Vitamin B6, measured as pyridoxal 5-phosphate (PLP) is a coenzyme in the trans-sulfuration pathway of homocysteine metabolism. Vitamin B6 levels are significantly reduced in sideroblastic cell anemia, in diabetic neuropathy and in chronic renal failure, and reduced PLP has been associated with immunological changes observed in HIV-infected patients. Few procedures have been developed for direct measurement of PLP and few comparative reports are available. We have compared three PLP assays: 1) vitamin B6 HPLC (Immunodiagnostik, Bensheim, Germany); 2) vitamin B6 3H-REA (Buhlmann Laboratories AG, Allschwil, Switzerland); and 3) a home-made radioenzymatic assay (REA) based on the selective extraction of [3H]tyramine and its subsequent quantification by liquid scintillation counting after the decarboxylation of high-specific-activity [3H]tyrosine by tyrosine apodecarboxylase enzyme. At variance with the home-made method, the commercial REA assay does not include a deproteinization step. Intra- and inter-assay imprecision (CV) of the 3 methods ranged from 4.6% to 9.6%. The study was performed on plasma samples collected in sodium citrate from 99 donors (age range: 30-60 years), including healthy subjects (n = 40) and patients with (n = 15) or without (n = 44) vitamin B6 supplementation. Median and (ranges) were: 34.5 (4.0-415), 37.9 (1.0-508 and 31.0 (1.0-660) for assay 1, 2 and 3 respectively. PLP levels were similar with the 2 commercial procedures and significantly higher (p<0.0001) than those recorded with the home-made assay. Correlation coefficients between methods ranged from 0.948 to 0.979. Agreement among the 3 methods was evaluated in samples from subjects not on vitamin B6 supplementation (n = 84). Limits of agreement ranged from 80% to 350%. Thus, despite comparable average values, agreement of PLP levels with the different assays was very poor. Since PLP depletion has been suggested as an independent risk factor for venous thromboembolism, PLP has gained importance as a marker to guide patient care. Given the results obtained in this study, standardization of PLP assays is urgently needed.

LONG-TERM OUTCOMES OF POST-SURGICAL DEEP VEIN THROMBOSIS IN ASIA: THE AIDA-EXTENSION STUDY
U.O. Malattie Tromboemboliche, IRCCS Policlinico San Matteo, 27100 - Pavia, Italy

Background: We have recently demonstrated in a large multinational, multiethnic study that the incidence of venographic deep-vein thrombosis (DVT) in Asian patients undergoing major orthopedic surgery of the lower limbs and not receiving thromboprophylaxis was similar to that observed in orthopedic patients in Western countries. Overall, 407 patients (20-99 years) undergoing THR (n=175), TKR (n=136) or HPS (n=96) were recruited in 19 centres across Asia (Indonesia, South Korea, Malaysia, Philippines, Taiwan and Thailand). 72.5% of the enrolled patients had adequate venograms. Total DVT was diagnosed in 121 of 295 evaluable patients (41.0%, [95% confidence interval: 35.4-46.7%]). Proximal DVT was found in 30 patients (10.2% [7.0-14.2%]). However the long-term outcomes following deep vein thrombosis such as recurrence of DVT, incidence of pulmonary embolism (PE), and post-thrombotic syndrome (PTS) remain unknown. PTS has received little attention in the literature, and particularly in Asia. Objectives: To assess the long-term complication rates (at 1 and 2 years) of patients with objectively assessed DVT compared to those observed in patients without post-operative DVT. Methods: Annual visits were performed to record the occurrence of DVT/PE, incidence of PTS, cumulative incidence rates and related predictive factors. PTS scoring were assessed using the Villalta scale (signs and symptoms). In addition in a subset of 18 out of 27 (66%) patients from South Korea with centrally adjudicated venographic DVT, a second venogram was performed at 1 year in order to verify possible spontaneous modifications of the thrombus (none of these patients had undergone any anticoagulant treatment). Results: From the 332 patients having completed the AIDA study in the 19 centres involved in the extension study, 236 patients (71.1%) agreed to participate in the extension phase and were assessed at year 1. Year 2 follow up visits were completed in May 2005. Complete data analysis of the final study results are ongoing and should be completed by September 2005. Of the 18 patients who underwent a second follow-up bilateral or unilateral ascending venography at year 1 year Â± 3 months post-operatively, 11 (61%) patients demonstrated resolution of the thrombus, with 6 (16%) patients showing incomplete resolution or persistence of the venous thrombosis. The venograms of the remaining four (23%) patients presented with development of collateral circulation confirming the progressive state of PTS. The majority of patients with centrally adjudicated venographic DVT demonstrated positive resolution following venographic assessment at year 1. The high incidence of thrombus resolution may have been due to the fact that most patients were diagnosed with distal DVT, which would suggest that a high percentage of DVT of the distal veins may resolve spontaneously compared to other types of DVT.
RATES OF CLINICALLY OVERT VENOUS THROMBOEMBOLISM AND DEATH RESULTING FROM PULMONARY EMBOLISM AMONG UNSELECTED HOSPITALIZED ACUTELY ILL MEDICAL PATIENTS VS. RATES PREDICTED FROM CLINICAL STUDIES

Piovella F, Anderson FA, Jr., Decousus H, FitzGerald G, Bergmann J-E, Kakkar AK, Spyropoulos AC, Zott RB, Spencer FA, Turpie AGG, for the IMPROVE Investigators

Servizio Malattie Tromboemboliche, IRCCS Policlinico San Matteo, Pavia, Italy; Center for Outcomes Research, University of Massachusetts Medical School, Worcester, USA; Centre Hospitalier Universitaire de Bellevue, Saint-Etienne, France; Hôpital Lariboisière Clinique Thérapeutique, Paris, France; Center for Surgical Science, Barts and the London Medical School, London, United Kingdom; Lovelace Medical Center Clinical Thrombosis Center, Albuquerque, USA; Universitätsklinikum Düsseldorf, Düsseldorf, Germany; University of Massachusetts Medical School, Worcester, USA; Hamilton Health Sciences General Hospital, Hamilton, Canada

Background: Without evidence from autopsies, the majority of deaths resulting from pulmonary emboli (PE) are indistinguishable from deaths due to other cardiovascular diseases. This has led to a gap in perceptions between the benefits and risks of providing venous thromboembolism (VTE) prophylaxis. In this study, we estimated the incidence of clinically apparent VTE in hospitalized acutely ill medical patients in The International Medical Prevention Registry on Venous Thromboembolism (IMPROVE), and compared this with the expected incidence derived from clinical studies that used autopsy or prospective venographic confirmation of clinically important VTE.

Methods: Beginning in July 2002, a consecutive, unselected sample of patients who were aged ≥18 years and hospitalized for ≥3 days with an acute medical illness, were enrolled in this observational cohort from 49 hospitals in 12 countries. Up to 31 March 2005, 6946 patients were enrolled.

Results: Based on autopsy series of all-cause in-hospital deaths reported in the literature, PE is associated with 10% of deaths. Up to 31 March 2005, 6946 patients were enrolled.

Heparin anticoagulants function by enhancing the inhibition of coagulation proteases by the serpin antithrombin (AT). A direct evaluation of the specific anti-factor Xa (fXa) activity of therapeutic heparins in the physiologically relevant plasma-based clotting assays has not been feasible since thrombin, the final protease of the cascade, is the primary target for inhibition by AT in the presence of heparin. To circumvent this problem, we developed an assay in which the native AT in plasma was replaced with an AT mutant which exhibits identical affinity for heparin and near normal reactivity for fXa, but does not react with thrombin and other coagulation proteases in either the absence or presence of heparin. This assay was used to distinguish the anti-fXa activity of different molecular weight heparins from their anti-thrombin activity in clotting assays which were initiated by the triggers of either the extrinsic or intrinsic coagulation pathway. The results suggest that the acceleration of fXa inhibition by AT exhibits a marked heparin chain-length dependence, with fondaparinux (a therapeutic pentasaccharide) having the lowest and unfractionated heparin having the highest effect. Interestingly, comparative studies revealed that the fondaparinux-catalyzed acceleration of thrombin inhibition by AT also contributes to the prolongation of the clotting time, possibly suggesting that, unlike the traditional view, the anticoagulant function of the therapeutic pentasaccharide is mediated though the inhibition of both fXa and thrombin.

EVALUATION OF POLYMORPHISMS OF GENES INVOLVED IN FOLATE METABOLISM IN PATIENTS AFFECTED BY DOWN SYNDROME

Sampietro F, Scala I, Granese B, Fermo I, D’Angelo A, Andria G, Coagulation Service & Thrombosis Research Unit, Scientific Institute H. S. Raffaele, Milano, and Dept of Pediatrics, Federico II University, Naples, Italy

Down syndrome (DS) is characterized by an extra copy of genes located on chromosome 21. The cystathionine beta-synthase (CBS) and the reduced folate carrier genes (RFC1) map on chromosome 21 and are involved in homocysteine/folate metabolism. We evaluated total fasting homocysteine (tHcy) plasma levels and the prevalence of polymorphisms in methylenetetrahydrofolate reductase (MTHFR C677T, A1298C), methionine synthase (MTR A2756G), methionine synthase reductase (MTRR A66G), CBS (844ins68) and RFC1 (A80G) genes in a large series of subjects with DS (n = 131, 54% males, median age 5.0 years, range 0.25-24 years) and in healthy control subjects (n = 90, 53% males, median age 7.0 years, range 1-23 years). Increasing age had a strong positive influence on tHcy levels. After correction for age and gender, tHcy levels were significantly lower in DS subjects than in controls (median values 5.8 µmol/l versus 7.2 µmol/l, p = 0.002). Allele frequencies for MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G were 0.44, 0.36, 0.13, 0.45 and 0.45, 0.33, 0.14, 0.44 in DS and control subjects, respectively (p > 0.4). The CBS 844ins68 mutation (heterozygous) was detected in 23 DS subjects (18%) and in 17 controls (19%, p = 0.8). Wild type and mutated homozygotes for the RFC1 A80G variant were 54 (41%) and 20 (15%) among DS subjects and 38 (42%) and 28
(31%) among healthy controls (p = 0.08). However, the frequency of subjects heterozygous for the RFC1 A80G polymorphism was significantly higher among DS (44%) than controls (27%, p = 0.02). No polymorphism showed a relation with tHcy levels either in DS or in controls (p > 0.21). Plasma folate levels were similar in DS and controls (p = 0.95). In both DS and controls, increasing age (p < 0.0001) and MTHFR 677TT genotype (p = 0.04) had a strong negative influence on plasma folate levels. Two DS subjects and one control had tHcy levels greater than 20 µmol/l; two of them had the MTHFR 677TT genotype and all had plasma folate levels lower than 1.4 ng/ml.

PHARMACOKINETIC PROPERTIES OF ANTITHROMBIN CONCENTRATE (ATII KEDRION®) SUPPLEMENTATION IN PATIENTS WITH CONGENITAL ANTITHROMBIN DEFICIENCY

Della Valle P, Tornene D, Guazzini S, Bacci M, Simioni P, D’Angelo A

Coagulation Service & Thrombosis Research Unit, Scientific Institute H. S. Raffaele, Milano, Kedrion S.p.A., Castelvecchio Pascoli, Lucca, and Department of Internal Medicine, University of Padova, Italy

In human beings, the fractions of total-body AT in plasma-, vascular-associated-, and extravascular-pool are about 40%, 10% and 50% respectively. The daily catabolic rate of total-body AT averages 23%, with a fractional 46% contributed by vascular-associated AT. Thus, vascular-associated AT distributes between plasma and an endothelial receptor (heparan-sulfate), and its amount is a function of the AT concentration measured in plasma. As a result, upon infusion of AT concentrates, and depending on the amount of AT infused, most likely to affect the 50% elimination time, healthy subjects and congenital AT deficient patients may show differences in the vascular-associated pool of AT. A single infusion of the concentrate AT III Kedrion (nanofiltered) was infused at a dosage of 40, 100 and 50 U/kg b.w. in 18 healthy volunteers (HV1, mean AT activity 93±10%, mean AT antigen 92±%9, mean AT antigen 91±10%) and 12 patients with congenital AT deficiency (ATD) respectively. Among patients, 9 had type I AT deficiency (mean AT activity 51±6%, mean AT antigen 47±7%), 3 type II AT deficiency (mean AT activity 49±14%, mean AT antigen 92±11%), and 8 were on oral anticoagulant treatment. Net pharmacokinetic values were obtained by subtracting the baseline values from those relative to the post-infusion phase. By non-compartmental analysis, recoveries of AT activity and antigen were similar in HV1, HV2 and ATD. The 50% elimination time [= t1/2, beta, but considering the time points until 24 hours only] for AT antigen was not significantly different in the three groups, but the 50% elimination time for AT activity was longer in the ATD group (50±11 hours) and more so in patients with type II deficiency (58±11 hours) than in HV (20±9 hours, p < 0.05), suggesting competition of active and inactive AT species for the binding to heparan-sulfate. To rule out product specificity of these findings, we compared the affinity of Fondaparinux® and of Idraparinux® for different commercial antithrombin concentrates, as a function of the rate of factor Xa neutralization in purified systems. The Ki for Xa neutralization (7.5 and 15 nM) at 0.3 U/ml AT (from Kedrion, Baxter, Grifols, Pharmacia, Aventis) with increasing pentasaccharide concentrations was similar with all AT preparations and was two-fold higher with Fondaparinux than with Idraparinux (p < 0.0001).

LIPOPROTEIN(A), FIBRINOGEN AND VASCULAR MORTALITY IN AN ELDERLY NORTHERN ITALIAN POPULATION


Coagulation Service & Thrombosis Research Unit, Epidemiology Unit and Vascular Biology Unit, Scientific Institute H S. Raffaele, Milano, Italy

High lipoprotein a (Lp(a)) and fibrinogen levels are suggested risk factors for coronary heart disease (CHD) and stroke morbidity and mortality. Experimental data strongly suggest a fibrinogen-Lp(a) interaction in mechanisms of atherothrombosis, but little clinical evidence of a synergism between these two parameters has been reported. Within the frame of a prospective population study conducted in the area of Cremona (Lombardia, Italy), 345 women and 216 men aged ≥65 years were evaluated for clinical and biochemical cardiovascular risk factors. Lp(a) levels ≥ 30 mg/dl were observed in 27.2% and 23.9% of men and women. Fibrinogen levels were higher in women (p<0.0001). After a median follow up of 6.3 years 107 deaths were recorded, 35 due to CHD or ischemic stroke. The combined incidence rate of CHD and stroke mortality increased from 10.8 (per 1000 person-yrs) for subjects with either Lp(a) ≥ 30 mg/dL and fibrinogen within the 5th quintile of the gender-specific distribution to 38.4 for subjects with both Lp(a) ≥ 30 mg/dL and fibrinogen within the 5th quintile. Age (p<0.0001), insulin (p<0.0002) and the combination of high Lp(a) and fibrinogen (hazard ratio = 5.11, p = 0.014), but not fibrinogen or Lp(a) levels in isolation, were independent predictors of CHD and stroke mortality. In a subgroup of 447 subjects who had C-reactive protein measurements, CRP levels were not predictive of the combined CHD and stroke mortality. The association of high Lp(a) and fibrinogen levels carries an increased risk of pooled CHD and stroke mortality in elderly subjects.

DISAPPEARANCE OF ANTI-PF4/HEPARIN ANTIBODIES UNDER PROLONGED FONDAPARINUX ADMINISTRATION IN A PATIENT WITH DVT ASSOCIATED WITH LMWH-INDUCED THROMBOCYTOPENIA

Crippa L, Della Valle P, Fattorini A, D’Angelo A

Coagulation Service & Thrombosis Research Unit, Scientific Institute H S. Raffaele, Milano, Italy

Heparin-induced thrombocytopenia (HIT) is an antibody-mediated, adverse effect of heparin, which is diagnosed when HIT antibody formation is accompanied by an otherwise unexplained platelet count fall. The neoepitopes recognized by HIT antibodies are located on platelet factor 4 (PF4), and are formed when PF4 binds to heparin. Low-molecular weight heparin (LMWH) carries a lower, but not negligible, risk of HIT than standard unfractionated heparin (UFH). Non-heparin anticoagulants have been studied for treatment of HIT-associated thrombosis, but they have not been directly compared in prospective studies. A 63 years old male patient on oral anticoagulation was admitted to our Institution for a bladder biopsy. He was shifted to LMWH (enoxaparin) for 7 days (20 mg/d). Two months later he was readmitted (platelet count 246,000/microL) and shifted again to LMWH (20 mg/d). The patient underwent radical surgery after 16 days of hospitalization, and LMWH was increased to 40 mg/d postoperatively. The platelet count was 185,000/microL on day 21, but progressively decreased to 58,000/microL over 4 days, when the patient developed left ileo-femoral-popliteal deep vein thrombosis (DVT), and then to 33,000/microL on the following day. On day 35 the
platelet count was 47,000/microL and LMWH administration (120 mg/d) was interrupted. Platelet counts increased to 240,000/microL by day 39. Fondaparinux administration (7.5 mg/d s.c.) was started on day 40 and continued for 28 days, with platelet counts in between 249,000/microL and 325,000/microL. Anti-PF4/heparin antibody titers (Asserachrom HPIA, Diagnostica Stago) were determined on citrated plasma and results expressed as percent of the absorbance measured at 450 nm with a positive control (1.94). Antibody titers declined from 55% to 21% from day 39 through day 80 (cut-off for a positive value = 23.5%). The patient underwent additional minor surgical explorations 5 and 9 months later and was shifted to Fonda- parinux (2.5 mg/d) and then again to warfarin on both occasions. Platelet counts increased from 283,000/microL to 291,000/microL before surgery to 347,000/microL and 358,000/microL after surgery. Anti-PF4/heparin antibody titers on these occasions ranged between 2% and 9% of the positive control. We show for the first time the time-course of anti-PF4/heparin antibody disappearance under Fonda- parinux administration, contributing additional evidence for the safety of Fondaparinux administration in the treatment of HIT-associated thrombosis.

RARE CASES OF CONGENITAL DYSFIBRINOGENEMIAS AND THROMBOPHILIA IN CHILDREN WITH DEEP VEIN THROMBOSIS

Sturov VG, Chuprova AV
Faculty of Clinical pediatrics and Pediatric Clinic Regional Clinical Hospitals, State Medical University, Novosibirsk, Russian Federation

Dysfibrinogens can be grossly divided in two groups: (1) defective thrombin-catalyzed conversion of fibrinogen to fibrin monomers, and (2) defective fibrin polymerization due to structural alterations in polymerization sites. Congenital dysfibrinogenemias are more often associated with bleeding and less often with a tendency to thrombosis. Among 256 cases of a dysfibrinogenemia (DFG), 35% were asymptomatic (casually observed), 57% had a bleeding tendency, and only at 8% had deep vein thrombosis (DVT). Ninety-two percent of all patients with DFG had attributes of a systemic mesenchimal dysplasia. Protein C, protein S deficiency ans APC resistance were not found in DVT patients, in whom familial DFG was confirmed. The prevalence of dysfibrinogenemia in patients with history of a venous thrombosis is low, that is 0.8% as derived from 9 observations in 7 countries on 2576 patients. Homozygotes have been established in 3 cases (Marburg, Bethesda and Tokyo II), hypofibrinogenemia (an antigen on less than 1.3 mg in ml) in 2 cases. Cases of a heterozygote carriage are detected in clinically asymptomatic subjects. The purpose of work was studying frequency of occurrence dysfibrinogenemia (DFG) at children. The primary diagnosis at which was hereditary blood platelets disorders (HBPD). Various patho- genetic variants, and also at children with secondary thrombophilia on a background of a chronic DIC-syndrome. 330 children various are surveyed age and sex. Group I (n=112) was made by patients at which blood platelet dysfunction was combined with other disturbances in system hemosta- sis, also it was accompanied typical displays of a systemic dysplasia of connective tissue (mesenchimal dysplasia) that kept within frameworks of a syndrome hemorrhagic hematomesenchimal dysplasias (HMD). The group II (n=198) included patients with various variants HBPD without attributes HMD. Group III (n=20) – patients with chronic the DIC-syndrome on background of the basic patholo- gy. Group of comparison – 43 adult patients (consanguinity relatives of these children). Control group – 50 healthy teenagers. At patients I of the group, the taped disturbances auto-and gene polymerization of fibrin-monomers (<0.01), distinct disturbances Echitox-time (Echis multisquamatus) and Agkistrodon-time (Agkistrodon halys halys) (<0.001) at the normal maintenance of a fibrinogen (<0.1) in a blood specify presence of the background (latent) dysfibrinogenemia, and in some cases and dysprothrombinemia, that is one of authentic laboratory attributes HMD. Over patients of II group in a laboratory picture dominated combined blood coagulation defects (at 31.3% of patients). At a part of patients given groups took place platelets disorders with primary disturbance of adhesion to a collagen and fiber glass and deficiency of activity of von Willebrand factor. Studying of tests with snake venoms coagulates has shown, that at sick children at presence of a chronic DIC-syndromes it was most often and constantly taped hypercoagulation in
Echitox-time (<0.05). At an estimation of not enzymatic phase of a final stage of blood coagulation authentic acceleration autopolymerization of fibrin-monomers (MF) (<0.001) is taped and stimulating influence of plasma sick of a chronic DIC-syndromes on assembly donor fibrin-monomers (<0.01) in reaction heteropolymerization the last, that authentically specified occurrence at the given category of patients with thrombosis variant of DFG. From carried out research it is possible to draw a conclusion, that most the informative tests reflecting hypercoagulative changes at chronic DIC-syndromes, assays with use coagulating are snake venom test *Echis multisquamatus* and tests of an estimation of rate auto-and heteropolymerization of MF. By comparison of laboratory parameters it has been certain, that at patients at whom HBPD proceeded are common with HMD, have appeared more enlarged Lebetox-time (Vipera lebetina turanica) and Echitox-time of coagulation. The specified fact can be bound to a combination. Platelet disorders with anomaly of coagulating factors, including Fibrinogen. The specified fact confirms underestimation of a dysfibrinogenemia at the given category of patients, and meanwhile, in itself DFG aggravates current platelet dysfunction also demands to itself more steadfast attention by way of therapeutic correction and development of a complex rehabilitation actions. Among all surveyed patients in 27.88% of cases were recorded attributes of an authentic dysfibrinogenemias, and more often specified changes affected patients, complicated current integrated hemorrhagic hematomesenchimal dysplasias (21.43%). The first place among a cohort of the verified pathology (47.12%) have made DFG with disturbance of eliminating under action of thrombin and a snake venom *Agkistrodon halys halys* of fibrinopeptide A; 2 place – 34.61% – DFG with disturbance of processes polymerizations MF in γ-chain of fibrinogen; 3 place (12.5 %) have made DFG with presence of inhibitors of polymerization of fibrin-monomers in plasma, to found out thicket at patients with a chronic DIC-syndromes; 4 place - DFG with a hypersensibility of an abnormal fibrinogen to a plasmin (t-PA), taped in activation of processes of a XII°-dependent fibrinolysis and the accelerated degree dissolution of fibrin clot at the normal contents plasminogen.

In conclusion: 1) high occurrence DFG at children’s is certain, especially in presence of HMD (21.45%); 2) proven importance of snake venoms tests and methods of estimation of MF polymerization rates in the diagnosis of DFG; 3) observed phenomena DFG demand carrying out of a course corrective cure, use cells merman stabilization drugs, modulators of synthesis glycosaminoglycans, and also stimulators generation of collagen, with the purpose corrections of arising disturbances at a level platelet-endothelial contact and activation of an endothelial metabolism; 4) DFG and other anomalies of an over stage of coagulation should be surveyed as potentially dangerous by way of development thrombo-haemorrhagic displays at patients with mesenchimal dysplasias; 5) the excavation of scientifically-practical researches in given sees important areas with use of methods of molecular biology and genetics, cytomorphology and clinical biochemistry with the purpose of optimization of scientific search.