-H). The blood of patients passed the filters J. R. O'Brien - E. Wenzel. The quantity of adhesive platelets corresponded with level complex's activity "von Willebrand Factor glycoprotein IIb/IIIa". The patient in the I group (n = 33) with angina pectoris and diabetes mellitus type 2 (DM2) (9 male and 24 female aged 39 - 77, averaged 62,9 ± 8,9 years), II group (n =18) with angina pectoris, myocardial infarction in anamnesis more 6 month and DM2 (3 male and 15 female aged 48 - 78, averaged 67,2 ± 7,9 years), III group (n = 19) with angina pectoris, peripheral artery disease and DM2 (7 male and 12 female aged 49 - 74, averaged 63,4 ± 8,3 years. In the IV group (n = 35) with diabetes mellitus type 2 without clinical display of atherothrombosis- 6 male and 29 female aged 39 - 75, averaged 55,8 ± 7,7 years. The control group included practically healthy persons (n = 20)- 5 male and 15 female aged 24 – 53, averaged 34,6 ± 7,2 years).
Results. In the I group the complex's activity "von Willebrand Factor - glycoprotein IIb/IIIa" compiled 30,2 ± 1,9 %, in the II group - 32,0 ± 1,6 %, in the III group - 35,9 ± 4,1 %. In the IV group the complex's activity "Factor of Willebrand - glycoprotein IIb/IIIa" compiled 27,0 ± 1,8 %, was higher (p<0,001) in control group (21,3 ± 0,8 %). The complex's activity "Factor of Willebrand - glycoprotein IIb/IIIa" in all groups was statistic higher (p<0,001), than in control group.
Conclusions. The high level of the complex's activity "von Willebrand Factor - glycoprotein IIb/IIIa" in patient with atherothrombosis of cardiac (Coronary Artery Disease) and peripheral arteries (Peripheral Artery Disease) was progressed on background of diabetes mellitus type 2 compared with healthy persons, which statisticaly increased in ranks «Angina pectoris - angina pectoris and myocardial infarction - coronary artery disease and peripheral artery disease». In patients with diabetes mellitus type 2 without clinical signs of atherothrombosis the complex's activity of "von Willebrand Factor - glycoprotein IIb/IIIa" was higer than at the healthy persons.

PP 107

ANTIOXIDATIVE PROPERTIES OF MOMORDICA CHARANTIA ON OXIDATION OF NATIVE AND MODIFIED LDL

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Momordica charantia (bitter gourd) is an herbal tropical plant (Thailand), which exerts a very wide action *in-vivo* as hypoglycaemic, antioxidant, hypocholeresterolemic and anticancer agent, among others. We investigated *in-vitro* its antioxidant action on oxidation of native (nLDL) and modified (glycated) LDL, known as a highly atherogenic process. The copper mediated oxidation end product malondialdehyde (MDA) formation, as well as the influence on LDL phospholipids (PC) oxidative degradation and lyso-phosphatidylcholine (LPC) formation by mass spectroscopy (MALDI) was estimated. The concentration of the drug was calculated in respect to maximal daily dose of 1500 mg corresponding to in-vivo of 0.3 mg/ml and according to a possible metabolism of 0.1 mg/ml. Also 0.03 mg/ml and 0.01 mg/ml were applied to investigate the interaction of very low dosages. The drug at a concentration of 0.3 mg/ml reduced the time-dependent (2-24h) nLDL oxidation based on MDA-generation by 98.3-80.0%, respectively. At a lower concentration of 0.1 mg/ml the reduction was only slightly lower by 94.9-78.9%, up to 6h. Even at very low concentrations of 0.03 mg/ml and 0.01 mg/ml the inhibition was still pronounced by 13.7-4.8%, respectively up to 6h. Analyzing gLDL (diabetes) oxidation (goxLDL), we found an inhibition of maximal oxidation at 4h amounting to 38.8% at 0.3 mg/ml and to 4.7% at 0.1 mg/ml. Phospholipid profiles were established based on MALDI measurements and changes of individual PC species containing polyunsaturated fatty acids (PUFA) and corresponding LPC formation, calculating relative signal intensity of nLDL during oxidation and glycation. The formation of LPC in goxLDL was decreased between 15.7-33.4% adding the drug at a concentration of 0.1 mg/ml by detection of LPC (16:0) and (18:0) formation between 15.7-33.4%, respectively. Breakdown of PC containing sn-2 docosahexaenoic acid (22:6) was very strongly prevented by 2.4-fold as compared to goxLDL. Degradation of PC containing linoleic acid (18:2) was reduced in the presence of the drug at both concentrations of 0.03 mg/ml and 0.01 mg/ml between 20.5-37.1%, respectively. No significant effect on PC degradation containing sn-2 arachidonic acid (20:4) in goxLDL was observed at both concentrations. On the other hand using a dosage of 0.3 mg/ml an increase in LPC formation was found. We conclude that momordica charantia exerts very strong antioxidative properties (reduced MDA-formation) as well as protective effects on the specific LDL profile. Its application *in-vivo* might therefore be beneficial for patients suffering from hypocholeresterolemia and/or diabetes.

PP 108

ANTI BETA 2 GLYCOPROTEIN I ANTIBODIES AT PATIENTS WITH DVT, CVI AND AMI

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Background: Anti beta 2 glycoprotein I (a-b2GPI) antibodies belong of antiphospholipids antibodies (APA). The understanding of their biology, laboratory diagnosis and clinical manifestation is very important because their strong correlations with deep venous thrombosis (DVT) and artherial thrombosis: cerebrovascular insult (CVI) and acute myocardial infarction (AMI).
Aim: To determine and correlate the concentrations of three classes a-b2GP I antibodies at patients with DVT, CVI and AIM and to make the algorithm for theirs following and treatment.
Method: 100 patients with DVT, 50 patients with AIM and 150 patients with CVI selected with request for own and family anamnesis for thrombosis complications. The concentration of a-b2GP I antibodies were determined with standardized home made micro-ELISA plates coated with purified human b2GP I.
Results reported in antiphosholipid units (APU). The results > 20 APU were assigned as positive. Increased IgG a-b2GPI were determined at 33 % patients with DVT (60.18 +70.11), 6 % with AIM (32.67 SAU +6.43) and 21 % with CVI (42.06 +32.35). Increased IgA a-b2GPI were determined at 33 % with DVT (58.82 + 36.06), 8 % with AIM (39 SAU + 19.32) and 20 % with CVI (30.87 + 17.72). Increased IgM a-b2GPI were determined at 35 % with DVT (42.74 + 24.84), 16 % with AIM (28.5 SAU + 5.18) and 14 % with CVI (53.52 + 41.26)
Conclusion: At patients with DVT the concentrations of IgG and IgA a-b2GPI antibodies are the highest and the most percent have elevated level. The concentration of IgM is the highest at patients with CVI, but the most percent of patients with DVT had elevated level of IgM. An elevated level of any class of a- b2GPI antibodies correlated with current clinical state (thrombosis) or it means increased risk for developing of thrombosis. So the determination of APA is introduced as obligatory laboratory test in addition to others to make exactly diagnosis for thrombosis and ordinate proper therapy.

PP 109

ELEVATIONS IN SOLUBLE CD40 LIGAND IN PATIENTS WITH HIGH PLATELET AGGREGABILITY UNDERGOING PCI

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Background. High aggregatory responses despite antiplatelet treatment are associated with an increased risk of thrombotic complications following (PCI). In the present study we investigated the relationship between platelet aggregatory responses to ADP and the release of CD40L (sCD40L) – an immunomodulatory compound involved in atherothrombosis - in patients undergoing PCI.
Methods. ADP-induced platelet aggregation, sCD40L and soluble P-selectin (sP-selectin) were determined before and 24 hours after PCI, in samples from 52 patients receiving aspirin and thienopyridines. Platelet aggregation to ADP above the median was defined as "high aggregation", and aggregation below the median as "low aggregation". Data below are medians and interquartile ranges.
Results. Patients with "high platelet aggregability" had a significantly higher increase in both sCD40L (Δ-values: 0.78 (-0.19 - 3.18) vs. -0.65 (-2.10 - 0.00) ng/ml, p=0.002) and sP-selectin (Δ-values: 8.0 (-2.00 - 16.00) vs. 4.50 (-13.00 - 0.50) ng/ml, p=0.001) compared to patients with "low platelet aggregability". In a multivariate linear regression analysis adjusted for clinical characteristics and type of pre-intervention therapy, the only independent predictors of sCD40L and sP-selectin were platelet aggregation to ADP before PCI ($p<0.001$) and the combination of platelet aggregation to ADP before PCI and urgency of PCI ($p<0.001$)
Conclusion. Circulating CD40L is more markedly increased after PCI in patients with high ADP-induced platelet aggregation.

PP 110

INTRAVENOUS TISSUE-TYPE PLASMINOGEN ACTIVATOR THERAPY FOR ISCHAEMIC STROKE: NIS STROKE TEAM EXPERIENCE

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Background. To present the preliminary experience of implementing intravenous thrombolytic therapy for acute ischaemic stroke in Stroke unite, Department of neurology, Clinical center Nis, South Serbia.
Methods. This prospective and observational study included 65 consecutive patients with an ischaemic stroke treated in our Stroke unit within 3 hours from the onset of symptoms, between November 2006 and January 2009. Patients were selected and treated in accordance with the American Heart Association guidelines. Primary safety and outcome variables were on MRI performed at 24-36 hours, mortality and independence at 90 days. Intracranial and systemic haemorrhagic complications were recorded.
Results. 65 patients (35 men and 30 women) with a median age of 69 years +/- 13.2 years (range 24-79) received thrombolytic treatment (approximately 3,4% of 910 patients with ischemic stroke). The median time from stroke onset to t-PA therapy was 110 minutes (range 20-180) and from arrival in the emergency room to the start of thrombolysis 80 minutes. Baseline median NIHSS was 16 (range 4-44). Forty-one patients exhibited early clinical improvement defined as a decrease in NIHSS score. Median NIHSS before discharge was 4,2 points. At 3 months, 80% (95% Ci, 47,9-64,1) of patients were functionally independent. One patient developed a haemorrhage. Within 3 months of ischaemic stroke 16.8% patients died.
Conclusion. The use of intravenous t-PA by experienced neurologists in Stroke unites, is safe and it is associated with a favorable outcome, without excess risk and similar to that observed in clinical trials. Successful experience with this therapy depends on organization of the treating team and adherence to published guidelines.

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PP 111

DISSEMINATED INTRAVASCULAR COAGULATION AT AN EARLY PHASE OF TRAUMA

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Background and aim: Disseminated intravascular coagulation (DIC) with an antifibrinolytic phenotype is characterized by thrombotic occlusion of the microvasculature by fibrin leading to multiple organ dysfunction and poor outcome of patients during the late-stage of trauma. The aim of the present study was to test the hypothesis that DIC with a fibrinolytic phenotype at an early stage of trauma also contributes to a poor outcome due to severe bleeding.
Methods: This is a retrospective, cohort study. The study subjects included 314 consecutive severe trauma patients classified into 289 survivors and 55 nonsurvivors. A systematic review of the computer-based medical records of the patients was conducted to provide the base line characteristics, coagulation and fibrinolytic markers and DIC-related variables. The worst data of these variables were obtained at 4 time points within 24 hr after arrival to the ED; Time Point 1, immediately after arrival to the ED to 4 hr after arrival; Time Point 2, 4 to 8 hr after arrival; Time Point 3, 8 to 16 hr after arrival; Time Point 4, 16 to 24 hr after arrival.
Results: Almost all nonsurvivors (87.3%, 48/55) met the Japanese Association for Acute Medicine (JAAM) DIC criteria showing lower fibrinogen levels, an increasing prolonged prothrombin time, and higher fibrin/fibrinogen degradation products (FDP) and D-dimer levels in comparison to those of the survivors. The FDP/D-dimer ratio and lactate level were significantly higher in the nonsurvivors than those of the survivors. A stepwise logistic regression analysis showed that the JAAM DIC score, levels of fibrinogen, FDP and lactate at Time Point 1 are independent predictors of death for trauma patients. In addition, low levels of fibrinogen and high FDP but not D-dimer predict massive bleeding at an early stage of trauma.
Conclusions: DIC wit a fibrinolytic phenotype modified through fibrinogenolysis at an early phase of trauma contributes to poor prognosis due to massive bleeding. Trauma-induced tissue hypoperfusion may be involved in the pathogenesis of this type of DIC.

PP 112

HAEMOSTATIC DISORDERS IN PATIENTS WITH MASSIVE TRANSFUSION

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Introduction: Patients with massive traumatic haemorrhage inevitably develop coagulopathy as a result of hemodilution and consumption of platelets and clotting factors. This acquired coagulopathy is further exacerbated by hypothermia and acidosis -"the bloody vicious cycle". Using laboratory-guided replacement therapy to correct this coagulopathy is not feasible in trauma patients with rapid ongoing haemorrhage and blood replacement. Empiric strategies, such as massive transfusion protocols, concurrently infuse red blood cells, plasma, and platelets in fixed ratios without waiting for laboratory results. Massive transfusions, however, reinforce existing coagulopathy and absorb more than half of all of the blood used in injury care in a trauma centre.
Aim: To review the pathophysiology of coagulopathy in massively transfused adult patients with politrauma, previously hemostatically competent, and to recommend the most appropriate treatment strategies.
Material and Methods: Primary haemostasis, the formation of a platelet plug, was studied in 22 injured patients receiving an average of 21 transfusions during the operation for control of bleeding. The storage age of the blood averaged 15 days; no platelet transfusions were given.
Findings and Discussion: Platelet counts (PLT) and bleeding time (BT) were studied intraoperatively; postoperatively at 6 hours, 25 hours, day 2, day 4; and during convalescence (8 days to 3 months). Serial PLT and BT levels were correlated with the number of transfusions and age of blood. During operation, the PLT fell to 109,000/mm3 and the BT was greater than 15 minutes. Thrombocytopenia did not correlate with the number of transfusions or age of blood. The PLT averaged 106,000/mm3 at 6 hours and then fell significantly to 73,000/mm3 at 15 hours and to 76,000/mm3 on day 2. The PLT rose significantly to 110,000/mm3 by day four and increased to supernormal levels by convalescence. The BT remained elevated at 6 hours, 15 hours, day 2, and day 4, and declined to normal by convalescence. Thrombocytopenia and prolonged BT after massive transfusion for injury indicate platelet dysfunction which may protect against disseminated intravascular coagulation. Correction of the thrombocytopenia should be reserved for patients with bleeding.
Conclusions: Coagulopathy associated with massive transfusion remains an important clinical problem. It is an intricate, multifactorial and multicellular event. Treatment strategies include the maintenance of adequate tissue perfusion, the correction of hypothermia and anaemia, and the use of haemostatic blood products to correct

microvascular bleeding. Consensus about the optimal transfusion protocol is lacking so in perspective the trauma team and transfusion services regarding strategies for resuscitation and haemostatic control in trauma patients with ongoing massive haemorrhage will have to cooperate closely to establish protocols and strategies for massive transfusion.

PP 113

ANTIPHOSPHOLIPID SYNDROME AND HELICOBACTER PYLORY INFECTION - CASE REPORT

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Introduction: Autoimmune disease has a multifactorial etiology. General opinion is that microbial agents or viruses can induce autoimmune diseases by a variety of pathogenetic mechanisms. Helicobacter pylorus is a causative agent in autoimmune gastritis, immune thrombocytopenic purpura, Sjogren's syndrome, ulcerative colitis, atherosclerosis, autoimmune pancreatitis. A few reports point to the effectiveness of antibiotics in APS associated with Helicobacter pylori. Clinical and immunological evaluation of the patterns of disease is the presence of antiphospholipid antibodies which bind target phospholipid molecules, mainly through β2 glycoprotein I (β2GPI).
Case report: We report the case of a 49-year-old woman admitted to our hospital, first with leukopenia, blood in expectoration, pain in the joints of hands and feet, four years before the deterioration of the disease. At the time, clinical examinations excluded systemic disease and lung disease. Deterioration of health manifested after four years with blood in expectoration, day to day chest pain and loss of feeling in the right hand. Laboratory investigation: WLB 3,4x109/l, Er 4,34x1012/l, Hgb 131, Hct 39,4, PLT 246x109/l, CRP 0,6, Vitamin B-12 over 1200 pg/ml, folic acid 10,8ng/ml. Screening coagulation tests: fibrinogen 4,03g/l, PT 96%, APTT 22,6 sec, Ddimer 142, LAC 1,57 moderately positive VWF 124%, FIX 98%, FXI 275%, FXII 108%, FX 166%, FII 146%, FV 179%, FVII 29%, FVIII 441%, LAC 1,96 moderately positive. Thrombophilia tests PS 83%, ATIII 120%, PC 150%, APCR 1,62, LAC 1,96 positive after 4 months. Immunological examination IgG 11,20 g/L, IgA 1,31 g/L, IgM 0,65 g/L, C3 1,58, C4 0,161. RF lower 0,29. ANA negative. LE cells not found. Direct Coombs tests negative. Bone marrow examination and variety of laboratory examinations we performed to differentiate the condition.
Conclusion: The infection is not always apparent in the case of antiphospholipid syndrome. Although the role of Helicobacter pylori in a patient with APS cannot be excluded, we found no evidence of an association between Helicobacter pylori infection and antiphospholipid syndrome. Moreover, spontaneous remissions cannot occur in APS.

PP 114

THE MOST COMMON COAGULATION DISORDERS IN PATENTS WITH SOLID TUMORS - OUR EXPERIENCES

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Background: Solid tumors are known to be followed by coagulation disorders. Although mechanisms of these disorders are not completely explained, it is supposed it may be associated with tumor exocrine secretion, which increases during the progression of disease, as well as tumor angiogenesis.
Aim: Aim of this work was to show the most frequent coagulation disorders in patients with solid tumors.
Methods: We examined coagulation status in 43 patients with solid malignant tumors, with different localizations, before they were treated with chemotherapy: 21 with colorectal adenocarcinomas, 7 with head and neck neoplastic diseases, 4 with stomach neoplasm, 5 with ovarian cancers, 2 patients with uterus malignancies, and 1 with testis malignancy. We identified the next parameters: PT, INR, TT, APTT, fibrinogen, D-Dimmer.
Results: 14/43 patients (32,56%) did not have any disorders of the mentioned parameters, and in 29/43 (67,44%) patients pathological values of some of these parameters were noted. D-dimer was increased in 17/43 (39,6%) patients, fibrinogen in 1/43 patients (2,32%). D-dimer and fibrinogen simultaneously were increased in 8/43 (18,6%) and D-dimer and PT simultaneously were increased in 3/43 patients (6,98%). Disorders of TT and APTT were not noted.
Conclusion: The most frequent disorder in the group of patients with different types of solid tumors was increased D-dimer concentration.

PP 115

USE OF PLATELETS IN PATIENTS WITH MALIGNANT DISEASES

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Introduction: Intensive therapies producing severe and sustained thrombocytopenia are used routinely in patients with hematological malignancies and they are applied to many patients with solid tumors as well.
Aim: The aim of this work was to determine a number of platelets representing the indication for transfusion, to establish the average use of platelets by malignant patients and to estimate the influence of the type of therapy on the level of thrombocytopenia.
Materials and Methods: Retrospectively, we analyzed the use of platelets at the Oncology Clinic of the Clinical Centre Nis, from November 1st 2007 till November 1st 2008. Patients received single-donor platelet concentrates or pooled platelet concentrates. We determined the platelet count at the beginning of and during the therapy. Patients were divided into three groups, according to the therapy treatment (I - only radiotherapy, II - only chemotherapy, III - radio and chemotherapy).
Results: In the period of investigation, 19 patients received platelet transfusion. An average use of platelet concentrates was 5,94 single-donor concentrate or approximately one pooled platelet concentrate per oncological patient who received transfusion, ranging from 4 to 12 concentrates. Hemorrhagic syndrome occurred in 9 patients. Among tested group of patients, 89,2% received transfusion of platelets when the platelet count was less then 20 x109 /l, 56,8% of patients had a severe form of thrombocytopenia (10 x109 /l). An average platelet count is less for 78,80 x109 /l after the treatment (z=4,83, p-0,001), and differs according to the type of therapy (the highest decrease in group II). Among 19 patients who received platelet concentrates, 7 patients are from group I, 9 from group II and 3 patients from group III.
Conclusions: An average use of platelet concentrate is 5,54 per oncological patient who received transfusion. Statistically, a significant decrease of platelet count was observed in patients treated only with radio-therapy who are the greatest consumers of platelet concentrates. For the purpose of efficient transfusion support in patients with malignant diseases, we have to follow the latest knowledge and attitudes about clinical use of blood products.

PP 116

DEVELOPMENT OF THE DISEASE AND FATAL OUTCOME IN A PATIENT WITH BERNARD-SOULIER SYNDROME

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Bernard-Soulier Syndrome (BSS) is a congenital platelet function disorder that impairs platelet adhesion to von Willebrand factor. The disorder results from deficiency or dysfunction of the GPIb-IX-V receptor on platelets. We report the development of the disease and fatal outcome of a female patient with BSS. The diagnosis was confirmed by macrothrombocytopenia, absence of ristocetin-induced platelet aggregation and profound decrease of GPIb-IX on the platelet surface. She had frequently skin haematomas and epistaxis. At the age of 20 she had a life threatening bleeding after delivery. At the age of 47 profound melenas occurred with multiple erosions in GIT. She was treated with H2 antagonists and massive transfusions of packed red blood cells, platelets and FFP. Surgical intervention was inevitable, she went through two interventions: first partial and two months later, total gastrectomy. Recombinant factor VIIa in standard doses was used prophylactically without any complication. The clinical state improved transiently but shortly, profound melena continued. Platelet transfusions were not useful any more because of the development of antiplatelet antibodies. Soon the patient died in coma after intracranial hemorrhage. Conclusion: BSS is a rare congenital platelet disorder where specific treatment of bleeding episodes with platelet transfusions is recommended. However, alloimmunization by HLA antigens frequently occurs that limits future responses to platelet transfusions. Recent patient studies and also our experience suggest the use of recombinant factor VIIa as alternative treatment of acute bleeding episodes and surgical interventions.

PP 117

THE TREATMENT OF CRITICAL BLEEDING EPISODES IN PATIENTS WITH SERIOUS HAEMATOLOGICAL DISORDERS WITH RECOMBINANT FACTOR VIIa

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Recombinant factor VIIa (rFVIIa) has been used in many clinical situations other than bleeding in patients with haemophilia and inhibitor. Due to efficacy and safety it is considered by some authors as universal haemostatic agent. Use of rFVIIa in patients with intractable bleeding and other serious haematological disorders, has not been studied in a larger cohort of patients. The aim of this retrospective study is to investigate conditions and efficacy of rFVIIa in haematological patients with severe bleeding. The study included all off-label patients since 2005, which were treated in the Clinic of haematology Novi Sad. In the assessment of efficacy, our scoring system which combines clinical and laboratory, radiological or endoscopic data, was used. There were 18 patients (9 with acute leukemia, 3 with nonHodgkin lymphoma, 1 with chronic lymphocytic leukemia, 2 with aplastic anaemia, 1 with paroxysmal nocturnal haemoglobinuria, 1 with vonWillebrand disease and 1 with adult-onset Still disease). The most of them, 9 patients (50%) had severe gastrointestinal bleeding, 4 (22%) had soft-tissue haemathomas (neck, diffuse skin or perianal), 3 (17 %) had intracranial bleeding and 2 (11%) respiratory tract bleeding. When the usual dose of rFVIIa (80-100 μg/kg BM) had been used, good efficacy was registered in more then 80% of patients. Low dose regimen (40 μg/kg BM or less) was associated with treatment failure. Although almost all patients received platelet transfusions, rFVIIa has been effective, even in severely thrombocytopenic patients. In our small group of haematological patients with severe haemorrhagic diathesis, rFVIIa proved effective, if given in usual dose and with platelet transfusions.

PP 118

DIFFUSE CAVERNOUS HEMANGIOMA OF THE LEFT LEG, VULVA ET UTERUS IN A PREGNANT WOMAN: REPORT OF A RARE CASE

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Background: Diffuse cavernous hemangioma of the whole left leg which involved vulva and uterus in a pregnant woman is an extremely rare condition. Although it is a benign condition but it can have serious consequences for the mother as well as the baby.
Method: Here we describe a unique case of 33-years old women, a primigravida at 24 weeks gestation with congenital diffuse cavernous hemangioma of whole left leg: regio pedis, cruris, femoris et glutei, with involvement of vulva, and just in pregnancy, by ultrasonic investigation, founded abnormal vessels of the left side of uterus resembling cavernous hemangioma. Cavernous hemangioma of uterus and placenta was cause of intrauterine fetal death confirmed by histopathological examination of specimen after cesarean section.
Results: Cavernous hemangioma of the uterus and fetal malfunction was diagnosed sonographically at 24 weeks gestation of pregnant women with congenital cavernous hemangioma of left leg and vulva. In coming 4 weeks she developed low grade disseminated intravascular coagulation with arised D-dimer and in therapy was included low- molecular-weight heparin in prophylactic doses of 50 U/kg twice a day. At 28 weeks gestation was diagnosed intrauterine fetal death, the patient was undergone of laparatomy and secion cesarean, hysterectomy was avoided. Histopathological examination showed cavernous hemangioma with massive thrombosis of uterus and placenta's vessels.
Conclusions: This case highlilights that huge congenital cutaneous cavernous hemangioma, in this case left leg, could exist mutual with visceral hemangioma such as uterus. In pregnant women, these conditions required increased attention for women's health and outcome of pregnancy.

PP 119

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

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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 heavy chain (A1-A2-B domains) and a light chain (A3-C1-C2 domains), which are held together by a metal ion dependent linkage. Recent studies have identified FVIII light chain region Glu1811–Lys1818 as being involved in FIXa binding and in the assembly of the FX-activating FIXa/FVIIIa complex.
Aim: We have synthesized linear analogue peptides (AP) of the 1811-1818 loop of the A3 subunit of the light chain of FVIIIa, in order to examine their anticoagulant activity.
Method: 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 OK buffer +300μl NP. B) 300μl AP (1mg/ml in OK buffer) +300μl pure recombinant FVIII (1u/ml in OK buffer). We test the mixtures in IL coagulation instrument, measured the aPTT and the FVIII activity. The results were a prolongation of aPTT varying more >6sec for the mixture of analogue Ac-ETKTYFWK-NH2 and an important reduction of FVIII activity (> 40%).

PP 120

THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR IN PATIENTS WITH DIABETES MELLITUS TYPE 2

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Background: Patients with diabetes mellitus (DM) have a tendency to hypercoagulation and to the decreased fibrinolysis. Thrombin activatable fibrinolysis inhibitor (TAFI) is a principal regulator of initial fibrinolysis.
Aim: Aim of the study was to find the relation of TAFI to abnormalities of fibrinolysis and endothelial dysfunction markers in patients with DM type 2.
Methods: Eighty-four patients with DM type 2 were randomized in two subgroups according to microalbuminuria (MAU more than 30 mg/24 h.). The subgroup with MAU (n=42) and the subgroup with normoalbuminuria (n=42) (NAU less than 30 mg/24 h.). The plasma levels of TAFI antigen (Ag), endothelial and fibrinolytic markers, prothrombin fragments (F1+F2) and metabolic parameters were measured.
Results: Patients with DM type 2 and MAU showed the increased TAFI Ag level 115.2% (IQR: 94.4-195.2%) compared to patients with DM type 2 and NAU 101.6% (IQR: 76.8-140.8%) and the significantly increased TAFI in comparison to healthy controls 75.2% (IQR: 57.7-88%) p less than 0,001). There was a significant correlation between TAFI and F1+F2 in patients with DM type 2 and MAU.
Conclusions: The results of our study support the significantly increased TAFI levels in patients with DM type 2 and MAU. Mechanism of the increased TAFI plasma levels can be secondary due to the activation of coagulation. We did not find any significant correlation between TAFI and the endothelial dysfunction markers.

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PP 121

RADIONUCLIDE THERAPY WITH 153-SAMARIUM-EDTMP TEMPORARILY DECREASES PLATELET COUNT PARALLELED BY AN INCREASED CELLULAR THROMBOXANE FORMATION

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Background: Radionuclide therapy for bone pain palliation is associated with a temporary decrease in peripheral platelet count. Platelets are the blood cells mainly affected. A peripheral platelet count of < 1.10^5/µl is widely considered a contraindication for 153-Samarium (153Sm)-EDTMP treatment. No data, however, as to the functional capacity of platelets in this condition exist.
Methods: We examined 55 patients undergoing repeated treatment with 153Sm-EDTMP (^{153}Sm-EDTMP) according to the Vienna protocol. They were treated in 3 months intervals for a total of 5 treatments within one year. Blood was drawn before each therapy as well as in regular intervals in between (week 1 and week 12) for peripheral platelet count and parameters of thromboxane synthesis (serum thromboxane B2 [TXB2], plasma TXB2, malondialdehyde and plasma 11-dehydro-TXB2).
Results: Pretreatment platelet count showed a wide range in between 249 - 327 x 10^3/µl. There was a significant drop in peripheral platelet count showing its maximum at 327 x 10^3/µl., however, balanced by a higher activity per platelet resulting in some patients even in a totally increased TX available. The kinetic behavior both of cellular number and activity during the five consecutive treatments is comparable.
Conclusions: These findings indicate that even in temporary thrombocytopenia induced by ^{153}Sm-EDTMP in the great majority of patients a decrease in peripheral platelet count is balanced by an increased cellular TX which may minimize in the overwhelming majority of patients the risk of bleeding. The approach to measure TXB2 on a prospective base in patients scheduled for the treatment with a critical platelet count of less than 100 x 10^3/µl might be promising to assess eventual later bleeding risk. How low to go there with the platelet count? What would be a cut-off for platelet functional activity?

PP 122

PLATELET LABELLING EFFICIENCY IS INFLUENCED NOT ONLY BY QUANTITATIVE BUT ALSO QUALITATIVE LIPOPROTEIN COMPOSITION

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Background: 111Indium labelling has been accepted long ago as the method of choice to assess platelet life-span being the most reliable in-vivo indicator for platelet function. It is also used successfully for radioimaging of thrombosis and atherosclerosis. Long ago we discovered that total and LDL-cholesterol (LDL-CH) show an inverse relationship to labelling efficiency (LE) as well as recovery. Analyzing the influence of cells (platelets) vs. plasma, it was clearly shown that this effect is due to a change in the cellular membrane lipid composition. Data on the influence of lipoprotein particle size and density on LE, however, are not yet available.
Methods: We examined the LE of platelets from donors with a total CH ranging between 160 and 500 mg/dl. Platelets derived from donors with identical LDL, but different LDL size and density and different oxidative states of LDL (determined spectrophotometrically as well as by the isoprostane 8-epi-PGF2) were radiolabelled. Platelets from normolipemics (total CH < 200 mg/dl, LDL-CH < 130 mg/dl) were incubated in the respective anticoagulated blood. Platelets were labelled for in-vitro purpose only with 10 Ci of 111 Indium-oxine at 37°C in a total volume of 1 ml. Cross-incubation of platelets with plasma from normolipemic controls or lipoproteins of different size and density was also performed.
Results: The findings indicate a significantly ($p < 0.01$) strong negative correlation between LE and extent of mortification being the closer the higher the LDL levels are. At the identical LDL level blood derived from patients with small dense LDL show a lower LE as do patients with higher oxidation state. In contrast, platelets from volunteers incubated in plasma with qualitatively or quantitatively abnormal LDL did now show a significant change of the labelling results.
Conclusions: These findings confirm once again that the plasma membrane of platelets is the key determinant for LE. In patients with small dense LDL and high oxidation state, LE may be even more impaired. These findings should be considered especially in the ever growing number of patients with metabolic syndrome and diabetes characterized in part by a greatly mortified population of all the lipoproteins, in particular LDL.

PP 123

IMPACT OF VALPROATS ON THE PLATELET NUMBER AND FUNCTION

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Backgound: Epilepsy is a highly prevalent illness and 0,5-1,5% population on our planet suffers from it. Valproats (VPA) are among the most frequently used antiepileptics which exhibit a certain undesirable influence on blood picture components.
Aim: To determine the impact of VPA on the platelet number and aggregation capacity.
Material and Methods: The research was being conducted in the time range of two years (May 2000 - May 2002) at the Pediatrics clinic in Kragujevac, wherein 38 children using VPA and 30 healthy children of specific sex and age were examined. Platelets were counted on the amount of the marginal sample and platelet aggregation was established by Born method. ADP and colagen (COL) were utilized as aggregating agents. The Valproat level in blood was determined by HPLC (by using the liquid chromatography method) in order to examine the connection between the negative influences of VPA dosage and level in blood on platelets.
Results: By the use of ANOVA test, a statistically significant difference in platelet number values was noted between the children group treated with VPA
(155,23 +/- 27,06 x 109/l) and the healthy children group (265,27 +/- 69,55 x 109/l). With VPA level increase in blood the average platelet number value decreases, but that difference did not show statistical importance by using ANOVA test. It was observed a statistically significant difference between the average ADP platelet aggregation value of the children group using VPA (53,06+/-10,83%) and the healthy children group (76,5+/- 7,42%). There was also remarked a statistically significant lower average COL platelet aggregation value of the children group with VPA (59,74 +/- 4,23%) in comparison with the healthy children group (80,57 +/- 5,63%). There was observed a statistically important lower average ADP and COL platelet aggregation value depending on VPA level increase in blood.
Conclusion: VPA indicate statistically significant negative impact on platelet number and aggregation. This impact is in negative correlation with VPA dosage and level in blood.

PP 124

HAEMOVIGILANCE AND PHARMACOVIGILANCE IN MEDICATION OBTAINED FROM HUMAN PLASMA

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Background. Haemovigilance comprises a set of organized surveillance procedures relating to serious adverse or unexpected events or reactions in blood donors or recipients and the epidemiological follow-up of donors. The term pharmacovigilance comprises all scientific and data gathering activities relating to the detection, assessment, and understanding of adverse events. This includes the use of pharmacoepidemiologic studies.
Aim. To find the connection between Haemovigilance and Phramacovigilance.
Methods and Results. To find a serious adverse event is defined as an unintended occurrence associated with the collection, testing, processing, storage and distribution of blood or blood components that might lead to death or life threatening, disabling or incapacitating conditions for patients or which results in, or prolongs, hospitalization or morbidity. A serious adverse reaction is defined as an unintended response in a donor or in a patient associated with the collection or transfusion of blood components that is fatal, life-threatening, disabling or incapacitating, or which results in or prolongs hospitalization or morbidity. Pharmacovigilance activities are undertaken with the goal of identifying adverse events and understanding, to the extent possible, their nature, frequency, and potential risk factors.
Conclusion: Preparation and usage of medication obtained from human plasma could be connected to haemovigilance through documents that report on unwanted side effects. In cases where medication obtained from human plasma is used, traceability is an important element that could be achieved by linking information obtained through haemovigilance with information obtained through pharmacovigilance.

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