Thrombosis and vascular biology 2

P1067

VENOUS THROMBOEMBOLISM (VTE) PROPHYLAXIS IN HOSPITALIZED OBSTETRIC PATIENTS: A MULTICENTRE CROSS-SECTIONAL STUDY

Maeve P Crowley[1,*], Caroline Noone[1], John R Higgins[2], Susan O'Shea[1]
[1]Haematology, Cork University Hospital, [2]Obstetrics, Cork University Maternity Hospital, Cork, Ireland

Background: VTE complicates 1 - 2/1000 pregnancies, and the risk increases with age, mode of delivery, and presence of co-morbidities. It continues to be one of the leading causes of maternal death. Prophylaxis with low molecular weight heparin (LMWH) is safe. Despite having a population of only 4.5 million, Ireland has 20 maternity hospitals. Given this large number, it is difficult to standardize practice with international best practice.
Aims: To assess the prevalence of VTE risk in pregnant women in the hospital setting and to determine the proportion of at-risk patients who receive effective prophylaxis.
Methods: The study period was September 2011 to November 2012. All patients admitted to the participating hospitals on the day of investigation were assessed for risk of VTE on the basis of hospital chart review. Risk was assessed in accordance with the 2009 Royal College of Obstetricians and Gynaecologist Guidelines. Patients undergoing procedures or on the labour ward at the time of review were excluded. Ethical approval was obtained from the ethics committees governing all centres.
Results: 540 pregnancies were reviewed across 16 centres. The average age of was 31+/- 5.65yrs (Range 16-47), with 21.87% (117/535) aged over 35. 22% (118/535) had a parity of 3 or more. The average weight was 71.51kg (Range 42-134kg, SD 14.482kg). Data on BMI was available for 77% - 34% were overweight and 21% were obese. 1% (6/420) had a BMI>40. 31% (168/540) were antenatal and 69% (372/540) were postnatal. 63% (105) of antenatal patients were low risk (<2 risk factors), 35% (59) were intermediate risk (2 or more risk factors, prophylaxis should be considered) and 2% (4) were high risk. All the high risk patients were on prophylaxis at an appropriate dose. 4% (6) of the low risk patients were on prophylaxis unnecessarily. Only 7% (4/59) of the intermediate risk patients were on prophylaxis (3/4 were on too low a dose) Among postnatal patients, 41% (153) were low risk (<2 risk factors), 58% (217) were intermediate risk (2 or more risk factors, require prophylaxis) and <1% (2) were high risk. 80% (296) were appropriately risk stratified and put on LMWH if necessary. 59% (219) of patients should have been on LMWH but only 42% (157) were (92% Tinzaparin and 8% Enoxoparin). This included 8 patients who were on LMWH unnecessarily. 38% (59/157) were on too low a dose.
Summary and Conclusions: VTE prophylaxis is an important issue in obstetrics given its prominent role in maternal morbidity and mortality and the increasing prevalence of risk factors such as obesity and increasing maternal age. It is clear that while there is good awareness of the risk in the postnatal period, there is less emphasis on risk assessment in antenatal patients where prophylaxis is rarely used. Those on prophylaxis are also likely to be on too low a dose. Given the number of maternity hospitals in Ireland, there is a role for a national guideline to standardize care for all pregnant women.

P1068

INVESTIGATION TO IMPROVE DETECTION OF LUPUS ANTICOAGULANT IN A LOCAL POPULATION EXPERIENCING RECURRENT MISCARRIAGE AND INTRAUTERINE DEATH

A McMahon[1,*], C Holt[1], D Keeffe[2]
[1]Haematology Laboratory, [2]Haematology, HSE, Limerick, Ireland

Background: It is widely reported that antiphospholipid syndrome (APS) is detectable in approximately 15% of women with recurrent miscarriage in the absence of any other abnormalities. The detection rate in our laboratory is much lower, at 1-2%. All screening is carried out according to international guidelines published by the Scientific Standards Committee of the International Society for Thrombosis and Haemostasis which caused us to question why the detection rate is so low. As lupus anticoagulant (LA) detection is the most complicated part of the APS screening process, with numerous tests and interpretation methods available, this became the focus of our investigation.
Aims: To investigate the use of alternative interpretation methods for dilute Russell's viper venom (dRVVt)-based screening tests, to investigate the use of integrated screening and confirmatory testing methods and to determine the usefulness of factor assays in LA screening, with the ultimate aim of improving detection of LA in our laboratory.
Methods: Factor assays for VIII, IX, XI and XII were carried out at serial dilutions- 1:10, 1:20, 1:40 and 1:80 on 50 patients. The results were than analysed for a pattern of inhibitory activity, *i.e.* presence of LA; and compared to the PTT-LA results from the routine LA screen. dRVVt and dRVV Confirm assays were carried out on 80 samples and interpreted using 3 different methods, namely the dRVVt/Confirm ratio, the normalised ratio and the calculation for the percentage correction of clotting time. The use of the dRVVt and Confirm together as an integrated test was also compared to the dRVVt alone as a screening test. All results were compared using Chi-Square analysis where a $P<0.05$ was considered statistically significant.
Results: Factor assays were found to be of no additional benefit to the LA screening process, as was the percentage correction of clotting time calculation for interpretation of the dRVVt and Confirm tests; both methods were shown to increase equivocal results. Integrated testing using the normalised ratio as the interpretation method of choice showed an improvement in sensitivity detecting three new weak-titre LAs. A more significant development occurred on further analysis of the data when it was noted that 15% of patients showed increased factor VIII activity, indicative of an acute phase, which one would expect to witness in pregnancy; the mean dRVVt was also lower for this study population than for the general population. This study population, though no longer pregnant appear to share characteristics with pregnant women in relation to their coagulation assays. If this is the case then these patients should not be compared to reference ranges derived from the general population but to reference ranges relevant to their own specific population. To investigate this discovery further new study population reference ranges were generated. Comparing our results to the new reference ranges produced one weak positive from the three previously detected. More significantly, the dRVVt for this sample was actually prolonged using the new reference range, therefore this LA would be detected without the use of integrated testing.
Summary and Conclusions: This study has shown for the first time that population-specific reference ranges increase the sensitivity of LA screening assays and may provide a more specific alternative to integrated testing, as illustrated by the dRVVt results.

P1069

PRACTICAL EXPERIENCE OF THROMBOPROPHYLAXIS AFTER LOWER LIMB REPLACEMENT–RIVAROXABAN VERSUS ENOXAPARIN

J Won[1,*], H Park[1], J Yun[2], K Kim[1], H Kim[3], S Kim[2], S Lee[3], H Kim[2], S Bae[3], C Kim[2], N Lee[1], K Lee[3], S Park[2], Dae2, Y Kim[1], N Ham[1]
[1]Soonchunhyang University Hospital, Seoul, [2]Soonchunhyang University Hospital, Bucheon, [3]Soonchunhyang University Hospital, Cheonan, Korea, Republic of Korea

Background: Since October 2010, rivaroxaban has been approved by Korean government to be covered by national health insurance reimbursement system as thromboprophylaxis after total hip arthroplasty and total knee arthroplasty. However, there is no available data on outcomes of rivaroxaban as thromboprophylaxis in Korea.
Aims: We performed a retrospective study to compare the efficacy and safety of venous thromboembolism prophylaxis with enoxaparin or rivaroxaban in 195 consecutive patients undergoing major orthopaedic surgery at our center.
Methods: This study retrospectively reviewed the medical records of the 195 patients who underwent a total hip replacement arthroplasty or total knee replacement arthroplasty and received thromboprophoylaxis with enoxaparin or rivaroxaban at Soonchunhyang University Hospital between March 2009 and May 2012. Each patient's medical records included information on age, sex, comorbidities (active malignant disease, renal insufficiency), treatment details (type of surgery, type of anesthesia, duration of surgery,), duration of prophylaxis, efficacy (death, pulmonary embolism, deep vein thrombosis), safety (major bleeding, cerebrovascular accident), cause of drug interruption.
Results: Of 195 patients, 129 patients received thromboprophylaxis with enoxaparin (group 1; our hospital standard since March 2009), 66 received rivaroxaban (group 2; our hospital standard since February 2011). Symptomatic venous thromboembolism was found in 0.7% of patients in the group 1 (1/129 patients) compared to 1.5% of group 2 (1/66 patients; P=0.627). No significant differences in the rates of symptomatic VTE were found. However, patients with received rivaroxaban had significantly more rates of major bleeding (0 in group 1 *vs* 3% (2/66 patients) in group 2; P=0.047). Although group 1 patients were planed receiving thromboprophylaxis with rivaroxaban from day one post operatively, mean time from the end of surgery to first rivaroxaban intatake was 4.2 days.
Summary and Conclusions: Despite lower compliance in rivaroxaban group, venous thromboembolism prophylaxis with rivaroxaban is not inferior to prophylaxis with enoxaparin with regard to the prevention of symptomatic venous thromboembolisms. But, more bleeding complications and wound problems revealed in rivaroxaban group. Further studies and experiences are needed to assess the efficacy and safety of rivaroxaban in clinical practice.

P1070

THROMBOSIS IN CHILDHOOD ACUTE LEUKEMIA

H Şahin[1], Y Yaman[2,*], G Özek[2], Ö Cartı[2], B Güneş[2], E Albudak[2], B Demirağ[2], A Yıldırım[3], Y Oymak[4], F Özkınay[5], U Karaarslan[1], C Vergin[1]
[1]Dr.Behçet Uz Children Hospital, izmir, Turkey, [2]Pediatric hematology, Dr.Behçet Uz Children Hospital, izmir, [3]Pediatric hematology, Diyarbakır Chil-

drens Hospital, Diyarbakır, [4]Pediatric hematology, Harran University Medical Faculty, Şanlıurfa, [5]Medical Genetics, Egean University Medical Faculty, izmir, Turkey

Background: Thromboembolism can occur during acute leukemia, especially acute lymphoblastic leukemia (ALL) treated with L-asparaginase and steroids and acute promyelocytic leukemia (AML M3). The presence of central venous lines, usage of Escherichia coli asparaginase, sepsis and hereditary thrombophilic abnormalities are risk factors for thrombosis in children.
Aims: To evaluate the risk of thrombosis in children with ALL and AML.
Methods: One hundred and ninety-seven consecutive patients with acute leukemia were recruited for this study from April 2005 to October 2010 retrospectively. Ages of patients at time of diagnosis, symptomatic thromboembolic events, type of leukemia, chemotherapy protocol, time of thromboembolic events during chemotherapy, site of thromboembolic events, hereditary and acquired risk factors for thromboembolic events had been examined from the patients' records retrospectively.
Results: One hundred seventy-five patients with ALL, 10 patients with AML M3 and 12 patients with non-M3 AML were detected. All were in treatment according BFM protocols; ALL BFM 95 and AML BFM 2004 protocols for ALL and AML patients, respectively. Sixteen patients of the 197 patients had tromboembolic events (8,1%). Fourteen of sixteen were patients with ALL, one with AML M3, one with non-M3 AML. Thromboembolic events were more common in follow-up of patients with high risk ALL (23,8%) than patients with standart and intermediate risk. A half (50%) of thromboembolic events were seen in patients who were under treatment for standart and intermediate risk ALL during the induction phase and reinduction phase of treatment. Site of thrombus were iliac vein, catheter tip, finger tip, arteria dorsalis pedis, spleen, femoral vein, renal and central nervous system. The use of central vein increased risk of tromboembolic events to 20,3%. Hereditary thrombophilic factors were positive in nine of patients with thrombosis. Factor V Leiden heterozygosity, MTHFR heterozygosity, prothrombin 20210 heterozygosity, Factor V Leiden heterozygosity+MTHFR heterozygosity were found in3,4,1,1 of these patients respectively. None of these patients were elevated activated protein C resistance and homocystein level of these patients were not evaluated. Three of the 16 patients died due to sepsis during thromboembolic events.
Summary and Conclusions: The incidence of thromboembolic event in patients with leukemia is not negligible. Guidelines for leukemia specific risk factors, thrombosis prevention and treatment strategies in acute leukemia patients are required.

P1071

PROTHROMBIN TIME AND ANTIPHOSPHOLIPID ANTIBODIES: THE NUMBER OF POSITIVE TESTS PREDICTS PROTHROMBIN TIME PROLONGATION

Marta Pereira[1,*], Gilberto Marques[2], Margarida Lourenço[2], Rosário Cunha[2], Fernando Rodrigues[2], Graça Ribeiro[2]
[1]Clinical Hematology Department, [2]Clinical Pathology Department, Coimbra University Hospitals, Coimbra, Portugal

Background: The presence of antiphospholipid antibodies (aPLA) - lupus-like anticoagulant (LAC), anti-Beta2-glycoprotein I (B2G) or anti-cardiolipin (aCL) - is one of the necessary criteria for the diagnosis of antiphospholipid syndrome (APS), a prothrombotic state. LAC prolongs the activated partial thromboplastin time (aPTT), *in vitro*; in contrast, in the majority of patients, LAC does not prolong the prothrombin time (PT) - this finding is clinically relevant, underlying the rationale of using PT (through the INR) to monitor anticoagulation in patients with APS, in whom PT results are assumed to reflect the effect of warfarin, and not an interference of the underlying disease. Nevertheless, some authors have noted a prolonged PT in a selection of patients with LAC; in these patients, it can be postulated that there is a risk of undertreatment with warfarin, with PT prolongation being in part due to an *in vitro* artifact, and not solely to the effect of treatment. The identification of patients with a prolonged PT ab initio will help optimize patient management and reduce the risk of undertreatment. The characterization of the subset of patients in whom aPLA associate with a prolonged PT is the first step towards this goal.
Aims: To determine whether the nature or the number of positive aPLA are determinants of PT prolongation.
Methods: We reviewed all lab requests from 01-01-2000 to 12-31-2012, selecting patients with simultaneous diagnostic PT, aPTT, LAC, G2B and aCL determinations (IgM and IgG considered together). Patients were grouped according to the type (LAC, B2G and aCL) and number of positive results; the mean PT and aPTT (normalized to control) were compared across the groups.
Results: Inclusion criteria were fulfilled by 1669 results. The difference between PT and control was 1.3±3.3s when all tests were negative, and increased to 2.1±3.9s, 3.4±6.3s and 4.4±7.3s when one, two or all three tests were positive (P<0.001). When only one test was positive, the difference was 1.6±4.3s with B2G, 1.7±2.7 with aCL and 2.6±4.2 with LAC (P=NS); when two tests were positive, the difference was 2.7±6.0 for B2G and LAC, 3.5±6.4 for B2G and aCL, and 3.5±6.6 for LAC and aCL (P=NS). The ratio of aPTT to control was 1.0±0.2 when all three tests were negative, increasing to 1.3±0.5, 1.4±0.6 and 2.4±1.3 when one, two or all three tests were positive (P<0.001). When only one test was positive, the ratio was 1.0±0.2 with B2G, 1.1±0.3 with aCL (P=NS) and 1.5±0.6 with LAC (P<0.001); when two tests were positive, 1.2±0.4 for B2G and aCL, 1.6±0.6 for aCL and LAC, and 2.0±0.7 for B2G and LAC (P<0.001).
Summary and Conclusions: Considering the three APS-associated tests (B2G, aCL and LAC), we found a significant increase in PT and aPTT with an increase in the number of positive tests. For aPTT, not just the number, but also the type of test that was positive influenced the ratio: single positivity for B2G or aCL was comparable to negativity for all tests, but positivity for LAC had the strongest impact on aPTT; two-positive-test combinations including LAC were also associated with higher aPTT ratios than the B2G-aCL combination. These findings are in accordance with the known mechanism of LAC interference on lab testing. On the other hand, for PT, we found that only the number of positive tests was significant, with no differences between test combinations; positivity for LAC did not impact on PT results. These results can contribute to our understanding of PT-prolongation in LAC-positive patients: our data suggests that increased PT, in these patients, could be due to simultaneous positivity for B2G and/or aCL. We propose that in patients with positivity for two or, especially, all three of the aPLA, special care should be taken to identify an ab initio increased PT, and INR results obtained during anticoagulation should be interpreted in its light.

P1072

ULTRASTRUCTURAL ANALYSES OF THE NOVEL CHIMERIC HEMOSTATIC AGENT GENERATED VIA NANOTECHNOLOGY, ABS NANOHEMOSTAT, AT THE RENAL TISSUE LEVEL

E Huri[1], I Haznedaroglu[2,*], M Hayran[3], M Ergun[3], A Firat[3], Y Beyazit[4], R Mammadov[5], M Guler[5], M Dadali[1], H Goker[2]
[1]Ankara Training and Research Hospital, Urology, [2]Hematology, [3]Anatomy, Hacettepe University Medical School, [4]Gastroenterology, YIH hospital, [5]Nanotechnology, UNAM, Ankara, Turkey

Background: Biomaterials used as tissue engineering scaffolds have specific physical properties and might form fibrous networks similar to collagenous extracellular matrix. They also can be programmed to carry chemical and physical cues to provide bioactivity for cell-materials interactions. In the search for more improved bioactive materials for tissue engineering purposes, peptide amphiphile (PA) molecules are good candidates to bring scaffold properties and bioactivity together.
Aims: We have generated a chimeric hemostatic agent, ABS Nanohemostat, via combining self-assembling peptide amphiphile (PA) molecules with the traditional ABS (Ankaferd Hemostat). The synthesis of the specific self-assembling peptide molecules capable of being a part of the combined ABS Nanohemostat compound and the assembly of the peptide nanofibers and ABS to generate the ABS Nanohemostat have already been published (Int J Biomaterials 2013, ID 949460, http://dx.doi.org/10.1155/2013/949460). The aim of this study is to assess renal tissue effects of the ABS Nanohemostat formed by the combination of self-assembling PA molecules and ABS.
Methods: Peptides were constructed on Rink Amide MBHA resin. Amino acid couplings were done with 2 equivalents of Fmoc protected amino acid, 1.95 equivalents HBTU and 3 equivalents of N,N-diisopropylethylamine (DIEA) for 2 hours. The PA was synthesized by Fmoc Solid Phase Peptide Synthesis (SPPS) method. It is composed of a lauryl (C12) group, hydrophobic region of the PA, and a peptide sequence. VVAG peptide sequence is used as β-sheet-inducer that causes nanofiber formation, while the lysine (K) residue is protonated at physiological pH and increase solvation of PA molecule in aqueous solution at pH 7. Renal artery and vein was revealed by hilar vascular dissection in 24 Wistar rats weighing 200 to 300 g. Subsequently renal artery and vein were clamped with Rommel vascular clamp. The lower third of the left kidney was resected in guillotine fashion with a single stroke of an amputating knife. Scanning Electron Microscopy (SEM) experiments were performed with FEI Nova NanoSEM 230, using the ETD detector at low vacuum mode with 30 keV beam energy to assess renal tissue alterations.
Results: SEM analyses revealed that significant erythroid aggregation are present inside the capillary bed of the renal tissue. However, neither the signs of necrosis nor any other sign of a tissue damage are not evident in the surrounding renal tissue supplied by those microcapillary vasculature. Furthermore, the appearance of the nucleus, cytoplasm of the vascular endothelial cells and their organelles are completely normal.
Summary and Conclusions: In previous investigations, histopathological examination of the damaged vascular structures revealed ABS-induced red blood cell aggregates supporting the hypothesis that ABS-induced formation of the protein network with vital erythroid aggregation covers the entire physiological hemostatic process (Critical Reviews in Oncology/Hematology 83 (2012) 21–34). We have observed the same structures in the kidney tissue in the present study via SEM analyses. In the current study, ABS Nanohemostat has lead to a more pronounced erythroid aggregation at the renal tissue level in comparison to the traditional ABS (Ankaferd hemostat).

P1073

MORTALITY OF PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND RELATION TO COAGULATION AND INFLAMMATORY BIOMARKERS

S Elmoamly[1,*], M Mattar[1], S Amin[2], M Yacoub[1]
[1]Internal Medicine & Hematology department, Faculty of Medicine, Cairo University, Cairo, [2]Clinical pathology department, Faculty of Medicine, Cairo University, Cairo, Egypt

Background: Patients with hematological malignancies often have a hypercoagulable state due to the production of substances with procoagulant activity. Also the role of inflammatory cells and pathways in the pathogenesis of cancer has become well established. Venous thromboembolism and inflammatory events are considered imortant causes of mortality in cancer patients. The impacts of vascular and inflammatory markers on the prognosis of hematological malignancies are still to be studied.
Aims: to study whether vascular and inflammatory biomarkers (as well as clinical variables) can be used as predictors of mortality in patients with hematological malignancies.
Methods: This study is a prospective observational cohort study; it was conducted on a group of 86 patients with malignant haematological conditions. They have been followed up for an average period of 314.45 days with an endpoint of mortality. **Exclusion criteria included** Overt bacterial or viral infection within the last 2 weeks, Venous or arterial thromboembolism within the last 3 months, Continuous anticoagulation with vitamin K antagonists or low molecular weight heparin (LMWH). Hypercoagulability and inflammation were assessed at the initiation of the study by measuring the circulating levels of the following parameters: Markers of coagulation and fibrinolysis activation (D-dimer, Fibrinogen, Thrombin, plasminogen activator inhibitor 1 [PAI-1]); Markers of endothelium and platelet activation (von Willebrand Factor [vWF], soluble P-selectin); and Markers of inflammation (Tumor necrosis factor alpha [TNF-α], Interleukin-6 [IL-6]).
Results: In our study, the mean age was 48.8 years. Our study included 38 (44.19%) female patients and 48 (55.81%) male patients. Out of 86 patients, 23 (26.74%) were diagnosed to have Lymphoproliferative disorders, 26 (30.23%) were diagnosed to have Myeloproliferative neoplasms, 19 (22.09%) were diagnosed to have AML (or MDS progressed to AML), 9 (10.47%) were diagnosed to have ALL, 9 (10.47%) were diagnosed to have Paraproteinaemias. Thirty two (37.21%) patients died during follow up. Twenty four (75%) patients died within 6 months after diagnosis. Eighteen patients (56.25%) died of disease progression, 7 patients (21.88%) died of infection, 4 patients (12.5%) died suddenly with suspected pulmonary embolism, 1 patient (3.13%) died of each of heart failure, bleeding, liver cell failure. There were statistically significant associations between mortality and ECOG performance status (P value: 0.001), duration of hospital stay (P value: 0.006), platelet count (P value: 0.033), transfused blood units (P value: 0.002), transfused platelet units (P value: 0.04), PTT (P value: 0.014), serum albumin (P value: 0.004), total bilirubin (P value: 0.007) Antithrombin (P value: 0.016), soluble P-selectin (P value 0.038), vWF (P value: 0.009), IL-6 (P value: 0.042). For prediction of mortality, ROC Curve of Albumin level showed that a level of 3.35 g/dl showed the highest likelihood ratio (LR) of 2.04 with sensitivity of 60% and specificity of 72.5%. For prediction of mortality, ROC Curve of total bilirubin level showed that a level of 0.58 mg/dl showed the highest likelihood ratio (LR) of 1.67 with sensitivity of 65.6% and specificity of 60.8%. ROC Curve of Antithrombin level showed that a level of 16.25 mg/dl showed the highest likelihood ratio (LR) of 1.8 with sensitivity of 61.3% and specificity of 66%. ROC Curve of soluble P-selectin level showed that a level of 28.28 ng/mL showed the highest likelihood ratio (LR) of 1.8 with sensitivity of 61.3% and specificity of 66%. ROC Curve of vWF level for showed that a level of 2.525mU/mL showed the highest likelihood ratio (LR) of 1.9 with sensitivity of 61.3% and specificity of 68%. ROC Curve of IL-6 level showed that a level of 3.35pg/mL showed the highest likelihood ratio (LR) of 1.62 with sensitivity of 61.3% and specificity of 62.3% (Figure 1).

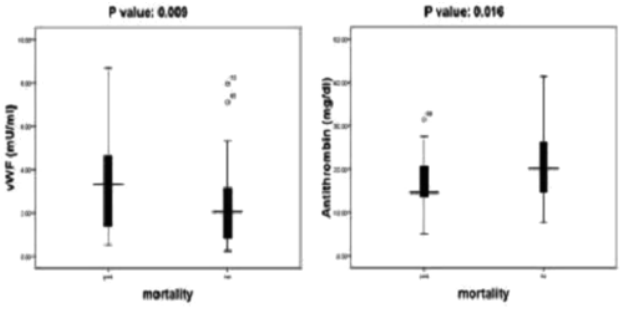

Figure 1. Relation between coagulation and inflammatory biomarkers and mortality in hematological malignancy.

Summary and Conclusions: our study concluded that initial levels of Albumin below 3.35g/dl, Total bilirubin above 0.58 mg/dl, Antithrombin below 16.25 mg/dl, soluble P-selectin below 28.28 ng/mL, vWF above 2.525mU/mL and IL-6 above of 3.35pg/mL are associated with poor outcome with increased mortality. these biomarkers can be included in a larger pridective model for mortality in patients with hematological malignancies

P1074

EPCR SER219GLY POLYMORPHISM AND SUSCEPTIBILITY TO VENOUS THROMBOEMBOLISM IN THE POPULATION OF NORTH-WESTERN RUSSIA

S Kapustin[1,*], A Demyanenko[2], V Kobilyanskaya[1], J Sidorova[1], T Morozova[1], A Polyakova[1], P Chechulov[2], N Saltykova[1], V Kargin[1], V Soroka[2], V Shmeleva[1], L Papayan[1]
[1]Russian Research Institute of Haematology and Transfusiology, [2]Emergency Research Institute, Saint-Petersburg, Russian Federation

Background: Venous thromboembolism (VT) is a multifactorial disorder. The genetic basis of predisposition to VT is not fully understood. Endothelial protein C receptor (EPCR) plays an important role in protein C anticoagulant pathway. An amino acid change Ser219Gly is associated with increased plasma levels of soluble EPCR and can affect thrombin degradation rate by activated protein C. The data on the role of EPCR Ser219Gly substitution in susceptibility to VT are still scarce.
Aims: To assess the role of EPCR Ser219Gly polymorphism in susceptibility to VT in the population of the North-Western region of Russian Federation.
Methods: We examined 300 patients with VT (147 men and 153 women, mean age 39.9±12.4 years) and 172 age- and sex-matched healthy controls (HC). In 200 patients, the first episode of VT was diagnosed at young age (before 45 years of old). All individuals originated from the North-Western region of Russia and gave informed consent for participation in the study. Allelic variants of the EPCR gene corresponding to Ser219Gly polymorphism were discriminated by PCR-RFLP method. The differences in genotype distributions between the groups were estimated by Fisher`s exact test. Odds ratios (OR) with their 95% confidence intervals (CI) as well as P-values were calculated by using GraphPad Prism software, version 4.0.
Results: The EPCR Gly219 variant was more frequently detected in the VT group (25.7% *vs.* 18.6% in controls, OR=1.5, 95% CI: 0.9-2.4, P=0.09). Notably, both hetero- and homozygosity for the EPCR Gly219 were more prevalent in patients than in HC, although not statistically significant (24.0% *vs.* 18.0%, and 1.7% *vs.* 0.6%, respectively). In the group of patients under 45 years of old, the frequency of individuals positive for the EPCR Gly219 variant was 2-times higher than in those with late-onset VT (31.0% *vs.* 15.0%, respectively, OR=2.5, 95% CI: 1.4-4.8, P=0.003). When comparing to HC, we found an increased risk of early-onset VT development in persons having EPCR Gly219 (OR=2.0, 95% CI: 1.2-3.2, P=0.008). Moreover, this variant of EPCR was present in 52 (33.5%) out of 155 young patients without factor V (G1691A) and factor II (G20120A) gene mutations (OR=2.2, 95% CI: 1.3-3.7, P=0.002, compared to control group).
Summary and Conclusions: Our data suggest that the EPCR Gly219 variant could independently increase the risk of VT development at young age in the population of North-Western Russia.

P1075

POLYMORPHISM OF VASCULAR TONE REGULATING GENES AND THE RISK OF VENOUS THROMBOEMBOLISM IN INDIVIDUALS WITH INHERITED THROMBOPHILIA

A Polyakova[1,*], V Shmeleva[1], V Soldatenkov[1], N Saltikova[1], V Kargin[1], M Blinov[1], S Kapustin[1]
[1]Russian Research Institute of Hematology and Blood Transfusion, Saint-Petersburg, Russian Federation

Background: Venous thromboembolism (VT) is one of the most actual multifactorial diseases in the world. Genetic predisposition plays a significant role in pathogenesis of VT. Mutations in the factor II (FII G20210A) and factor V (G1691A, FV Leiden) genes are the most frequent inherited risk factors for VT and could be detected in about 9% and 20% of VT cases, respectively, in the population of North-Western Russia. Endothelial dysfunction is an important mechanism underlying thrombosis, and it frequently occurs as a result of imbalance between vasoconstriction and -dilatation processes. Variations in the genes coding for components of the renin-angiotensin system (RAS) and endothelial NO-synthase (eNOS) can lead to changes in their structure and/or functional activity and modulate the risk of VT.
Aims: To investigate the role of angiotensinogen (AGT), angiotensin II receptor type 1 (AGTR1), angiotensin-converting enzyme (ACE) and eNOS genes polymorphism in the development of VT at young age in individuals with inherited thrombophilia.
Methods: Retrospective study involved 181 patients with early-onset VT (mean group age 34,0±8,6 years) and 156 sex- and age-matched healthy controls (HC). All individuals originated from the North-Western region of Russia and gave informed consent for participation in the study. Variations in the FII

(G20210A), FV (G1691A, Leiden), ACE (Ins/Del), AGT (T704C, Met235Thr), AGTR1 (A1166C) and eNOS (T−786C) genes were discriminated by PCR-RFLP method. The differences in genotype distributions between groups were estimated by Fisher`s exact test.
Results: The distributions of alleles and genotypes of the vascular tone regulating genes in patients without known inherited risk factors, as well as in those having FII G20210A mutation were not significantly different from HC. At the same time, the positive association between the FV G1691A and eNOS −786CC genotypes was observed in the VT group (OR=3,2; 95% CI:1,3-7,5; P=0,01). Homozygosity for the eNOS −786C allele was more frequently seen among carriers of FV Leiden mutation than in patients with normal FII and FV genotypes (29,7% *vs.*13,1%, respectively, P=0,024). The "unfavorable" variants of the RAS genes were also over-represented in patients with FV Leiden. In particular, the simultaneous presence of the ACE Del/Del and AGT 704CC genotypes was almost 5-times more frequently seen in these individuals than in patients with normal FV and FII genotypes (15,2% *vs.*3,1%, respectively, P=0,018) Interestingly, neither the eNOS −786CC variant nor the "ACE Del/Del-AGT 704CC" combination was detected in VT patients having FII G20210A mutation and normal FV genotype.
Summary and Conclusions: We suggest that polymorphism of the vascular tone regulating genes can affect the imbalance between vasoconstriction and vasodilatation processes and play a provocative role in the development of early-onset VT among patients with FV Leiden variant.

P1076

PROPHYLAXIS FOR VENOUS THROMBOEMBOLISM IN PATIENTS TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA–A SYSTEMATIC REVIEW

M Lauw[1,2,*], L Hubers[2], S Barco[2], C Van Ommen[3], B Hutten[4], B Biemond[1], S Middeldorp[2]
[1]Dept. of Hematology, [2]Dept. of Vascular Medicine, Academic Medical Center, [3]Department of Pediatric Hematology, Emma's Childrens Hospital/Academic Medical Center, [4]Department of Clinical Epidemiology & Biostatistics, Academic Medical Center, Amsterdam, Netherlands

Background: Venous thromboembolism (VTE) occurs frequently in patients with acute lymphoblastic leukemia (ALL). Reported incidences vary between 2 and 36%. Occurrence is often associated with treatment components, particularly L-asparaginase. Efficacy and optimal approach of VTE prevention during ALL treatment are unclear.
Aims: To investigate the efficacy and safety of systemic thromboprophylaxis, using blood-derived products, *i.e.* fresh frozen plasma, cryoprecipitate or antithrombin concentrate, or anticoagulant agents, *i.e.* (low-molecular-weight) heparin, fondaparinux or oral anticoagulants (vitamin K antagonists), on VTE incidence in pediatric and adult patients treated for primary ALL with L-asparaginase therapy. Also, impact of thromboprophylaxis on overall survival and treatment outcomes of ALL were investigated.
Methods: We systematically searched The Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*, Issue 7 2012), MEDLINE (January 1966 to August 2012; accessed via Pubmed) and EMBASE (January 1980 to August 2012; accessed via OVID). We handsearched conference proceedings and checked references of included studies. All randomized controlled trials (RCTs) that assessed the efficacy and safety of systemic thromboprophylaxis in patients treated for primary ALL with L-asparaginase therapy were eligible. Interventions included any dose of the above mentioned blood-derived products or anticoagulants, in comparison with no intervention or placebo, or a comparison of two different interventions. Three authors independently assessed eligible articles and systematically extracted the data from selected articles. Discrepancies were resolved by discussion or with the opinion of a fourth author. Risk of bias, quality of evidence, potential heterogeneity and reporting biases were explored.
Results: Of 304 identified citations, 44 articles were selected for full-text evaluation. Cross-referencing of articles yielded another 20 articles. Finally, one RCT enrolling 109 patients fulfilled our inclusion criteria and was analyzed for our review. This study assessed a randomization between antithrombin concentrate infusions (once weekly for 4 weeks) and no intervention in children treated for primary ALL. Outcomes were symptomatic and asymptomatic thrombosis (by radiographic screening following completion of the induction phase), and bleeding events. 7 of 25 analyzed children with antithrombin had thrombosis (28.0%) *versus* 22 of 60 patients without antithrombin (36.7%; OR 0.67; 95% CI 0.3-2.3, P=0.43). One major bleeding event (1.7%) occurred in the non-antithrombin arm, *versus* no major but two minor bleeds in the antithrombin arm. Impact of thromboprophylaxis on survival or ALL treatment outcomes was not assessed.
Summary and Conclusions: Only one RCT in children with ALL was identified, with a high risk of bias due to an open-label design and incomplete outcome data as a result of a per protocol analysis. In this study, no statistically significant effect of antithrombin infusions was seen on the outcomes of interest. However, the sample size was small with a skewed randomization ratio, and may have missed a clinically important effect. No RCTs were found addressing other blood-derived products or anticoagulants. Therefore, the efficacy and safety of thromboprophylaxis to prevent VTE during ALL treatment remain unclear. The use of thromboprophylaxis during ALL treatment, in particular during L-asparaginase therapy, needs to be assessed in randomized controlled trials.

P1077

THROMBOPHILIAS INCIDENCE IN PATIENTS WITH FETAL LOSS IN LAST TRIMESTER AND EVALUATION OF THE EFFECTIVENESS OF TREATMENT

L Martínez[1,*], R Herrero[1], R Martínez[1], I de Torres[2], B Sierra[1], C Cornacchia[1], J Hernández[1], A Mateo[1], S Castillo[1], RSantamaria[3], J Martín[1]
[1]Hematology, [2]Clinical Analysis, [3]Gynecology, General Hospital of Segovia, Segovia, Spain

Background: Up to 5% of women of reproductive age, have >2 fetal losses, one of the most common causes of female infertility. Several studies identify thrombophilia as a major cause of these events.
Aims: We set as our primary objective to analyze the incidence of thrombophilia in a population of women who had pregnancies with fetal deaths (defined as a gestational age>20 weeks). Secondly, we evaluate both the presence of hereditary thrombophilia(HT), as the existence of acquired variations, to estimate the influence of both hypercoagulable states over the course of pregnancy. Finally correlate the use of low molecular weight heparin(LMWH) with gestational viability in these patients.
Methods: Retrospective January1999/December2011 taking as its starting point the existing number of stillbirths during this period in the Gynecology Service of the Hospital of Segovia. Mothers who were evaluated after the event came to the Hematology Service to perform hypercoagulability´s study. Collect the incidence of HT, acquired or absence of these alterations in coagulation studies. In parallel we analyzed the characteristics of obstetric patients for the presence of abortions, bleeding events during birth and number of live births. Finally the women were assessed in held LMWH prophylaxis and treatment, collecting potential medication side effects and the success of this therapy.
Results: During the study period, we collected a total of 97 patients with at least one pregnancy, in which the result was a dead fetus, performing hypercoagulability in 50 patients (51,5%). The average age of women at the time of the event was 33 years (range: 20-42). 38% of these patients (n=19) had suffered a previous abortion, distributed by episodes: 1 abortion (n=13;68%), 2 abortions (n=4;21%), 3 abortions (n=1;5%) and 5 (n=1;5%). Stresses that 36% (n=18) of women in the study had at least one living child prior to the event, not objectified in neither case malformations in children born. Regarding coagulation studies, 64% (n=32) were diagnosed with various syndromes of hypercoagulability (SH). The percentage distribution of final diagnoses are shown in Table 1. Since one of the most common diagnoses in the population was the mutation at the gene level of homocysteine(C-677-T), we performed a subanalysis in which we evaluated the mean serum levels of homocysteine in patients with a homozygous mutation *vs.* heterozygous, no statistically significant differences (8,61*vs.*7,96;P>0.5). 78% patients (n=25) diagnosed with SH, were treated with LMWH and folic acid as needed. In all cases we chose Bemiparin for easy administration (once daily).Close monitoring was performed during pregnancy and postpartum by measuring anti-Xa and platelet count to detect possible side effects. The mean platelet count at baseline was 270,000/mm³(range:135-402), and monitoring functions at3, 6 and 9 months (mean: 252, 243 and 207 respectively) demonstrated the absence of thrombocytopenia. Of all patients with SH 78%(n=25) had a pregnancy after the event, when assessed by subgroups was observed that 92%(n=23) of patients treated getting a viable fetus compared to 28%(n=2) of the untreated group.

Table 1.

DIAGNOSTIC	CASE NUMBER	% TOTAL
MUTATION HOMOCYSTEINE HOMOZYGOUS	13	41%
MUTATION HOMOCYSTEINE HETEROZYGOUS	3	10%
FACTOR V LEIDEN MUTATION	2	6%
ANTIPHOSPHOLIPID SYNDROME	2	6%
PLASMINOGEN DEFICIT	1	3%
MIXED PATTERNS	11	34%
—	N=32	100%

Summary and Conclusions: The incidence of fetal deaths in our population is similar to that reported in the literature. The most common types are the inherent SH and combined. Hypercoagulability studies identify patients with high-risk pregnancies, candidates for the implementation of therapeutic strategies. The use of LMWH in these patients achieved pregnancy with good fetal viability, with success rates similar to those reported.

P1078

IS THERE AN ASSOCIATION BETWEEN ABO BLOOD GROUP AND MICROANGIOPATHIC HEMOLYTIC ANEMIA?

Z Mozaheb[1,*]
[1]hematology, mashhad university of medical science, mashhad, Iran, Islamic Republic of Korea

Background: Bachground: Several studies have reported the relationship between ABO blood groups and thrombosis and hemorrhagic disorders, most of them showing that non-O blood groups have a high risk for thrombosis and O blood groups have a high risk for hemorrhage. However, there are no studies about the relationship between the microangiopathic hemolytic anemia (MAHA) and the ABO blood groups.
Aims: Due to the different risks for hemorrhage and thrombosis in relation to ABO blood groups in different studies, in this study we evaluate the relationship between ABO blood groups and MAHA.
Methods: A prospective case-control study was conducted in the ICU centers of the Hasheminejhad and the Imam Reza Hospital of the Mashhad University of Medical Science in Mashhad, Iran between May 2011-Desember 2012. Patients admitted to the ICU with different etiology showed symptoms and signs of microangiopathic hemolytic anemia. There were 80 patients (age: 20-70 years) and 100 controls in this study. Controls were selected at random from a laboratory.
Results: In this study, we show that there is a significant difference in all blood groups between patients with microangipatic hemolytic anemia and the control group (P. value=0.009), with a significant difference between O and non-O blood groups (P. value=0.023).
Summary and Conclusions: previous studies confirm the historical linkage between some vascular disorders and non-O blood group status. In this study we show the significant relationship between microangiopathic hemolytic anemia and non-O blood groups; therefore, we recommended non-O blood group consider as a risk factor for this group of disease.

P1079

THE NOVEL NOX INHIBITOR 2-ACETYLPHENOTHIAZINE IMPAIRS COLLAGEN-DEPENDENT THROMBUS FORMATION IN A GPVI-DEPENDENT MANNER

D Vara[1,*], M Campanella[2], G Pula[1]
[1]Pharmacy and Pharmacology, University of Bath, Bath Spa, [2]Department of Veterinary Basic Sciences, Royal Veterinary College, London, United Kingdom

Background: Besides classical agonist-induced signal transduction pathways, platelet activation is also regulated by reactive oxygen species (ROS). In particular, superoxide ions from exogenous and endogenous sources increase collagen-dependent aggregation and thrombus formation. NADPH oxidases (NOXs) play a critical role in the generation of superoxide ions in platelets and contribute to platelet activation, although their mechanism of action remains largely unknown. Therefore, NADPH inhibitors may represent novel potential candidates for the development of anti-platelet agents.
Aims: In this project, we studied the effect of the novel NOX inhibitor 2-acetylphenotiazine (2-APT) on human platelet functional responses and intracellular signalling pathways.
Methods: The generation of superoxide ions was assessed by single cell imaging on adhering platelets using dihydroethidium (DHE), while cumulative ROS generation was detected with 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (CM-H2-DCFDA). Whole blood thrombus formation, washed platelet aggregation, integrin αIIbβ3 inside-out signalling, Syk phosphorylation, and protein kinase C (PKC) activation were analysed to understand the functional consequences of NOX inhibition by 2-APT in platelets.
Results: Superoxide ion generation stimulated by platelet adhesion on collagen and fibrinogen was significantly inhibited by 2-APT in concentration-dependent manner within the submicromolar range, whereas this pharmacological agent did not affect cumulative ROS generation. 2-APT impaired washed platelet aggregation in response to collagen but not thrombin and abolished whole blood thrombus formation stimulated by collagen but not fibrinogen. The activation of integrin αIIbβ3 and protein kinase C in response to the GPVI-specific agonist collagen-related peptide (CRP) was significantly reduced, whereas the same responses to thrombin were not significantly affected by 2-APT. Finally, Syk activation in response to collagen but not thrombin was inhibited by 2-APT, which suggests a stimulatory role for NOX-generated superoxide ions in the early events of the signalling cascade of GPVI.
Summary and Conclusions: Taken together, our results suggest that 2-APT attenuates GPVI-specific signalling and is a novel inhibitor of collagen-induced platelet activation. Therefore, 2-APT can represent a novel candidate for the development of anti-thrombotic drugs and NOXs are promising new targets for anti-thrombotic drug discovery.