Haemostasis

Cod: 0722

ANTI-PLATELETS ACTIVITY IN AFRICAN WOMEN ON PROGESTIN INJECTABLE CONTRACEPTIVES

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BACKGROUND: The usage of various contraceptives are on the rise globally, especially in African women. The various consequences of contraceptives usage on socio- economics, psychological, epidemiological, cellular and possible genetics effects are well documented in developed world constrary to Africa. The role of platelets and Anti- platelets activity in haemostasis are extentively researched in cacusian women. Literatures are very scanty on the effects of hormonal contraceptives on Anti- platelets activity in health and diseases in Africa. This research work is aim at investigating the effects of progestin injectable contraceptive and oral contraceptive pills on Anti- platelates activity, platelates count, and plasma fibrinogen concentration.What effects doec it have on these parameters on long trem usage on African women.

METHODS: A total of 300 apparently healthy female subjects were used for this study{100 Control, 100 Progestin injectables contraceptives[PIC], 100 Oral contraceptives pills[OCP]}, Platelets Count{PC}, Plasma Fibrinogen Concentration{PFC} and Anti-Platelets Activity estimated using Platelets Factor 3 Availability{PF-3}. The various parameters were estimated using regerence methods

RESULTS: We observed a significant decrease in PC {P<0.005} and a significant increase in PF-3 and PFC{P<0.005} between controls and PIC, OCP respectively. The results also shows a significant increase in PF-3{P<0.005} between PIC and OCP. However, there was no significant difference in PC and PFC between PIC ans OCP. We also observed a cummulative increase in PF-3 and PFC with increase in age and duration of usage in PIC and OCP users.

CONCLUSIONS: The usage of both OCP and PIC has a possible an Anti-platelets activity, thrombocytopenia and hyperfibrinogenaenia effects, particularly on the long term on African women

Haemostasis

Cod: 0723

COMPARISON OF CALIBRATED CHROMOGENIC ANTI-XA ASSAY (DIXAI) FOR RIVAROXABAN (RXA), FIRST ANTI-XA DOAC, WITH A MODIFIED CALIBRATED CHROMOGENIC ANTI-XA ASSAY (DIXAI.L+H) AND WITH A ROUTINELY USED ANTI-XA ASSAY FOR LMWH (AXA) AS SURROGATE MARKER FOR RXA

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BACKGROUND: Measuring RXA was offered originally by DiXaI assay (Biophen DiXa-I®, Hyphen, France). Douxfils et al. have demonstrated that results of DiXaI <100 ng/ml compared with mass-spectrometry showed no good correlation (r^2= 0.83). They recommend the use of DiXaI only in RXA >30 ng/ml (Thromb Haemos 2013;110). Recently we could investigate a modified DiXaI assay (DiXaI.L+H) using an own standard curve for RXA levels ≤100 ng/ml plasma. Routine monitoring of RXA is not necessary, therefore DiXaI is not easily available when needed in urgent situations, such as stroke or bleeding. Hence, the capability of a routine AXa assay for LMW-Heparin (Biophen, Heparin, Hyphen, France) as "surrogate marker" for RXA was evaluated in comparison with the DiXaI and the DiXaI.L+H assay.

METHODS: 39 plasma samples (RXA 0 - 481 ng/ml) of patients under and after withdrawal of RXA were included and were analysed on BCS XP (Siemens, Germany) using DiXaI, DiXaI.L+H (research only assay; standards& controls were kindly supplied by Hyphen, France) and AXa assay. Spearmans nonparametric correlation was calculated for all samples and also for two groups RXA plasma levels ≤100 ng/ml (n=25, lower detection limit (<DL) <1ng/ml) and >100 ng/ml (n=14) by DiXaI.L+H assay, respectively.

RESULTS: Correlation of DiXaI with DiXaI.L+H and AXa as well as DiXaI.L+H with AXa was r^2=0.9282, r^2=0.9016 and r^2=0.9653, respectively. Correlation of DiXaI with DiXaI.L+H and AXa and DiXaI.L+H with AXa calculated for RXA ≤100 ng/ml and >100 ng/ml* was r^2=0.7295 (r^2=0.9868*), r^2=0.6682 (r^2=0.7503*), r^2=0.9102 (r^2=0.7569*), respectively ($p<0.05$). Five of 25 AXa tests were <DL; one was <DL by all tests, 4 were positive by DiXaI.L+H and two by DiXaI. This is also reflected in the poor correlation of DiXaI.L+H with DiXaI (RXA <100 ng/ml). AXa however, correlated only well in samples <100 ng/ml with DiXaI.L+H (but losing 4 RXA positive samples).

CONCLUSIONS: Correct detection of low RXA levels is mandatory for an interventional stroke therapy, but is limited by AXa assay as "surrogate marker" and also by DiXaI assay. A positive AXa assay result however, gives a fast and sure indication for RXA blood levels. Modified DiXaI.L+H test seems to offer an improvement for this purpose.

Haemostasis

Cod: 0724

MULTIVARIATE MODEL FOR PREDICTING TRANSFUSION OF ALLOGENEIC BLOOD PRODUCTS IN ADULTS UNDERGOING ORTHOTOPIC LIVER TRANSPLANTATION. PRELIMINARY RESULTS

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BACKGROUND: Liver transplantation (LT) is a surgical procedure that can lead to massive blood loss and consequently result in transfusion of blood products. The evidence suggests that the use of blood products during OLT is associated with morbidity and mortality, and has been identified as an independent risk factor for adverse postoperative outcomes. The primary objectives of our study were to identify preoperative predictors of blood products transfusion in LT, and to develop a risk index to predict blood products transfusion in LT.

METHODS: We performed a retrospective, observational study. A hundred and twenty five LTs were included during the study period. The following variables were recorded for each patient: age; thromboelastometry´s variables (CT (clotting time); A10 (amplitude clot after 10 minutes); CFT (clotting formation time); MCF (maximum clot of firmness); alpha); INR (international normalized ratio); aPTT (activated partial thromboplastin time); Fg (fibrinogen); RBC (red blood cells); Hb (hemoglobin) and platelets. Independent predictors of blood products transfusion were identified by multivariable logistic regression analysis. We have developed a risk index of blood products transfusion with the total of the quartile´s value and the diagnostic performance was established by calculating area under the ROC curve, sensitivity, specificity, and 95% confidence intervals (CI).

RESULTS: Multivariable logistic regression analysis revealed that CT (OR=1.036; IC 95%, 1.003-1.069; p=0.030); A10 (OR=0.765; IC 95%, 0.612-0.956; p=0.018); MCF (OR=1.275; IC 95%, 1.012-1.605; p=0.039); Hb (OR=0.942; IC 95%, 0.894-0.992; p=0.024) were associated with an overall risk of transfusion. We obtained an area under the ROC curve of 0.77, 95% IC (0.68-0.84; p<0.001) a sensitivity of 76.6%, 95% IC (66.7%- 84.7%) and a specificity of 65.4%; 95% IC (44.3% - 82.8%).

CONCLUSIONS: This index showed sufficient sensitivity and specificity to predict which patients would require a transfusion and, as a result, the use of index will enable optimization of hospital blood product resources.

Haemostasis

Cod: 0725

PROFILE AND PREVALENCE OF LABORATORY ASPIRIN RESISTANCE IN ALGERIEN PATIENTS: COMPARISON OF THE PLATELET FUNCTION ANALYZER PFA-100 WITH OPTICAL AGGREGOMETRY

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BACKGROUND: Aspirin provides satisfactory protection against thrombotic episodes. However, clinical and laboratory evidence demonstrates diminished or no response to aspirin in considerable fraction of the patients that is called aspirin resistance.We determined the prevalence and factors associated with aspirin resistance among Algerians patients. We also compared the detection of aspirin resistance with optical platelet aggregation, a widely accepted method, with a newer, more rapid method, the platelet function analyzer (PFA-100).

METHODS: The study was performed on a sample of 200 adult patients who were taking aspirin but no other antiplatelet agents. Blood samples were analyzed in a blinded fashion for aspirin resistance by optical aggregation arachidonic acid, and by PFA-100 using collagen /epinephrine (CEPI) to measure aperture closure time (CT). Possible associations between aspirin resistance and the different variables were analyzed by chi-squared test and checked by a logistic regression analysis. The Spearman correlation coefficients and kappa statistics and the respective p-values were calculated to assess correlation and agreement between the two tests.

RESULTS: Prevalence of aspirin non responders measured by PFA-100 and by optical aggregation was 48 % and 45.5% respectively. The results exhibited higher agreement between the 2 tests (K = 0,98 P< 0, 000001) and very good correlation was found (r = 0,891 P< 0, 000001).There were no differences in aspirin sensitivity by Age, sex, doses of aspirin, ABO blood group, thrombocytosis, hypertension, diabetes, renal disease, treatment with heparin and statin therapy but a strong association was found between non-response to aspirin and smoking status by PFA-100 (OR=9,56 CI = 4,40 to 20,74, p<0.000001) and also by arachidonic acid-induced aggregometry (OR=7,47, CI=3,70 to 15,10, p<0.000001).

CONCLUSIONS: The prevalence of aspirin resistance is high in the Algerian population. It occurs more frequently in smokers.The PFA-100 system appears to be a reliable and rapid method in the assessment of aspirin's antiplatelet effect in patients than aggregometry which is labor-intensive and time-consuming.

Haemostasis

Cod: 0726

PRECISION STUDY OF A LATEX-ENHANCED TURBIDIMETRIC IMMUNOASSAY FOR D-DIMER

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BACKGROUND: Many studies used D-dimer cut-off value of 500 ng/mL for pulmonary embolism or deep vein thrombosis. We wanted to confirm the efficiency of our in house laboratory assay around this decision concentration.

METHODS: Our method was a latex-enhanced turbidimetric immunoassay HemosIL D-Dimer on ACL TOP (Italy) automated analyzer. We evaluated the precisions for different concentrations. We used the manifacturer supplied D-dimer reference material with a concentration of 3370 ng/mL. We prepared dilutions from this level. We tested the within-run coefficients of variations (CV's) according to EP5A2 protocol. We also calculated the limit of blank according to EP 17-A protocol.

RESULTS: The within-run CV's were found as 3.14 %, 3.01 %, 4.03 %, 10.2 %, 42.7 %, and 47.2 % for 3585, 1712, 839, 375, 86.8 and 52.4 ng/ml concentrations respectively. Limit of blank of the assay was found as 25 ng/ml.

CONCLUSIONS: The allowable total error (TEA) opposed by CLIA 2014 for D-Dimer was 28%. This study demonstrates that D-Dimer HS assay below 375 ng/mL showed a bigger random error than allowed even in the first step of method evaluation. Altough this may not contribute a problem to clinical decision, D Dimer test results should be reported carefully after evaluating total analytical error of any system and compairing ıt with TEA.

Haemostasis

Cod: 0727

ACQUIRED RESISTANCE TO ACTIVATED PROTEIN C IN THE ELDERLY WITH AND WITHOUT COGNITIVE IMPAIRMENT

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BACKGROUND: A prothrombotic state has been reported as an important factor in exacerbating the neurodegenerative process. Thrombin generation assays (TGA) have been widely used to assess the hemostatic status due to procoagulant and anticoagulant forces, while the test of resistance to activated protein C (aPC) based on the generation of thrombin identifies individuals at risk for thrombotic events due to acquired or genetic factors that affect the aPC pathway.

METHODS: In this context , the present study aimed to evaluate the hemostatic system of elderly individuals with and without cognitive impairment, with a focus towards aPC by using the TGT (CAT - Calibrated Automated Thrombograph). From the results obtained and described as ETP (Endogenous Thrombin Potential) it was calculated the nAPCsr index (Activated Protein C Sensitivity Normalized Ratio) to assess the level of acquired resistance to aPC in older adults with mild cognitive impairment (MCI, n = 55) and with probable Alzheimer's disease (AD, n = 65), compared to those without cognitive impairment (controls, n = 30). TGT was performed in the presence and absence of APC. nAPCrs ranges from 0 to 10, and increases with the resistance level of the sample.

RESULTS: After analyzing the data for nAPCsr it was possible to observe a much more evident acquired resistance to the aPC in elderly people with MCI and AD, compared to the control group. Considering as resistant those individuals with nAPCsr > 2, from 55 elderly patients with MCI, 25 (45.4%) were resistant to aPC, whereas from 65 with AD, 19 (29.2%) were resistant. For the control group of 30 elderly people without cognitive impairment, only 3 (10%) had nAPCsr > 2 . FV Leiden did not explain the increased values of this index, as only two cases were detected, one in the control group and one in the group with MCI .

CONCLUSIONS: It was concluded that older adults with cognitive impairment, compared to controls, displayed a clear picture of acquired resistance to aPC, which indicates a hypercoagulable state, a condition that may be contributing to cognitive decline and dementia.

Haemostasis

Cod: 0728

ASSOCIATION BETWEEN THE RESPONSE TO ACETYLSALICYLIC ACID AND PLATELET ACTIVATION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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BACKGROUND: Type 2 diabetes mellitus (DM2) is a metabolic disorder associated with hyperactivation of platelets and increased formation of platelet microparticles (PMP) that are related to the development of atherosclerosis in DM2. Acetylsalicylic acid (ASA) is an antiplatelet agent used in the prevention of atherothrombotic events by inhibition of platelet cyclooxygenase-1, blocking the formation of thromboxane A2. The aim of this study was to evaluate the effect of ASA by means platelet activation in patients with DM2 using ASA for primary prevention of atherothrombotic events.

METHODS: We collected blood samples of 81 patients with DM2 in two distinct moments, the first immediately prior to initiation of treatment with ASA and the second, at the fifteenth day of treatment with 100 mg of this medication daily. These samples were analyzed to determine the plasma levels of 2,3-dinor-thromboxane B2 (2.3 dinorTXB2) and PMP. Plasma levels of 2,3-dinor-TXB2 were quantitatively measured using a commercially available enzyme immunoassay and to isolation and quantification of PMP was used the flow cytometric technique. Data were analyzed by Wilcoxon test and p value less than 0.05 was considered significant.

RESULTS: A significant decrease ($p < 0.001$) was observed in the levels of 2,3-dinor-TXB2. However, no significant difference ($p = 0.403$) was found between the % PMP before and during the use of ASA.

CONCLUSIONS: This results may be attributed to high levels of PMP in diabetics patients and, moreover, it is important to emphasize that different from 2,3-dinor-TXB2 (marker of platelet activation), PMP can also be formed from aging or apoptosis of platelets. In the study by Bulut et al. (2011) a significant reduction ($p < 0.005$) of the number of PMP was observed after treatment with 100 mg of ASA for 8 weeks in patients with coronary artery disease. One possible explanation for the difference between these results was the time of administration of ASA (8 weeks X 15 days). It is also important to consider that the response to ASA is influenced by epidemiological, clinical and genetic factors. This strengthens the need for further studies with a larger number of patients aiming to reduce the occurrence of arterial thrombotic events, once ASA are widely prescribed.

Haemostasis

Cod: 0729

PREECLAMPSIA: INTEGRATED NETWORK MODEL OF PLATELET BIOMARKERS INTERACTION AS A TOOL TO EVALUATE THE HAEMOSTATIC/IMMUNOLOGICAL INTERFACE

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BACKGROUND: Studies suggest that preeclampsia (PE) is associated with a relevant disturbance in the haemostatic/immunological systems leading to hypercoagulable state. Despite platelets have an active role in the coagulation and inflammatory events; the use of platelet activation status in PE is still controversial. In this study, the platelet activation status along with the frequency of platelet-leukocyte aggregates (APL) and monocyte tissue factor expression were evaluated in preeclamptic women (severe and mild forms) and compared with normotensive pregnant (NP) and non-pregnant women (nonP).

METHODS: Mean fluorescence intensity of CD41a, CD61, CD42a, CD62P and percentage of CD62P+, platelets-monocytes aggregates (APM), platelets-neutrophils aggregates (APN) and TF+-monocytes were evaluated by flow cytometry in 35 PE women (20 mild PE and 15 severe PE), 31 NP and 31 nonP. Data were analyzed by ANOVA/LSD and Kruskal-Wallis/Mann-Whitney. Differences were significant at $P \leq 0.05$ and $P \leq 0.017$ (Bonferroni). To evaluate the association between peripheral blood cell populations, the Spearman correlation coefficient and significance were used.

RESULTS: The data demonstrated that platelet counts and CD41a expression by platelets were lower in NP and severe PE as compared to nonP. Moreover, the expression of CD61 was lower during pregnancy despite the occurrence of PE. No significant differences in the APL and TF+-monocytes were observed amongst the clinical groups. Analysis of biomarkers network pointed out that PE displayed a multifaceted haemostatic system characterized by expanded connecting axes involving platelets activation linked to APL and TF+-monocytes.

CONCLUSIONS: There is a clear expansion in the haemostatic biomarker network connections in PE, towards the participation of APL and the monocytes-TF+ poles. Moreover, this complex and imbricate network showed relevant dissimilarity between severe and mild PE forms supporting the multifactorial nature of this syndrome.
Support: CNPq/FAPEMIG/CAPES

Haemostasis

Cod: 0731

QUANTIFICATION OF PROTHROMBIN FRAGMENT 1+2 (F1+2) IN HUMAN CITRATED PLASMA BASED ON LOCI TECHNOLOGY*

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BACKGROUND: As a marker of thrombin generation, F1+2 has the clinical potential for assessing thrombotic risk and monitoring anticoagulation therapy. Here, we describe an automated prototype immunoassay for quantification of F1+2 based on LOCI® technology* on a prototype analyzer*.

METHODS: The assay is based on two latex bead reagents (sensibeads and chemibeads) containing photosensitive / chemiluminescent dyes. The analyte F1+2 forms bead-aggregated immune complexes via a primary neoepitope specific monoclonal antibody and an immune complex specific secondary monoclonal antibody. Illumination by light at 680 nm generates singlet oxygen from sensibeads, which diffuses to adjacent chemibeads to trigger a chemiluminescent reaction that is measured at 612 nm. The intensity of light emitted is related to the amount of analyte in the sample.

RESULTS: Preliminary performance data demonstrate a good correlation to the Enzygnost® F1+2 (monoclonal) ELISA ($r = 0.99$, $y=1.034 x + 3.954$). Repeatability ranged from 1.2 % CV to 3.3 % CV. Linearity was shown over the entire range tested (4.65 - 4799.35 pmol/L F1+2). The Limit of Quantification was determined with 11.44 pmol/L F1+2 (total error +/- 18.19%) by using 5 µl sample volume. No high dose hook effect was observed up to 20,000 pmol/L F1+2.

CONCLUSIONS: We conclude that the assay delivers reliable results across a dynamic assay range from 11.44 pmol/L to 5,000 pmol/L by using low sample volume.
*Product under feasibility evaluation. Not available for sale and its future availability cannot be guaranteed.

Haemostasis

Cod: 0732

ESTABLISHING APTT THERAPEUTIC RANGE FOR PATIENTS RECEIVING STANDARD UNFRACTIONED HEPARIN

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BACKGROUND: Thromboembolic complications of large number of patients in departments of cardiology, pulmonology and vascular surgery of "Zvezdara" University Medical Centre (UMC) are treated with unfractioned heparin (UH). Laboratory monitoring with the activated thromboplastin time (aPTT) test is used for precise dosing of UH. The aim of this study was to establish aPTT therapeutic range in Haemostasis Laboratory of "Zvezdara" UMC.

METHODS: Samples from patients treated with UH manufacturer "Galenika" for thromboembolic disease were studied. Samples were collected before administrating UH, 6 hours after bolus or dose change and 24 hours after adequate dose. At least two samples of each patient were taken and analyzed. We used Instrumentation Laboratory (IL) reagents: aPTT-SP Liquid, Liquid Heparin, IL automatic coagulometar ACL Elite Pro and data base of Laboratory Information System. For calculating aPTT therapeutic range according to therapeutic level of UH we used samples with UH concentration 0.3-0.7 IU/mL. Calculation of therapeutic ranges were made according to patient's basal aPTT, mean aPTT of normal plasma, upper limit of reference ranges aPTT and mean aPTT of IL normal lyophilisated plasma.

RESULTS: Therapeutic ranges were: 1) according to therapeutic level UH, 50–99 s, 2) according to patient's basal aPTT, 41–68 s, 3) according to mean aPTT of normal plasma (28 s), 42-70 s, 4) according to upper limit of reference ranges aPTT (35s), 52–88 s, 5) according to mean aPTT of IL normal lyophilisated plasma (32s), 48–80 s.

CONCLUSIONS: Different aPTT therapeutic ranges using standard criteria according to aPTT therapeutic range using therapeutic level UH, showed that patients could be UH subtherapeutic. APTT therapeutic range according to therapeutic level of UH using IL reagents and instruments in Haemostasis Laboratory of "Zvezdara" UMC were similar with the results of other authors. Establishing aPTT therapeutic range, using IL reagents, instruments and UH manufacturer "Galenika" in conditions of "Zvezdara" UMC is enabling precise dosing of patients with UH and decreasing a risk of thrombosis or hemorrhage. Also, adopted standardized protocols for initiation and monitoring of UH therapy are enabling more rational use of laboratory tests.

Haemostasis

Cod: 0733

HEMOSTASIS INDICATORS AS PREDICTORS OF CANCER-SPECIFIC SURVIVAL IN PATIENTS WITH LUNG CANCER

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BACKGROUND: Systemic hemostasis activation is often observed at oncological patients in lack of thrombosis. This activation is involved in processes of a tumor progression, angiogenesis and metastases development. In recent years a lot of experimental evidence of participation and influence of hemostatic system on tumor development is obtained. The purpose of this research is studying of the predictive importance of some hemostasis indicators concerning cancer-specific survival of the patients having a lung cancer.

METHODS: The 3-year event-free survival (EFS) of 67 radically treated patients with lung cancer where the death from cancer was considered as an event is estimated. Survival was compared in groups with fibrinogen concentration less and more than 4 g/l, D-dimer concentration less and more than 0.5 mkg/ml, vWf activity less and more than 150%. The blood sampling was carried out prior to special treatment. Indicators were defined on automatic analyzers of hemostasis: STA Compact (Diagnostica Stago, France) and Fluoroscan Ascent (Thermo, Finland). Log-rank test and Cox's regression analysis were applied for the statistics.

RESULTS: It is shown that lower indicators of EFS are registered at D-dimer concentration>0.5 mkg/ml (log-rank <0.01), vWf activity>150% (log-rank=0.04) and fibrinogen concentration>4 g/l (log-rank=0.04). With Cox regression analysis this parameters were analyzed as potential predictors along with the proved predictive factor – stage of lung cancer (χ2=9.2; p<0.01). The most statistically significant model contained such parameters as lung cancer stage (the most predictively important), D-dimer concentration and vWf activity. The χ2 criterion for the created model was 15.3 (p<0.01). Model characteristics showed that all parameters included in the model were independent predictive factors of cancer specific survival in lung cancer patients.

CONCLUSIONS: The obtained data testify to influence of a fibrinolysis system hyperactivity (high D-dimer concentration) and a vascular link (high activity of vWF) on lung cancer progressing that gives the chance to use these parameters together with clinical factors for forecasting of an outcome of a disease at a presurgical stage.

Haemostasis

Cod: 0734

NEW HIGHLY SENSITIVE LOCI D-DIMER METHOD FOR THE DETECTION OF VERY LOW CONCENTRATIONS IN HUMAN CITRATED PLASMA*

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BACKGROUND: As a marker of thrombotic events, d-Dimer has the clinical potential for assessing cardiovascular thrombotic risk and monitoring anticoagulation therapy. Here, we describe an automated prototype immunoassay* for quantification of d-Dimer based on LOCI® technology* on a prototype analyzer*.

METHODS: The assay* is based on two latex bead reagents (sensibeads and chemibeads) containing photosensitive / chemiluminescent dyes. The analyte d-Dimer forms bead-aggregated immune complexes via specific monoclonal antibodies. Illumination by light at 680 nm generates singlet oxygen from sensibeads, which diffuses to adjacent chemibeads and triggers a chemiluminescent reaction that is measured at 612 nm. The intensity of light emitted is related to the amount of analyte in the sample.

RESULTS: Preliminary performance data demonstrate a good correlation to the INNOVANCE® D-Dimer assay on the BCS® system (r = 0.99, y=0.980 x -0.047) and the VIDAS D-Dimer assay (r = 0.934, y=0.846 x -0.030). Repeatability ranged from 2.4 to 4.0% CV. Linearity was shown over the entire range tested (0 to 60,470 µg/L FEU). The limit of quantification was determined with 21.1 µg/L FEU (total error +/- 13.24%) by using 5 µl sample volume. No high dose hook effect was observed >600,000 µg/L FEU.

CONCLUSIONS: We conclude that the assay* delivers reliable results across a dynamic assay range from 21.1 to 50,000 µg/L FEU by using low sample volume.
*Product under feasibility evaluation. Not available for sale and its future availability cannot be guaranteed.

Haemostasis

Cod: 0735

INTRAPLATELET CYCLIC GUANOSINE MONOPHOSPHATE (CGMP) ASSESSMENT IN SEVERE PREECLAMPSIA

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BACKGROUND: Preeclampsia (PE) is characterized by hypertension and proteinuria occurring after the 20th week of pregnancy in women who have had no previous symptoms. Clinically, it is important to diagnose the severe form, in which blood pressure and proteinuria are much higher. According to gestational age (GA) at the onset of disease, PE has been classified as early (GA<34 weeks) and late (GA≥34 weeks). Although the etiology of PE is unclear, activation of platelet and inflammatory system is thought to play a crucial part in its pathogenesis. NO activates guanylate cyclase to convert guanosine triphosphate in cyclic guanosine-3',5'-monophosphate (cGMP). High cGMP levels result in Ca++ decreased, leading to vasodilatation and platelet activation inhibition. Since cGMP levels control the intraplatelet Ca++, the goal of this study was to investigate the platelet activation inhibition by intraplatelet cGMP levels assessment in severe PE/sPE (early and late) aiming to establish their role as a biomarker to help the PE diagnosis/prognosis.

METHODS: A total of 119 pregnant women; 34 with early sPE, 24 with late sPE and 61 normotensive pregnant were enrolled in this study. Five mL of venous blood were collected into EDTA tubes and centrifuged to obtain the platelet-rich-plasma, to which isobutylmethylxanthinewas added. These tubes were centrifuged again to obtain the platelet-pellet, which were stored at -80°C until use. cGMP levels were determined by ELISA (Amersham®/acetylationprotocol-3). Data statistical analysis was performed using SPSS 13.0 software, by Mann-Whitney test.

RESULTS: Intraplateletc GMP levels in early sPE [1.83 (0.85-3.27)] did not differ from late sPE [2.80(1.20-4.23)] (P=0.273) or normotensive pregnant [1.46(0.97-3.11)] (P=0.813). Similarly, no difference was observed comparing late sPE and normotensive pregnant (P=0.130).

CONCLUSIONS: The interpretation of intraplatelet NO-induced cGMP levels should be done with caution, since several interferences can mask the results. Concluding, although cGMP levels were not shown as a good biomarker for PE diagnosis/prognosis, differences in NO bioavailability in sPE should not be discarded considering the complexity of this disease.

Haemostasis

Cod: 0736

RESPONSE TO ACETYLSALICYLIC ACID IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ITS ASSOCIATION WITH GPIIIA GENE POLYMORPHISM

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BACKGROUND: Type 2 diabetes mellitus (DM2) is a metabolic disorder associated with cardiovascular complications and hyperactivation of platelets. Acetylsalicylic acid (ASA) is an antiplatelet agent used in the prevention of atherothrombotic events by inhibition of platelet cyclooxygenase-1, thus blocking the formation of thromboxane A2. The effect of ASA can be determined by the plasma levels of 2,3-dinor-thromboxane B2 (2.3-dinor-TXB2). The β3 subunit (GPIIIa) of the platelet glycoprotein GPIIb/IIIa can present a polymorphism at position 33 which consists in a replacement of a leucine (PIA1) by a proline (PIA2) resulting from a single nucleotide transition in the GPIIIa gene (C155T). Previous studies reported that this polymorphism is associated with increased platelet aggregation and may contribute to ASA resistance. However, this association is still controversial. Aim: To investigate the association between 2.3-dinor-TXB2 plasma levels and GPIIIa gene polymorphism in patients with DM2 using ASA for primary prevention of atherothrombotic events.

METHODS: Blood samples from 65 patients with DM2 were collected in two distinct moments, the first immediately prior to initiation of treatment with ASA and the second, at the fifteenth day of treatment with 100 mg of this medication daily. Patients with DM2 were selected in the Santa Casa Hospital, Belo Horizonte, Brazil. These samples were analyzed to determine levels of 2.3-dinor-TXB2. GPIIb/IIIa (PIA) gene polymorphism was studied using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. Statistical analysis was performed using Pearson Chi-Square test and $p< 0.05$ as significant.

RESULTS: During treatment with ASA, 29 patients (44.6%) had a higher or equal to 75% reduction in the 2.3-dinor-TXB2 levels. The PIA2 allele of GPIIIa gene was found in 25.9% of the participants (23.5% heterozygous and 2.5% homozygous). It was observed that the PIA2 allele of GPIIIa gene was present in higher frequency in patients who had a reduction of 2.3-dinor-TXB2 higher or equal to 75% ($p = 0.032$).

CONCLUSIONS: Results suggest that the presence of PIA2 allele in GPIIIa gene may be associated with a better response to ASA intake in patients with DM2.
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Haemostasis

Cod: 0737

EVALUATION OF HAEMOSTATIC PARAMETERS AND PLATELET AGGREGATION IN TYPE 2 DIABETES MELLITUS

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BACKGROUND: In diabetic patients, the metabolic disorder linked to hyperglycemia causes endothelial damage, platelet activation and imbalance of the coagulation cascade, leading to a relative prothrombotic state. Aim of the present study is to investigate common haemostatic parameters and platelet aggregation in patients with type 2 diabetes mellitus (T2DM), with the hypothesis that a sensitive laboratory marker can improve care for the prevention of diabetic thrombotic complications.

METHODS: We examined 69 adult patients with T2DM (35 untreated, and 34 treated with acetylsalycilic acid), and 14 healthy controls, aged between 40 and 85 years, enrolled at the phlebotomy centre of our Institution. Platelet ADP-induced aggregation with two different concentrations (5μM and 20μM) was assessed by Light Transmission Aggregometry using AggRam (Helena Laboratories). We also evaluated, by standardized procedures, platelet count, PT, aPTT, ATIII, fibrinogen and HbA1c. Statistical evaluation by Mann-Whitney and Student's t test for independent samples was performed using SPSS 20.0 for Windows.

RESULTS: Among the haemostatic parameters, fibrinogen showed a significant increase in T2DM patients vs healthy controls (370.90±92.2 vs 307.10±74.7, P=0.031). No association was shown between the haemostatic markers and HbA1c levels. Also, statistical analysis did not demonstrate any significant variation in all the haemostatic parameters tested among treated and untreated diabetic patients. When treated and untreated T2DM patients were compared, we did not observe any variation with both 5μM and 20μM ADP-induced platelet aggregation: treated vs untreated, 32% [IQR, 23-48] vs 32% [IQR,25-43], and 69% [IQR, 52-76] vs 66% [IQR, 52-75], respectively (P=0.88; P=0.63).

CONCLUSIONS: The association of fibrinogen with T2DM suggests that this marker could be appropriate in the evaluation of the prothrombotic state in these patients. The lack of variation in platelet aggregation status between treated and untreated patients may be partly due to the low responsiveness to the standard dose of aspirin in diabetics, indicating that conventional treatment may be not appropriate to prevent functional activation of platelets in these patients.

Haemostasis

Cod: 0738

PLATELET MORPHOLOGY PARAMETERS AS A ROUTINE DIAGNOSTICS AND PROGNOSTICS FACTORS IN THE COURSE OF CORONARY HEART DISEASE.

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BACKGROUND: Early diagnosis of coronary heart disease (CHD) complications risks plays essential role in the prevention and effective treatment of CHD. Platelets play important role in pathogenesis and progression of CHD. The aim of this study was to evaluate morphological parameters of blood platelets in correlation with markers of systemic inflammation and necrosis of cardiomyocytes in patients with stable angina (SA), unstable angina (UA) and acute myocardial infarction (MI).

METHODS: The study was included 154 subjects (129 patients with CHD and 25 healthy volunteers). The large platelet count (L-PLT), mean platelet volume (MPV) and mean platelet component concentration (MPC) were performed using the method of two-dimensional optical platelets analysis. The level of CD62P expression was assayed by the immunocytofluorometric method. The concentration of PF4 was demonstrated by ELISA immunoenzymatic assay, CRP and D-dimer - by immunoturbidymetric assay.

RESULTS: In opposite to CRP and DD level there were significant differences between SA and UA groups in levels of L-PLT (6,96±2,39 vs. 8,42±4,68 103/µl)) and MPC (28,02±2,15 vs. 26,89±3,09 g/L). Additionally MPV, L-PLT and MPC have shown a high correlation (r) with CD62P, known marker of platelet release (0,510; 0,580; 0,580 respectively). Additionally CD62P have shown very high correlation with PF4 (0,760). PF4 and L-PLT had highest diagnostic value in differentiation MI vs. control (AUC: 0,974 and 0,832, respectively) as well as MI vs. SA (AUC: 0,984).

CONCLUSIONS: Routine morphological parameters of platelets (MPV, L-PLT, MPC) are more sensitive indicators of inflammation associated with unstable atherosclerotic plaque than the level of CRP and D-Dimer. Taking together, platelet morphology parameters and recognized markers of platelet release (CD62P and PF4) may serve as attractive, routine diagnostic markers in patients with stable coronary artery disease as well as prognostic factors in patients with the risk of acute cardiovascular events.

Haemostasis

Cod: 0739

INTRA- AND POSTOPERATIVE COAGULATION STATUS OF CANCER PATIENTS

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BACKGROUND: Venous thromboembolism (VTE) belong to the most common death causes in cancer patients. Extensive surgical interventions increase the incidence of VTE. The aim of the study is to determine the nature of changes in the blood coagulation system of cancer patients under anticoagulant prophylaxis before and after surgery.

METHODS: The research was conducted on citrate plasma of 56 I-IV stage colorectal cancer (CRC) patients. All patients received perioperative low molecular weight heparins (LMWH) according to the international recommendations. In order to assess the routine parameters of the hemostasis and thrombin generation test, blood sampling was performed on admission to hospital as well as on the 1st and 7th postoperative day. STA Compact analyzer (Stago) and Fluoroscan Ascent fluorometer (ThermoFisher Scientific) were used to conduct the study. Calculations were made using nonparametric statistics.

RESULTS: On admission coagulation status of cancer patients tended to hypercoagulation, which is confirmed by high levels of endogenous thrombin potential ($p=0.03$), peak of thrombin ($p<0.001$) and velocity rate index ($p<0.001$) as well as increased von Willebrand factor activity ($p<0.001$), fibrinogen ($p<0.001$) and D-dimers ($p<0.001$) concentrations and reduced antiplasmin activity ($p=0.002$). Preoperative administration of LMWH leads to thrombin generation inhibition in 80.8% of cases. On the 1st postoperative day a statistically significant increase in the activity of von Willebrand factor was revealed ($p<0.001$), that persisted throughout the observation period. By the 7th postoperative day hypercoagulation manifested itself in increased fibrinogen ($p<0.001$) and D-dimer ($p<0.001$) concentrations and peak of thrombin ($p<0.001$), as compared with the preoperative values. By the end of the first postsurgical week a complete recovery of the fibrinolytic system function and a partial restoration of natural anticoagulants could be observed.

CONCLUSIONS: Preoperative administration of LMWH contributes to thrombin generation inhibition before surgery. Assessment of coagulation, anticoagulation and fibrinolytic status of cancer patients during postoperative period allows to evaluate hemostatic disorders and assign adequate VTE prevention after surgery.

Haemostasis

Cod: 0740

QUANTIFICATION OF THROMBIN-ANTITHROMBIN COMPLEXES (TAT) IN HUMAN CITRATED PLASMA BASED ON LOCI TECHNOLOGY*

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BACKGROUND: Conversion of prothrombin into active thrombin is a key event within the coagulation cascade. The inhibition of thrombin by antithrombin results in thrombin-antithrombin complexes (TAT) that can be measured quantitatively by enzyme immunoassay (Enzygnost® TAT micro ELISA, Siemens). Patients predisposed to thrombosis, e.g. patients with disseminated intravascular coagulation (DIC), multiple trauma, and septicemia are found to have elevated concentrations of TAT.

METHODS: Here, we describe an automated prototype immunoassay* for quantification of TAT based on LOCI® technology*. The assay* is based on two latex bead reagents (sensibeads and chemibeads) containing photosensitive/ chemiluminescent dyes. Plasma TAT forms bead-aggregated immuno-complexes via specific monoclonal antibodies. Illumination by light at 680 nm generates singlet oxygen from sensibeads, which diffuses to adjacent chemibeads to trigger a chemiluminescent reaction that is measured at 612 nm.

RESULTS: Preliminary performance data demonstrate an adequate correlation to the Enzygnost TAT micro ELISA (r = 0.97). Repeatability ranged from 1.6 % CV to 5.5 % CV. Linearity was good over the entire range tested (2 - 550 µg/ L TAT). Interference by rheumatoid factor and human anti-mouse antibodies is minimized by the use of an active blocking antibody as part of the reagent.

CONCLUSIONS: We conclude that the assay has the potential for excellent precision, dynamic range, and correlation to the Enzygnost TAT micro ELISA and therefore is suitable to reliably measure TAT levels in plasma.
*Under feasibility evaluation. Not available for sale and its future availability cannot be guaranteed.

Haemostasis

Cod: 0741

NEGATIVE AFFINITY CHROMATOGRAPHY AS THE METHOD PURIFICATION OF FACTOR VIII

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BACKGROUND: To correct factor deficiency or to prevent bleeding in patients with hemophilia A the replacement therapy is carried out. This therapy involves administrating plasma or recombinant preparations of factor VIII (FVIII). Coagulation FVIII preparations were obtained mostly using a cold precipitation of whole plasma called cryoprecipitation, followed by polyethylene glycol or glycine precipitation steps to partially remove protein contaminants such as fibrinogen. Additional, affinity chromatography has emerged as an efficient tool to purify FVIII. Synthetic ligands include dye molecules, which have been known for a long time and considered as one of the important alternatives to biologic ligands for specific affinity chromatography. Dye affinity chromatography is a protein purification procedure based on the high affinity of immobilized dyes for the binding sites on many proteins. Negative chromatography is particularly convenient for the rapid removal of degradative enzymes such as proteases and nucleases, and for the removal of very abundant proteins, such as serum albumin. The aim of our research: the method of negative affinity chromatography was investigated as an additional step for purification FVIII.

METHODS: Initial raw material was a preparation of cryoprecipitate. We used one-stage clotting method for FVIII activity determination. Chromatographic sorbents are using where the matrix was Diasorb- aminopropyl and triazine dyes as ligands. To 2.0 ml of each of the sorbents (equilibrium 0.05 M Tris-HCl buffer, pH 8.0) was added 2.0 ml of working solution of cryoprecipitate in the same buffer.

RESULTS: Studies have shown that FVIII is not adsorbing any of these sorbents. Many of the undesired proteins (FVIII) are retained by the column while the desired protein as well as some of the undesired proteins flows through the colomn. We received best results when used as ligands Procion blue HB and Procion blue MXR (factor activity increased on the order).

CONCLUSIONS: Triazine-dye affinity chromatography on immobilized Cibacron Blue HB and Procion blue MXR may be used on a pilot-scale to purify cryoprecipitate, obtained by a complete chromatographic procedure.

Haemostasis

Cod: 0742

APPLICATION OF SERUM HEMOLYSIS INDEX IN COAGULATION TESTS

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BACKGROUND: It is traditionally accepted that hemolytic samples are unsuitable for coagulation assays because of the release of hemoglobin, intracellular components, and thromboplastic substances from damaged blood cells. In hemolyzed samples, the repeated blood sampling and coagulation testing should be done, which is often time-consuming and expensive. Since the exact effect of hemolysis on the results of coagulation tests is not well established, we investigated its influence on global coagulation tests: prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen using serum indices (SI) as an indicator of the degree of hemolysis. Serum indices are calculations of absorbance measurements that provide a semi-quantitative representation of the level of hemolysis in patient samples.

METHODS: We analyzed 30 hemolyzed and 30 non-hemolyzed plasma samples of the same patients which remained after routine coagulation testing. In all samples, the indices of hemolysis were measured on biochemistry analyzer Roche Cobas c6000, and PT, APTT, and fibrinogen were measured on Siemens BCS® XP System by routine coagulation methods. The influence of hemolysis was also studied in plasma samples with known concentration of free hemoglobin and known values of SI to investigate the correlation between changes in values of PT, APTT and fibrinogen and concentrations of plasma hemoglobin.

RESULTS: The influence of hemolysis was demonstrated on the results of all examined coagulation tests, but in different way for each test. The minimum influence of hemolysis was detected on APTT results in samples with normal values. The major influence of hemolysis was demonstrated in samples with pathological PT results. The results showed also that the concentration of free plasma hemoglobin (or values of SI) does not correlate with the percentage of change in the tested parameters.

CONCLUSIONS: Hemolysis affected the results of examined coagulation tests, but in different way for each test. The measuring of serum indices in plasma samples before performing coagulation assays reduces the time for additional blood sampling and prevents repeated measurements.

Haemostasis

Cod: 0743

A NEW METHOD FOR DETECTION AND EVALUATION OF ANTITHROMBIN DEFICIENCY

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BACKGROUND: Antithrombin (AT) plays a pivotal role as a major inhibitor of coagulation; possessing a relatively low inhibitory activity in itself, but when bound to heparin (in vivo: heparan sulfate) the inhibitory activity is increased significantly. AT deficiency, i.e. a decreased level of AT antigen or decreased activity of AT, enhance the risk of venous thrombosis. Defects in the heparin binding site of AT are less thrombogenic than other AT defects. We have previously shown that for the heparin binding defects (HBD) AT Basel and Toyama, the β-isoform sustains some of its affinity towards heparin and thereby is partly able to compensate for the lack of affinity of the α-isoform. However, studies show that these defects are not without importance, but in some cases the detection is hindered by limitations in the design of the currently available assays. The aim of this study is to assess the potentiality of a novel thrombin generation assay, evaluating the thrombogenicity of different AT defects including AT variants with HBD.

METHODS: We use an in vitro Thrombin Generation Assay, a global assay of coagulation mimicking to some extent in vivo coagulation. To determine Endogenous Thrombin Potential (ETP) after 30 min, we compared AT deficient plasma (ATdp) to both purified α-AT from donor plasma to seven recombinant produced AT variants, both in the presence and absence of 0.2 U unfractionated heparin.

RESULTS: Initially, various concentrations of AT and heparin were tested for optimization. Day to day variation (CV) was around 10% for different tested AT concentrations. The ETP for HBDs in the presence of heparin was clearly higher than for α-AT comprising 85-95% of ATdp indicating a minimum of activity compared with an ETP around 15-20 % of ATdp for α-AT. AT variants with Reactive Center Loop defects (RCLD) showed a low activity with no significant difference compared to ATdp, both with and without heparin.

CONCLUSIONS: The results show that the method is able to identify the AT mutants in the presence of heparin where a higher ETP is obtained compared with α-AT. The effect is very clear in the presence of heparin for especially HBD. Further, a trend was observed for pleiotropic and RCL defects showing a higher ETP compared with HBD, indicating a higher thrombotic risk.