Clinical aspects of thrombosis

Wednesday 7th July 2010

P37

DIFFERENCES IN CLINICAL PRESENTATION OF PULMONARY EMBOLISM IN WOMEN AND MEN

M. Richini 1, H. Robert-Ebadi 1, G. Le Gal 3, M. Carrier 3, F. Coutoudra 1, A. Perrier 1, H. Bounameaux 1

1 Division of Angiology and Hemostasis, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland; 2 Department of Internal Medicine and Chest Diseases, EA 3878 GETBO, Breast University Hospital, Brest, France; 3 Division of Hematology, Department of Medicine, University of Ottawa, Ottawa, Canada; 4 Department of Internal Medicine, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland

Keywords: pulmonary embolism, gender, D-dimer, helical computed tomography

Background: Risk of recurrence of pulmonary embolism (PE) is higher in men than in women. Differences in clinical presentation of deep vein thrombosis (DVT) have been reported between the two genders but comparative data on PE are lacking.

Objectives: To compare the clinical characteristics between women and men presenting with suspected and confirmed PE and their impact on clinical probability prediction scores and on diagnostic work-up of PE, and to assess whether differences in clinical presentation could account for the increased recurrence rate in men.

Methods: Combined data from three prospective cohort studies including a total of 3414 patients with suspected PE were included. Respectively, prevalence of clinical characteristics, pretest probability of PE and diagnostic yield of non-invasive tests were compared between genders.

Results: The overall prevalence of PE was similar among women and men (22.3% vs 23.1%; p=0.05). The clinical probability prediction scores (both Geneva score and Wells score) performed equally well in both genders. A non invasive diagnostic work-up combining plasma D-dimer measurement and lower limb venous compression ultrasonography was more often possible in men. Finally, the proportion of PE-associated DVT was higher in men than in women (43% vs 33%; p=0.009).

Conclusions: In spite of some differences in the clinical presentation of PE between women and men, clinical probability prediction scores perform equally in both genders. A higher prevalence of PE-associated DVT in men could possibly indicate greater severity of PE episodes and partly account for the higher recurrence rate in men.

Corresponding Author: Marc Richini, Geneva University Hospital, 4, rue Gabrielle Perret-Gentil, Geneva, Switzerland, marc.richini@hcuge.ch

P513

LONG-TERM EVALUATION OF THE RISK OF RECURRENT AFTER CEREBRAL SINUS-VENOUS THROMBOSIS

L. Martinelli, P. Bucciarelli, S.M. Passamonti, T. Battagliotti, E. Previtati, P.M. Mannucci

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Dept of Internal Medicine/Fondazione IRCCS Ca’ Granda - Ospedale Maggiore Policlinico, Milano, Italy

Background: The clinical course of cerebral sinus-venous thrombosis (CSVT) is largely unknown because prospective studies are lacking with a long follow-up and with the goal to assess thrombosis recurrence rate and predisposing factors for recurrence.

Methods and results: 145 patients with a first CSVT were followed-up for a median time of 6 years after discontinuation of anticoagulant treatment. End points were recurrent CSVT or other clinical manifestations of venous thromboembolism. CSVT recurred in 5 patients (3%) and other manifestations of venous thromboembolism (deep vein thrombosis of the lower limbs or pulmonary embolism) in 10 additional patients (7%), for an overall incidence of recurrence of 2.03% patients-year (95%CI 1.16-3.14) and of recurrent CSVT of 0.63% patients-year (95%CI 0.20-1.30). Nearly half of the occurrences were occurred within the first year after discontinuation of anticoagulant therapy. Risk factors for recurrent venous thrombosis were male sex (adjusted hazard ratio 9.66, 95%CI 2.86-32.7) and, for thromboses other than CSVT, severe thrombophilia due to antithrombin, protein C, protein S deficiency, antiphospholipid antibodies or combined abnormalities (adjusted hazard ratio 4.71, 95%CI 1.34-16.5).

Conclusions: The risk of recurrent CSVT is low, being higher in the first year after discontinuation of anticoagulant treatment and among men. Mild thrombophilia abnormalities are not associated with recurrent CSVT, but severe thrombophilia entails an increased risk of deep vein thrombosis of the lower limbs or pulmonary embolism.

Corresponding Author: Ida Martinelli, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Dept of Internal Medicine, Fondazione IRCCS Ca’ Granda - Ospedale Maggiore Policlinico, Via Pace 9, Milan, Italy, martin@policlinico.mi.it

P387

D-DIMER AND ULTRASOND TO ESTABLISH THE OPTIMAL DURATION OF ANTICOAGULATION FOR VENOUS THROMBOEMBOLISM: PRELIMINARY RESULTS OF THE D-DIMER AND ULTRASONID IN COMBINATION ITALIAN STUDY (DULCIS)

B. Cosmi 1, G. Legnani 1, A. Ghirarduzzi 2, S. Testa 2, P. Frandoni 1, V. Pengo 1, E. Favaretto 1, G. Palareti 1 on behalf of the DULCIS Investigators

1 Dept Angiology & Blood Coagulation “Marino Golinelli”, S.Orsola-Malpighi University Hospital, Bologna, Italy; 2 Dept. Internal Medicine I, Angiology, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; 3 Haemostasis & Thrombosis Center, General Hospital, Cremona, Italy; 4 Dept of Cardiothoracic and Vascular Sciences, Thromboembolism Unit, University of Padua, Padua, Italy; 5 Dept. Clinical and Experimental Medicine, Division of Clinical Cardiology, University Hospital, Padua, Italy.

Keywords: D-dimer, recurrent venous thromboembolism, oral anticoagulants

Background/Aims: The purpose of this study is to evaluate the efficacy and safety of a procedure employing the evaluation of residual vein obstruction (RVO) and D-dimer to establish the individual risk of recurrence and thus the necessity to prolong or stop anticoagulation after deep vein thrombosis (DVT) and/or normal pulmonary arterial pressure with echocardio-graphy in case of previous PE and to have undergone additional 6 months of therapy for previously altered RVO, D-dimer is measured during anticoagulation. IF D-dimer is below age and gender cut-offs, anticoagulation is interrupted and D-dimer is then re-assessed after 15, 30, 60 and 90 days. If all the D-dimer measurements are below the cut-offs, anticoagulation is definitely interrupted and patients are followed-up for two years. If one of these D-dimer measurement is above the cut-off, anticoagulation is resumed for at least 6 months and patients are re-evaluated.

Results: as of 28th February, 206 out of 539 screened patients (38%) have been enrolled. Of these 123 (60%) have stopped anticoagulation because of a normal D-dimer and 6 had a recurrent event (4.9%). In 83 subjects anticoagulation was resumed and 1 major bleeding event was observed (1.2%). Additional data will be available in the near future.

Corresponding Author: Benilde Cosmi, S. Orsola-Malpighi Univ. Hospital, Via P. Albertoni 15, Bologna, Italy, benilde.cosmi@unibo.it

P541

CLINICAL PREDICTION OF VTE RECURRiENCE IN PATIENTS WITH PREViouS UNPROVOKED VENOUS THROMBOEMBOLiSM: RESULTs FROM AN INDIVIDUAL-LEVEL META-ANALYSIS

A. Tozetto 1, A. Iorio 2, M. Marucci 2, T. Baglin 3, M. Cushman 4, S. Eichinger 5, G. Palareti 1, D. Poli 1, R.C. Tait 8, J. Douketis 8

1 Dept Hematology, S. Bortolo Hospital, Vicenza, Italy; 2 Dept Medicine, University of Perugia, Perugia, Italy; 3 Dept Hematology, Addenbrooke Hospital, Cambridge, UK; 4 Dept Medicine, University of Vermont, Colchester, VT, USA; 5 Dept of Medicine, University of Vienna, Vienna, Austria; 6 Dept Medicine, University of Bologna, Bologna, Italy; 7 Thrombosis Center, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; 8 Dept Haematology, Royal Infirmary, Glasgow, UK; 9 Department of Medicine, McMaster University, Hamilton, Canada

Keywords: venous thrombosis, D-dimer, clinical prediction rule, prediction algorithm

Background/Aims: Several patient characteristics, including D-dimer after stopping anticoagulants, gender or thrombophilia, have been associated with recurrence of VTE after a first episode of unprovoked VTE. Very few data exist about their joint effect on the prediction of recurrent VTE because the sample size of individual studies does not allow such an evaluation. We aimed to develop a model that could optimize the prediction of disease recurrence, while retaining sufficient simplicity for clinical use.

Methods: We obtained individual patient data of 7 prospective studies. We selected all cases with unprovoked, proximal VTE as the primary event. A cross-sectional cohort was formed at 18 months of follow-up, by selecting all patients that had a VTE recurrence before 18 months (cases) together with all those patients without a VTE recurrence before 18 months of follow-up (controls). Using parametric and non-parametric methods we developed two models for the identification of subjects at low risk for VTE recurrence. Results: Of 1862 patients with a first unprovoked proximal VTE, 153 (8.2%) subjects were analyzed. 1,218 being defined controls and 188 that were defined as cases. D-dimer levels, gender and use of estrogепrogestin at time of first VTE were associated with recurrence, whereas BMI, presence of thrombophilia were not. Two independent prediction rules were able to identify patients with an annual risk of recurrence below 4%. The first one based on D-dimer testing in females suggested VKA suspension in females having normal D-dimer; the second one based on D-dimer testing in all patients suggested VKA suspension with normal D-dimer or estrogепrogestin-related VTE.

Conclusions: A prediction rule based on the combination of D-dimer and gender (or estrogепrogestin use) may identify patients at low risk of recurrence, potentially sparing VKA therapy in at least one-fourth of subjects having a first episode of unprovoked VTE.

Corresponding Author: Alberto Tozetto, S. Bortolo Hospital, Vicenza, Italy, tozetto@hemato.ven.it
ARE MEN AT HIGHER RISK FOR DISEASE RECURRENT THAN WOMEN

J. Doukeris 1, A. Tossi 2, M. Marcucci 1, T. Baglin 1, M. Cushman 1, S. Eichinger 1, G. Palareti 7, D. Poli 1, R. Campbell Tait 1, A. Iorio 1

1 Dept. of Medicine, McMaster University, Hamilton, Canada; 2 Dept. of Hematology, S. Bortolo Hospital, Vicenza, Italy; 3 Dept. of Medicine, University of Perugia, Perugia, Italy; 4 Dept. of Haematology, Addenbrookes Hospital, Cambridge, UK; 5 Dept. of Medicine, University of Vermont, Colchester, USA; 6 Dept. of Medicine, University of Vienna, Vienna, Austria; 7 Dept. of Medicine, University of Bologna, Bologna, Italy; 8 Centro di Riferimento Regionale per la Trombosi, Azienda Ospedaliero-Universitaria Cà Foscari, Florence, Italy; 9 Dept. of Haematology, Royal Infirmary, Glasgow, UK

Keywords: venous thrombosis; recurrence; sex; women issued

Background: In patients with a first episode of venous thromboembolism (VTE), the purported higher risk for recurrent disease in men may be spurious and attributable to a lower risk in women, some of whom have a low risk recurrence after hormonal therapy (HT)-associated VTE.

Methods: We did a patient-level meta-analysis of prospective studies in patients with a first VTE who were followed after anticoagulation was stopped for symptomatic recurrent VTE. We used Kaplan-Meier analysis to determine the cumulative incidence of recurrent VTE and multivariable Cox regression to adjust for patient sex and HT.

Results: We studied 2,554 patients with a first VTE who had follow-up for a mean (standard deviation) of 27.1 (19.6) months. The 3-year cumulative incidence of recurrent VTE was 9.1% (95% CI 7.3-11.3) in women and 19.7% (95% CI 16.5-23.4) in men. The table shows the results of Cox regression analysis.

Conclusions: In patients with a first unprovoked VTE, men have a 2.2-fold higher risk for recurrent VTE; this risk remained 1.8-fold higher in men than women after the exclusion of women with HT-associated VTE. These results are not confirmed in patients with a first provoked VTE.

Table

<table>
<thead>
<tr>
<th>Patient Groups</th>
<th>Risk for Recurrent VTE: HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprovoked VTE index event</td>
<td>Unprovoked VTE index event</td>
</tr>
<tr>
<td>men vs. all women (HT-associated VTE cases included)</td>
<td>2.2 (1.7-2.8)</td>
</tr>
<tr>
<td>men vs. all women (HT-associated VTE cases excluded)</td>
<td>1.8 (1.4-2.5)</td>
</tr>
<tr>
<td>HT users women vs. HT non-user women</td>
<td>0.90 (0.39-2.60)</td>
</tr>
<tr>
<td>Provoked VTE index event</td>
<td>Provoked VTE index event</td>
</tr>
<tr>
<td>men vs. all women (HT-associated VTE cases included)</td>
<td>1.2 (0.6-2.4)</td>
</tr>
<tr>
<td>men vs. all women (HT-associated VTE cases excluded)</td>
<td>1.2 (0.6-2.3)</td>
</tr>
</tbody>
</table>

Legend: HR, hazard ratio; VTE occurring in absence of a major antecedent risk factor (e.g., surgery, trauma); †VTE occurring in presence of at least one antecedent major risk factor.

Corresponding Author: Alfonso Iorio, Department of Internal Medicine, University of Perugia, Perugia, Italy, iorioa@unipg.it

THE COMPARATIVE STUDY BETWEEN PROXIMAL AND DISTAL TYPE OF CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION TREATED WITH PULMONARY THROMBOEMBOLARCTOMY

H. Gan, J. Zhang, S. Liu, Y. Gao, X. Zhang

Beijing Anzhen Hospital, Beijing, China

Aims: To retrospectively compare the difference of the effects of pulmonary thromboendarterectomy (PTE) between distal and proximal type of chronic thromboembolic pulmonary hypertension (CTEPH).

Methods: Seventy cases of CTEPH operated with PTE from March 2002 to March 2009 in Anzhen Hospital were retrospectively reviewed and were classified as proximal CTEPH group (n=51) or distal CTEPH group (n=19).

Results: There were no early deaths and no late deaths. 15 had residual pulmonary hypertension and 23 had pulmonary reperfusion injury postoperatively, all of reperfusion were recovered with the support of ventilation or ECMO. The pulmonary artery systolic pressure and pulmonary vascular resistance of the proximal type of CTEPH 72h after the PTE procedure is lower significantly than of the distal type of CTEPH 45±17.4 mm Hg vs. 67.8±21.3 mm Hg, 37.9±30.7 kPa/L·S vs. 52.8±32.1 kPa/L·S, S-1 vs. S-2±32.1 kPa/L·S, S-1), and the PaO2 of the proximal type of CTEPH are significantly higher than of the distal type of CTEPH (88.8±9.32 mm Hg vs. 76.7±8.66 mm Hg). With a mean follow-up of (32.7±13.6) (2-81) months (cumulative follow-up was 191.8 patient-years), 47 cases from the whole cohort have a complete data set of CTPA and isotope perfusion / ventilation scan, and the residual occlusive pulmonary artery segment in proximal type of CTEPH are significantly fewer than of distal type isotope perfusion / ventilation scan: 2.2±1.1 vs. 4.7±2.1 mm Hg (CTPA:3.5±1.4 vs. 4.9±2.0 mm Hg). The NYHA functional class and 6MWD in proximal type of CTEPH are significantly better than in distal type of CTEPH (1.7±0.5 vs 2.3±0.4; 479.2±51.2 vs. 438.6±39.5 mmHg). According to Kaplan-Meier actuarial curve, the freedom from reembolism at 3 years was (96.7±2.8%). The linear Bleeding rate related to anticoagulation is 2.47% patient-years, and the linear thromboembolic rate related to anticoagulation is 1.64% patient-years.

Conclusions: although the early and mid-long term survival rate of PTE procedure of both proximal and distal type of CTEPH is agreeable and the complication rate related to anticoagulation with warfarin is relatively low, the recovery of the SPA, PVR and 6MWD, and blood gases in proximal type of CTEPH are significantly better than of distal type of CTEPH. On the one hand, anticoagulation can singularly provide enough protection to proximal type of CTEPH, but on the other hand, diuretics and pulmonary hypertension alleviation drug should be added the treatment regimen of the distal type of CTEPH after the procedure of PTE.

Corresponding Author: Huili Gan, Beijing Anzhen Hospital, Anzhenli Andingmenwai Beijing Anzhen Hospital, Beijing, China, ganhuili@hotmail.com

VENOUS THROMBOEMBOLISM RECURRENT AFTER A FIRST EPISODE OF PROVOKED VENOUS THROMBOSIS DUE TO A TRANSIENT RISK FACTOR. A SYSTEMATIC REVIEW OF THE LITERATURE

A. Iorio 1, C. Kearon 2, E. Filippucci 3, M. Marcucci 1, V. Pengo 1, S. Siragusa 5, G. Palareti 6

1 Department of Internal Medicine, Internal Medicine/Stroke Unit, University of Perugia, Perugia, Italy; 2 Department Internal Medicine, McMaster University, Ontario, Canada; 3 ASL 3, Foligno, Perugia Hospital, Italy; 4 Department of Medicine and Surgery, University of Padua, Padua, Italy; 5 Cattedra ed U.O. di Ematologia, Dipartimento di Medicina Interna, Malattie Cardiovascolari e Neofruminologiche Universita degli Studi di Palermo, Palermo, Italy; 6 Angiology and Coagulation Disorders Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy

Keywords: venous thromboembolism, recurrence, oral anticoagulant therapy

Background: Venous thromboembolism (VTE) provoked by a transient risk factor is associated with a low risk of recurrence and it is usually treated with three months of anticoagulation. The rate of recurrence after provoked VTE could be taken as a cut off value to stop anticoagulation in unprovoked VTE.

Aims: Systematically appraise the risk of recurrence for VTE provoked by different transient risk factors.

Materials and methods: Data Sources. MEDLINE, EMBase and Cochrane CENTRAL up to June 2008. Study Eligibility Criteria. Cohort and randomized studies which included patients with a first episode of VTE provoked by a transient risk factor, treated for at least 3 months and prospectively followed after stopping anticoagulant therapy.

Study Appraisal and Synthesis Methods: Number of patients, patient-years of follow-up, and recurrent VTE in each subgroup during the 0-12 and 0-24 month intervals after stopping therapies; study design and risk factor characteristics. Annualized recurrence rates in individual studies were combined to obtain pooled estimates.

Results: In the 24 months after stopping therapy, the rate of recurrence was 3.3% per patient-year (95% CI 2.8% to 3.9%; 11 studies, 2268 patients) for any transient risk factor, 0.7% (95% CI 0 to 1.5%; 3 studies, 248 patients) for a surgical factor, and 4.2% (95% CI 2.8 to 5.6; 3 studies, 509 patients) for a non-surgical factors. The rate ratio for a non-surgical compared with a surgical factor was 3.0 (95% CI 1.1-8.1). The rate ratio for unprovoked thrombosis was 2.3 (95% CI 1.9 to 2.8) when compared with any transient risk factor, and 1.8 (95% CI 1.2 to 2.5) when compared with a non-surgical factor.

Conclusions: The risk of recurrence after stopping anticoagulant therapy is lower for VTE provoked by surgery than by a non-surgical transient risk factor. Both groups have a lower risk than unprovoked VTE.

Corresponding Author: Alfonso Iorio, Department of Internal Medicine, Internal Medicine/Stroke Unit, University of Perugia, Perugia, Italy, iorioa@unipg.it
COMPARISON OF RISK PROFILE AND CLINICAL OUTCOME OF PATIENTS AFTER ACUTE PULMONARY EMBOLISM IN UNIVERSITY AND NON-UNIVERSITY HOSPITALS

W. Zondag 1, F.A. Klok 1, M. Nijkeuter 1, M. Kruip 1, R.A. Douma 1, M.H.H. Kramer 1, M.V. Huismann 1

1 Leiden University Medical Centre, Section Vascular Medicine, Department of General Internal Medicine - Endocrinology, Leiden, Netherlands; 2 Department of Hematology, Erasmus Medical Centre, Rotterdam, The Netherlands; 3 Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands; 4 Department of Internal Medicine, VU University Medical Centre, Amsterdam, The Netherlands

Keywords: pulmonary embolism, university hospital, clinical outcome

Background: Current knowledge on diagnostic management and treatment of patients with acute pulmonary embolism (PE) is partly derived from outcome studies including patients from university hospitals alone. It is debatable whether these data are applicable to patients in non-university hospitals.

Aims: To compare baseline characteristics and clinical outcome of patients with PE treated in university hospitals versus patients treated in non-university hospitals.

Materials and methods: Post-hoc analysis on data derived from Christopher Study, a prospective multicenter management study.

Results: A total of 399 (59%) patients with PE presented to a university hospital and 275 (41%) to a non-university teaching hospital. The characteristics of patients from the university and non-university hospitals were different with respect to age (65 vs. 70 years, p<0.001), sex (85% vs. 67% male, p<0.001), and comorbidities (40% vs. 6%, p<0.001). In-hospital mortality in the university group was 9.4% compared to 12.8% in the non-university group (p=0.05).

Conclusions: Physicians should be aware of differences in patient characteristics and outcome between university and non-university hospitals when interpreting results from large clinical trials and applying these to their everyday medical practice.

Table Adverse clinical outcome in a three months follow-up period

<table>
<thead>
<tr>
<th>Outcome</th>
<th>University hospital</th>
<th>Non-university teaching hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total recurrences</td>
<td>13 (3.3)</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td>Fatal recurrent PE</td>
<td>6 (1.5)</td>
<td>5 (1.8)</td>
</tr>
<tr>
<td>Non-fatal recurrent PE</td>
<td>2 (0.5)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>All bleeding complications</td>
<td>17 (4.3)</td>
<td>6 (2.2)</td>
</tr>
<tr>
<td>Fatal bleeding</td>
<td>1 (0.3)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Major bleeding</td>
<td>7 (1.8)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Clinically relevant bleeding</td>
<td>9 (2.3)</td>
<td>4 (1.5)</td>
</tr>
<tr>
<td>All cause mortality</td>
<td>36 (9.0)</td>
<td>19 (6.9)</td>
</tr>
</tbody>
</table>

Data are displayed as No (%).

P198

KEY ROLE OF EARLY DIAGNOSIS IN REDUCING MORTALITY IN PATIENTS WITH HEPARIN-INDUCED THROMBOCYTOPENIA/HIT: A SINGLE-CENTER EXPERIENCE WITH FONDAPARINUX AS ALTERNATIVE ANTICOAGULANT

S.L. Barco 1, M. Barone 1, C. Beltrametti 1, M. De Amicis 2, V. Giunta 3, D. Iossub 4, F. Piovella 4

1 S.C. Angiologia - Malattie Tromboemboliche; 2 S.C. Pediatria; Fondazione IRCCS Policlinico “San Matteo”; 3 Fondazione Ospedaliero-Università Careggi, Florence, Italy; 4 Dept. of Haematology, Royal Infirmary, Glasgow, UK

Keywords: fondaparinux, heparin-induced thrombocytopenia, thrombosis

Background: HIT is an immune-mediated adverse reaction of heparins caused by platelet-activating anti-FP4/heparin antibodies. Fondaparinux, a selective inhibitor of factor Xa which does not react with HIT antibodies, could represent a potential alternative to manage this condition.

Methods: We treated 52 patients with strong suspect of isolated HIT (20 patients) or HIT and thrombosis, HITT (32 patients). In the HITT group, we applied therapeutic dosages of fondaparinux (7.5 mg/day) or lower, according with bleeding risk. The remaining patients with isolated HIT were given 2.5 mg/day. Fondaparinux was administered for 9.6±8 days before switching to warfarin. The mean of our patients 4Ts score was 5.8±1.5; the mean platelet count nadir was 36.7±26.5*109/L.

Results: Seven patients (13.5%) had thromboembolic complications, 6 of whom received a prophylactic fondaparinux administration, of which 6/32 with HIT (18.7%) and 1/20 with HIT (5%): in 2 patients (one with isolated HIT and one with HITT) an increased dose of fondaparinux (to 7.5 mg/day) allowed resolution of the thromboembolic event. Of the 7 patients with thromboembolic complications, 6 had a delayed diagnosis of HIT. Three episodes of major bleeding (5.8%) were recorded (no deaths for bleeding). Of these, 2 had mild or severe renal insufficiency (2/6, 12.5%). All cause mortality was 28.8% (n=15/52). No patient died due to HIT solely. Delay in assessing the diagnosis was pivotal element for the mortality rate. Excluding deaths due to comorbid conditions and restricting the analysis to HIT-related deaths only (n=11), one could evaluate a significant correlation with the diagnostic delay. We found the same correlation analyzing patients with high 4Ts score only. The ROC curve analysis, considering a 5 days delay cut-off, showed a sensitivity and specificity of 91% and 83% in predicting death.

Conclusions: This report provides further evidence supporting the potential role of fondaparinux administration in the management of strongly suspected HIT and underlines the key role of early diagnosis in reducing mortality.

Corresponding Author: Stefano L. Barco, S.C. Angiologia - Malattie Tromboemboliche, Fondazione IRCCS Policlinico “San Matteo”, Viale Golgi, 19, Pavia, Italy, ste.barco@gmail.com

P546

DOES THE CLINICAL PRESENTATION AND EXTENT OF VENOUS THROMBOSIS PREDICT LIKELIHOOD AND TYPE OF RECURRENCE? A PATIENT LEVEL META-ANALYSIS OF 2,554 UNSELECTED PATIENTS AFTER A FIRST THROMBOSIS

T. Baglin 1, J. Douketis 2, A. Tosetto 1, M. Marucci 4, M. Cushman 5, S. Eichberger 6, G. Palareti 7, D. Poli 8, R. Campbell Tait 9, A. Iorio 4

1 Dept. of Haematology, Addenbrookes Hospital, Cambridge, UK; 2 Dept. of Medicine, McMaster University, Hamilton, ON, Canada; 3 Dept. of Hematology, S. Bortolo Hospital, Vicenza, Italy; 4 Dept. of Medicine, University of Perugia, Perugia, Italy; 5 Dept. of Medicine, University of Vermont, Colchester, VT, USA; 6 Dept. of Medicine, University of Vienna, Vienna, Austria; 7 Dept. of Medicine, University of Bologna, Bologna, Italy; 8 Centro di Riferimento Regionale per la Trombosi, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; 9 Dept. of Haematology, Royal Infirmary, Glasgow, UK

Keywords: Venous Thrombosis; Pulmonary embolism; recurrence risk

Aims: To determine if the mode of presentation of a first episode of venous thromboembolism (VTE) predicts likelihood and type of recurrence.

Materials and methods: Patient-level meta-analysis of seven prospective cohort studies. Time-to-event analysis was performed by Kaplan-Meier estimates with cumulative recurrence rates reported at different years of follow up and annualized rates presented as events per 100 patient-years. Hazard ratios (HR) were calculated for clinical presentation and extent of disease and adjusting for other putative confounders (age, sex, provoked or unprovoked VTE, hormone therapy).

Results: In 869 patients presenting with symptomatic PE the cumulative rate of recurrence at 5 years was 22.0% and recurrence as PE was 10.6%. In 1,365 patients presenting with symptomatic DVT the cumulative rate of recurrence at 5 years was 26.4% and recurrence as PE was 36.6%. The risk of recurrence as PE was higher for patients presenting with symptomatic PE compared to proximal DVT (hazard ratio 3.1, 95% confidence interval [CI] 1.9 – 5.1) and tended to be greater in patients presenting with proximal DVT compared to distal DVT, even if without a statistical significance (hazard ratio 4.5, 95% CI 0.6 – 33.9). Patients with clot confined to the calf had an almost 5-fold lower cumulative recurrence rate than patients with proximal DVT (hazard ratio 3.0, 95% CI 1.7 – 5.3). Patients with clot confined to the calf had an almost 5-fold lower cumulative recurrence rate than patients with proximal DVT, even if without a statistical significance (hazard ratio 4.5, 95% CI 0.6 – 33.9). Patients with clot confined to the calf had an almost 5-fold lower cumulative recurrence rate than patients with proximal DVT, even if without a statistical significance (hazard ratio 4.5, 95% CI 0.6 – 33.9). Patients with clot confined to the calf had an almost 5-fold lower cumulative recurrence rate than patients with proximal DVT.

Conclusions: Whilst DVT and PE are manifestations of the same pathology the phenotype of the disease is predetermined. Patients presenting with symptomatic PE are 4-times more likely to suffer recurrence as PE compared to patients presenting with DVT alone. Patients presenting with DVT confined to the calf veins are at low risk of recurrence and of recurrence as PE.

Corresponding Author: Alfonsio Iorio, Dept. of Medicine, University of Perugia, Perugia, Italy, iorio@unipg.it
IS ATRIAL FIBRILLATION ASSOCIATED WITH PULMONARY EMBOLISM?

G. Gex 1, F. Gerstel 1, M. Righini 1, G. Le Gal 4, D. Aujesky 1, P.M. Roy 1, O. Sanchez 1, F. Verschuren 1, O. Rutschmann 1, T. Perneger 1, A. Perrier 1

1 Division of Angiology and Hemostasis, 2 Division of General Internal Medicine, 3 Department of Internal Medicine, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland; 4 Department of Internal Medicine and Chest Diseases, EA 3878 (GETBO), Brest University Hospital, Brest, France; 5 Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 6 Emergency Department, Angers University Hospital, Angers, France; 7 and Service of Pneumology

Keywords: atrial fibrillation, pulmonary embolism, new-onset dyspnea

Background: Pulmonary embolism (PE) is deemed to trigger atrial fibrillation (AF). Nevertheless, the association between PE and AF is based on weak data. We compared AF prevalence among patients with or without PE, in a cohort of patients with PE suspicion.

Methods and results: Data from two trials on PE diagnosis were analyzed. 2449 consecutive patients admitted for clinically suspected PE were included. ECG was systematically performed. PE was diagnosed in 551 (22%) patients by computed tomography. The prevalence of AF was 4.6% in patients with PE and 5.8% in patients without PE, a non-significant difference (p=0.28). After adjustment for confounding factors, AF tended to decrease PE probability (OR 0.47, CI95% 0.28-0.80). As AF can manifest as new-onset dyspnea, its presence could have misled to PE suspicion. Accordingly, AF significantly decreased PE probability (OR 0.68, CI95% 0.40-0.96). However, when PE was suspected because of a chest pain without dyspnea, AF tended to increase the risk of PE (OR 2.42, CI95% 0.97-6.07, p=0.059).

Conclusions: Despite common belief, presence of AF does not increase PE probability when this diagnosis is suspected. When PE suspicion is based on a new-onset dyspnea, AF decreases significantly the risk of PE, probably because AF can mimic its clinical presentation. On the contrary, when PE suspicion does not emerge from a new onset dyspnea, AF tends to increase PE probability. This observation is suggestive of a true association between these two conditions.

Table: Association between PE and AF, heart failure and COPD, accounting for dyspnea presence

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>New dyspnea</th>
<th>No new dyspnea</th>
<th>Test for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted OR of PE in case of AF (95%CI)*</td>
<td>0.68 (0.43-1.11)</td>
<td>0.47 (0.26-0.84)</td>
<td>2.42 (0.97-6.07)</td>
<td>p=0.093</td>
</tr>
<tr>
<td></td>
<td>p=0.122</td>
<td>p=0.010</td>
<td>p=0.059</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=133</td>
<td>n=104</td>
<td>n=29</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted OR of PE in case of COPD (95%CI)*</td>
<td>0.43 (0.28-0.65)</td>
<td>0.32 (0.20-0.51)</td>
<td>1.40 (0.51-3.87)</td>
<td>p=0.099</td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td>p=0.001</td>
<td>p=0.515</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=247</td>
<td>n=217</td>
<td>n=30</td>
<td></td>
</tr>
<tr>
<td>Heart failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted OR of PE in case of heart failure (95%CI)*</td>
<td>0.53 (0.31-0.88)</td>
<td>0.43 (0.25-0.73)</td>
<td>1.80 (0.36-8.99)</td>
<td>p=0.095</td>
</tr>
<tr>
<td></td>
<td>p=0.014</td>
<td>p=0.002</td>
<td>p=0.475</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=144</td>
<td>n=135</td>
<td>n=9</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and presence of AF, heart failure, COPD, stroke or cancer in the past and creatinine clearance

Corresponding Author: Eric Gerstel, Division of General Internal Medicine, Geneva University Hospital and Faculty of Medicine, Rue Gabrielle-Perret-Gentil 4, Geneva, Switzerland, eric.gerstel@hcuge.ch

FOLLOW-UP IN PULMONARY EMBOLISM WITH CT ANGIOGRAPHY: IS IT NECESSARY?

C. Fernández Capitán 1, M.I. Torres 2, G. Gallardo 1, M.A. Rodríguez Davila 1, V. Perez Dueñas 1, N. Iniesta 1, M. Fernández Velilla 1, S. Caro 1, J. Camacho 1, A. Lorenzo Hernández 1

1 Internal Medicine Department, Hospital Universitario La Paz, Madrid, Spain; 2 Radiology Department. Hospital Universitario La Paz, Madrid, Spain

Keywords: follow-up, CT angiography, thromboembolism

Aims: To assess the utility of 6-months follow-up CT pulmonary angiography (CTA), for controlling interruption of anticoagulant therapy and determine evolution to chronic pulmonary embolism (PE) and/or chronic thromboembolic pulmonary hypertension

Materials and methods: From March 2001 to January 2010 we reviewed the CT angiography of patients with acute pulmonary embolism at diagnosis and after 6 months of treatment. According to findings we divided patients in two groups: total resolution (group A) and non total resolution (group B). Group B was defined as: CT signs of persistence of the clot (filling defect or complete occlusion), evolution to chronic PE (severe arterial luminal narrowing or vessel occlusion of a stenosed artery) and pulmonary hypertension -PHT-(mosaic parenchyma pattern) at the level of the central and peripheral pulmonary arteries.

Results: 605 patients were studied with CT angiography both at diagnosis and at six months of treatment. Patients were treated with anticoagulant therapy during at least 6 months. 40% of patients did not achieve complete resolution. Persistence of clot was seen in 21%. In the other 19%, progression to chronic pulmonary embolism was seen. No case of pulmonary hypertension was observed in group A, but 10% of group B patients had CT signs of pulmonary hypertension. There were no differences in clinical symptoms between group A and group B.

Conclusions: CT is a useful tool in the follow-up of thromboembolism patients, in particular those who could develop pulmonary hypertension in the future. Complete resolution of pulmonary embolism should be confirmed before stopping anticoagulant treatment.

Corresponding Author: Alicia Lorenzo Hernández, Hospital Universitario La Paz, Paseo de la Castellana 261, Madrid, Spain, alicia.lh@terra.es

STATINS, FIBRATES, AND VENOUS THROMBOEMBOLISM: A META-ANALYSIS

A. Squizzato 1, M. Galli 1, E. Romualdi 1, F. Dentali 1, P.W. Kamphuisen 2, L. Guasti 1, A. Venco 1, W. Ageno 1

1 Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy; 2 Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands

Keywords: statins, fibrates, venous thromboembolism

Background/Aims: Recent data suggest a possible benefit of lipid-lowering drugs, in particular statins, in preventing venous thromboembolism (VTE). The aim of this systematic review of the literature is to assess the effect of lipid-lowering drugs on VTE occurrence.

Materials and methods: MEDLINE and EMBASE databases were searched to identify studies that evaluated the effect of lipid-lowering drugs, in particular statins, in preventing venous thromboembolism (VTE). The aim of this systematic review of the literature is to assess the effect of lipid-lowering drugs on VTE occurrence.

Results: Three randomized controlled trials (RCTs), three cohort, and eight case-control studies were included in our systematic review, for a total of 863,805 patients. Statins use significantly reduced VTE risk [OR, 0.81; 95% CI, 0.66-0.99, p=0.001] in the primary outcome across the studies. Other lipid-lowering drugs were lacking.

Conclusions: This meta-analysis of available literature suggests that statins may lower the risk of VTE, whereas fibrates may increase this risk. Due to several methodological limitations, this conclusion should be considered with caution, and additional, specifically designed RCTs are warranted.

Corresponding Author: Alessandro Squizzato, Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Medicina 1, Ospedale di Circolo, Viale Borri, 57, Varese, Italy, alessquizzato@libero.it
THE PROGNOSIS OF PULMONARY EMBOLISM DEPENDS OF ITS LOCALIZATION? RESULTS FROM THE RIETE

D. Nauffal Manzur, E. Ansótegui, D. Jiménez, R. Otero, M. Monreal, M. Perpiñá
Pneumology Service, Hospital Universitario La Fe, Valencia, Spain

Keywords: pulmonary embolism, recurrences, mortality

Background: The prognosis of pulmonary embolism (PE) depends mainly on mortality and recurrences. Mortality is associated with advanced age, renal failure, cancer and long previous hospitalization. Recurrences are related with a high level of plasmatic D-dimer and persistent signs of deep venous thrombosis (DVT) in the leg ultrasound, after anticoagulation is stopped. However it’s not known if the prognosis is worst when PE affects the main pulmonary artery or a lobar branch than when affects segmental or subsegmental branches.

Aims: To know if the prognosis of PE depends on its localization and if there are risk factors related.

Methods: Retrospective study of all patients with PE included in the RIETE between January 2001 and August 2004. The following variables were analyzed: age, gender, other diseases, previous PE and/or DVT, cancer, previous immobilization, shock, clinical signs of DVT, localization (main and lobar artery group 1, segmental and subsegmental branches group 2) d-dimer, respiratory failure, radiologic and electrocardiographic findings, mortality and recurrences. Statistics: descriptive, univariate and multivariate analysis. Significance was considered when p<0.05.

Results: Group 1 included 443 patients, mean age 68.5 years old, 222 male and 221 female. Group 2 included 97 patients, mean age 63.4 years old, 57 male and 40 female. The number of recurrences was small in the two groups. Fiftyfive patients (15%) of the group 1 and 5 (7%) of the group 2 died. Cancer and respiratory failure were found as only variables significantly related to mortality in both groups but they did not find differences either in mortality or in recurrences between the two groups. Variables found as significant in both groups in the univariate analysis are shown in the table. No significances were seen in the multivariate analysis.

Conclusions: With the limitations of the study - few patients in group 2 compared with those of group 1- we conclude that the prognosis of PE is independent of its localization. Cancer and respiratory failure can predict mortality but no recurrences of PE and it is also independent of its localization.

Corresponding Author: Dolores Nauffal Manzur, Pneumology Service, Hospital Universitario La Fe, Avenida Campanar 21, Valencia, Spain, dnauffal@sespar.es

Table

<table>
<thead>
<tr>
<th>Group</th>
<th>Cancer</th>
<th>Respiratory Failure</th>
<th>Other Diseases</th>
<th>Abnormal Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.004</td>
</tr>
<tr>
<td>2</td>
<td>p&lt;0.02</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Corresponding Author: Paolo Prandoni, Department of Medical and Surgical Sciences, University of Padua, Padua, Italy; paoloprandoni@tin.it
LONG-TERM OUTCOME OF VENOUS THROMBOEMBOLISM IN ELDERLY PATIENTS: FINDINGS FROM THE MASTER REGISTRY

W. Ageno 1, G. Agnelli 2, M. Verso 2, D. Imberti 3, M. Moia 4, G. Palareti 2, R. Pistelli 4 and the MASTER investigators

1 Department of Clinical Medicine, University of Insubria, Varese, Italy; 2 University of Perugia, Perugia, Italy; 3 Hospital of Piacenza, Piacenza, Italy; 4 BCCS Cà Granda, Milan, Italy; 5 University of Bologna, Bologna, Italy; 6 Catholic University, Rome, Italy

Background: Little information exists on the long-term clinical outcome of venous thromboembolism (VTE) in elderly patients.

Aims: To prospectively compare the long-term clinical outcome of VTE in a cohort of elderly patients aged >75 years and in a cohort of patients aged ≤75 years enrolled in a large, multicenter registry and to identify independent predictors of clinical outcomes in the elderly.

Patients and methods: Consecutive patients with symptomatic, objectively confirmed, acute VTE were included in the MASTER registry in 25 Italian centers. Patients were followed-up for 24 months. Major clinical outcomes were death, recurrence of VTE and major bleeding. Cox regression analysis was used to assess major determinants of outcomes.

Results: A total of 2119 patients (49.8% males) were enrolled in the study, of whom 440 (20.8%) were >75 years and 1679 (79.2%) ≤75 years. Information on mortality at 2 years was available for 2021 patients (413 >75 years and 1608 ≤75 years) and information on VTE recurrence and bleeding events was available for 1988 patients (404 >75 years and 1584 ≤75 years). The 2-year cumulative incidence of mortality was 13.1% in patients >75 and 7.0% in patients ≤75, hazard ratio (HR) 1.52, 95% CI 1.09-2.13. Cancer (HR 3.44, 95% CI 1.94-6.09) was the only independent predictor of mortality in the elderly. The 2-year cumulative incidence of recurrent VTE was 6.4% in patients >75 and 6.2% in patients ≤75 (HR 1.05; 95% CI 0.67-1.63). The 2-year cumulative incidence of bleeding was 4.0% in patients >75 and 2.2% in patients ≤75, Odds Ratio 1.84; 95% CI 0.97-3.50.

Conclusions: As expected, long term mortality rates after acute VTE are significantly higher in patients >75 years than in younger patients. Rates of recurrent thrombotic events were similar between the two groups, whereas bleeding events were nearly twice as frequent in the elderly.

Corresponding Author: Walter Ageno, Department of Clinical Medicine, University of Insubria, Varese, Italy, agenow@yahoo.com

PREVENTION OF VENOUS THROMBOEMBOLISM IN AT-RISK MEDICAL PATIENTS IN THE USA

A.N. Amin 1, J. Lin 2, D. Wiederkehr 3

1 School of Medicine, University of California-Irvine, Orange, CA, USA; 2 Sanofi-Aventis, Bridgewater, NJ, USA; 3 Quintiles Consulting, Hawthorne, NY, USA

Keywords: venous thromboembolism; prophylaxis; readmission

Background/Aims: In addition to the well-recognized risk among surgical patients, hospitalized medical patients are at risk of venous thromboembolism (VTE). We conducted a real-world analysis of VTE rates and thromboprophylaxis prescribing patterns in US medical patients in hospital, and for 30 days post-discharge.

Methods: Data were extracted from the US Premier Perspective™-i3 Pharma Informatics linked database for medical patients at-risk of VTE (e.g. cancer, heart failure, severe lung disease, infectious disease) and admitted between January 2005–November 2007. Included patients were aged ≥ 18 years, with at least 6 months’ continuous plan enrollment. Patients were excluded if discharged to an acute-care hospital of Piacenza, Piacenza, Italy; 4 BCCS Cà Granda, Milan, Italy; 5 University of Bologna, Bologna, Italy; 6 Catholic University, Rome, Italy

Background: Little information exists on the long-term clinical outcome of venous thromboembolism (VTE) in elderly patients.

Aims: To prospectively compare the long-term clinical outcome of VTE in a cohort of elderly patients aged >75 years and in a cohort of patients aged ≤75 years enrolled in a large, multicenter registry and to identify independent predictors of clinical outcomes in the elderly.

Patients and methods: Consecutive patients with symptomatic, objectively confirmed, acute VTE were included in the MASTER registry in 25 Italian centers. Patients were followed-up for 24 months. Major clinical outcomes were death, recurrence of VTE and major bleeding. Cox regression analysis was used to assess major determinants of outcomes.

Results: A total of 2119 patients (49.8% males) were enrolled in the study, of whom 440 (20.8%) were >75 years and 1679 (79.2%) ≤75 years. Information on mortality at 2 years was available for 2021 patients (413 >75 years and 1608 ≤75 years) and information on VTE recurrence and bleeding events was available for 1988 patients (404 >75 years and 1584 ≤75 years). The 2-year cumulative incidence of mortality was 13.1% in patients >75 and 7.0% in patients ≤75, hazard ratio (HR) 1.52, 95% CI 1.09-2.13. Cancer (HR 3.44, 95% CI 1.94-6.09) was the only independent predictor of mortality in the elderly. The 2-year cumulative incidence of recurrent VTE was 6.4% in patients >75 and 6.2% in patients ≤75 (HR 1.05; 95% CI 0.67-1.63). The 2-year cumulative incidence of bleeding was 4.0% in patients >75 and 2.2% in patients ≤75, Odds Ratio 1.84; 95% CI 0.97-3.50.

Conclusions: As expected, long term mortality rates after acute VTE are significantly higher in patients >75 years than in younger patients. Rates of recurrent thrombotic events were similar between the two groups, whereas bleeding events were nearly twice as frequent in the elderly.

Corresponding Author: Walter Ageno, Department of Clinical Medicine, University of Insubria, Varese, Italy, agenow@yahoo.com

PREVENTION OF VENOUS THROMBOEMBOLISM IN AT-RISK MEDICAL PATIENTS IN THE USA

A.N. Amin 1, J. Lin 2, D. Wiederkehr 3

1 School of Medicine, University of California-Irvine, Orange, CA, USA; 2 Sanofi-Aventis, Bridgewater, NJ, USA; 3 Quintiles Consulting, Hawthorne, NY, USA

Keywords: venous thromboembolism; prophylaxis; readmission

Background/Aims: In addition to the well-recognized risk among surgical patients, hospitalized medical patients are at risk of venous thromboembolism (VTE). We conducted a real-world analysis of VTE rates and thromboprophylaxis prescribing patterns in US medical patients in hospital, and for 30 days post-discharge.

Methods: Data were extracted from the US Premier Perspective™-i3 Pharma Informatics linked database for medical patients at-risk of VTE (e.g. cancer, heart failure, severe lung disease, infectious disease) and admitted between January 2005–November 2007. Included patients were aged ≥ 18 years, with at least 6 months’ continuous plan enrollment. Patients were excluded if discharged to an acute-care facility or diagnosed with atrial fibrillation. VTE rates, and prophylaxis status and duration were evaluated for inpatients and then assessed post-discharge for 30 days.

Results: Of the 15,721 at-risk patients analyzed, 39% received inpatient pharmacological or mechanical thromboprophylaxis, and 3% received pharmacological prophylaxis in the outpatient setting. The total mean duration of prophylaxis was 2.16 (standard deviation 5.74) days, with a mean length of hospital stay of 4.36 (3.64) days. VTE occurred in 3.02% of patients during hospitalization. After discharge, 1.09% of patients were rehospitalized for VTE or treated for VTE in the outpatient setting. Lower inpatient VTE rates were found to correlate with medical conditions with higher inpatient prophylaxis rates (R2 = 0.72) (Figure). A low VTE rate also seemed to be related to longer prophylaxis duration (R2 = 0.19).

Conclusions: Our analysis demonstrates the substantial burden of VTE both during hospitalization and post-discharge, and highlights the current underuse of thromboprophylaxis in both settings. A negative correlation was found between inpatient VTE rates and prophylaxis rates. There is a need for improved thromboprophylaxis prescribing across the continuum of care in order to reduce the burden of avoidable VTE events.
Corresponding Author: Marta González, Corresponding Author: P. Saracco 1, C. Gentilomo 2, A.M. Lavdera 1, M. Agostini 1, R. Bagna 1, B. Bassi 1, A. Falanga 1, P. Giordano 1, C. Molinari 1, L. Ramenghi 1, P. Simioni 1, on behalf of The Group for the Italian Registry of Pediatric Thrombosis GIRTI – Gruppo Italiano per il Registro Trombosi Infantili

Keywords: childhood, thrombosis, registry

Background: Thromboembolism (TE) in newborns and children is becoming a rapidly increasing condition burdened by mortality and high morbidity. A dramatic increase in TE (from an annual rate of 34 to one of 54 cases per 10,000 admissions) has been reported in tertiary care hospitals in US between 2001-2007. Risk factors, clinical features and prognosis are dependent on age as well as on optimal treatment strategy. However randomised controlled trials are not available and most current treatment recommendation are extrapolated by adult studies. National and international registries have been created in various countries aiming at developing clinical trials to better understand and improve outcomes in children with TE. In 2008, a multi-centre research network of Italian investigators, promoted by the national Pediatric Scientific Associations, has developed a national prospective on-line registry of childhood TE based on a secure web database (RITI-www.trombosinfantili.it). The initiative has been supported by an Italian onlus association (ALT).

Aims: To explore the clinical features leading to computed tomography pulmonary angiography (CTPA) and to compare the outcomes of patients with PE vs without PE.

Method: Retrospective study of clinical and radiographic records of consecutive ICU patients undergoing a CTPA for suspected PE during their ICU stay at 2 hospitals in Canada and Italy. Data were collected on baseline characteristics, features leading to a suspicion of PE, CTPA findings and hospital mortality.

Results: Among 97 patients, PE was confirmed in 16 out of 103 (15.5%) CTPAs. Most CTPAs (71 of 103, 68.9%) were performed to evaluate the reason for ICU admission. Overall, the only clinical features distinguishing patients with PE vs without PE was hypotension (68.8% vs 47.1%, respectively, p=0.011) and decreasing O2 saturation in ICU (18.8% vs 3.4%, respectively, p=0.047). Among the 31.2% of patients with Negative PE during their ICU stay, the only clinical feature distinguishing distinguishing patients with PE vs those without PE was the suspicion or presence of deep vein thrombosis (50% vs 3.8%, respectively, p=0.015). Regarding other CTPA findings, pneumonia were significantly less frequent in patient with PE vs without PE (18.8% vs 51.7%, p=0.027). Patients with PE were more likely to die in hospital than those without PE (50% vs 33.3%, p=0.01). These results suggest that CTPA are used in younger patients, but with higher comorbidities, higher risk of bleeding and poorer prognosis. Follow up studies are needed to know clinical balance between risk-benefits of these devices.

Conclusion: These results demonstrate that sPESI can select patients with very low adverse events (sPESI 4.6% [95%CI, 2.3% to 7.0%] versus SI 12.8% [95% CI, 10.9-14.8]. These results suggest that VCF are used in younger patients, but with higher comorbidities, higher risk of bleeding and poorer prognosis. Follow up studies are needed to know clinical balance between risk-benefits of these devices.

Keywords: venous thromboembolism, bleeding
Background: The risk factors that affect the development of post-thrombotic syndrome (PTS) are not fully recognized, and it is difficult to reliably predict which patients are likely to develop PTS in acute phase of deep vein thrombosis (DVT). Aims: To investigate changes in calf muscle deoxygenated hemoglobin (HHb) levels after DVT, and to determine the indicative parameters reflecting the progression of PTS.

Methods: Seventy-six consecutive patients with a first episode of unilateral DVT were prospectively enrolled. Clinical manifestations were categorized according to the CEAP (Clinical, Etiologic, Anatomical, and Pathophysiologic) classification, and the patients were divided into no-PTS (C0-3Es,As,d,p,Pr,o) and PTS (C4-6Es,As,d,p,Pr,o) groups. Near-infrared spectroscopy (NIRS) was used to measure calf muscle HHb levels at 6 months after diagnosis of DVT. The calf venous blood filling index (HHbFI) was calculated on standing, and the venous ejection index and the venous retention index (HHbRI) were then obtained after exercise. All patients were followed up for more than 24 months after the diagnosis of DVT.

Results: Out of 76 patients evaluated, 20 (26.3%) had PTS. The proportion of iliofemoral DVT was significantly higher in patients who developed PTS than in patients who did not (P=0.043). The NIRS-derived HHbFI and HHbRI were significantly increased in patients who developed PTS in comparison with those who did not (P=0.04 and P=0.0001, respectively). HHbRI was significantly increased in patients with iliofemoral DVT in comparison with patients with calf DVT (P=0.041). An optimal cut-off point of 2.9 for HHbRI showed the strongest ability to predict the development of PTS, with a sensitivity of 100% and a specificity of 82.1%.

Conclusions: HHbRI as measured by NIRS is significantly increased in patients with iliofemoral DVT as compared with those with calf DVT. Furthermore, HHbRI > 2.9 is a strong predictor of the development of PTS at 6 months.

Corresponding Author: Takashi Yamaki, Department of Plastic and Reconstructive Surgery, Tokyo Women’s Medical University, 8-1, Kawada-cho, Shinjuku-ku, Tokyo, Japan, yamaki@grs.nwmu.ac.jp

Keywords: deep venous thrombosis, ankle fracture
DEMOGRAPHIC AND GRAVITY ANALYSIS OF PATIENTS WITH PULMONARY EMBOLISM

A. Martin-Quiros 1, C. Navarro San Francisco 1, S. Caro Bragado 1, N. Iniesta Arandía 1, A. Lorenzo Hernández 1, M. Rodríguez Dávila 1, M. Torres Sánchez 2, J. Camacho Siles 1, C. Fernández Capitán 1

1 Medicina Interna, 2 Radiodiagnóstico, Hospital Universitario la Paz, Madrid, Spain

Keywords: diagnosis, imaging test

Background/Aims: It is important to know epidemiologic characteristics of patients with pulmonary embolism (PE). The objectives are to describe demographic characteristics of patients with PE and to analyze differences according to severity.

Material and methods: Clinical records were analyzed from the database of thromboembolic disease (TED) from January-02/July-09 from a Third Level Hospital. Patients with PE were selected and compared according to the severity of PE using the shock index (heart rate/systolic blood pressure>1).

Results: Out of 583 patients with PE, 52% were women. Mean age was 66.48±17.5. Shock index>1 was present in 15% (86 patients). Mean age of non-severe PE was 67.05 years and 63.32 in severe PE (p<0.05). Age distribution is showed in the table. Overweight or more existed in 70% and comorbidity in 69.67% (p>0.05). In 94%, PE was in outpatient, of which 14.53% was severe. In admitted patients (6%, 35 patients), PE was severe in 18.92% (7). Antithrombotic prophylaxis (ATP) was prescribed in 94.29% (33). Patients with ATP had, as risk factor for TED: 0.06% (2) cancer and surgery; 0.03% (1), hip surgery; 0.09% (3) non-surgical immobilization, 0.06% (2) severe acute infection. There was a total of 21 (4.1%) deaths (3.9% vs. 5.2%, p>0.05). Six deaths related with PE. In remaining died patients: 1 bleeding at 80 days, 8 infection (5 after 3 months), 1 cardiac failure at 15 days, 1 pulmonary hypertension after 7 years and 5 for neoplastic progression after 3 months.

Conclusions: 1. Mean age is 66.48 years with differences according to severity: 63.32 vs 67.05 years, p<0.05. 2. Overweigh exists in 70.24% without severity differences. 3. PE was severe in 14.5% of outpatients and 19% in admitted patients. 5. Mortality was 4.1% without severity differences. Most of them were later than 3 months after PE diagnosis.

Table: Distribution by age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Global</th>
<th>Non severe</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>20-39</td>
<td>58</td>
<td>49</td>
<td>9</td>
</tr>
<tr>
<td>40-59</td>
<td>109</td>
<td>88</td>
<td>20</td>
</tr>
<tr>
<td>60-80</td>
<td>283</td>
<td>244</td>
<td>39</td>
</tr>
<tr>
<td>&gt;80</td>
<td>104</td>
<td>108</td>
<td>16</td>
</tr>
</tbody>
</table>

Corresponding Author: Alejandro Martin-Quiros, Hospital Universitario La Paz, Paseo de La Castellana 261, Madrid, Spain, botikin@gmail.com

ETOLOGIES AND TIME TO DEATH IN 1,142 CONSECUTIVE PATIENTS WITH ACUTE SYMPTOMATIC PULMONARY EMBOLISM

D. Sánchez 1, C. Wagner 1, V. Gómez 1, M. Soto 1, D. Kopocena 1, C. Zamarro 1, A. Sam 1, A. Sueiro 1, D. Jiménez 1

1 Ramón y Cajal Hospital and Alcalá de Henares University, Madrid, Spain; 2 Respiratory Department, Ramón y Cajal Hospital, Madrid, Spain

Background: Information regarding etiologies and time to death is important for the identification of low-risk patients with acute symptomatic pulmonary embolism (PE) who might benefit from an abbreviated hospital stay or outpatient therapy, and for the identification of high-risk patients who might benefit from more aggressive therapies, such as lysis.

Methods: This study included 1,142 consecutive patients with acute symptomatic PE. Outcomes were assessed during the first month after the diagnosis of acute PE. The primary outcome of the study was all-cause mortality. Mortality was assessed by using patient or proxy interviews, and/or hospital chart review. Interviews were performed through telephone and administered by local study personnel. Two investigators (D.J. and V.G.) adjudicated the cause of all deaths as (1) definite fatal PE, and (2) possible fatal PE, or (3) death from other causes. Death was judged to be definite fatal PE if it was confirmed by autopsy, or if death followed a clinically severe PE, either initially or shortly after an objectively confirmed recurrent event, in the absence of any alternative diagnosis. Possible fatal PE consisted of death in a patient who died suddenly or unexpectedly.

Results: Mortality data were available for all patients at the conclusion of the study. Overall, 115 out of 1,142 patients died (10.1%; 95% confidence interval [CI], 8.3% to 11.8%) during the first month of follow-up, and 50.4% of these were due to PE. Fifty-eight patients (58 of 1,142 patients; 5.1%; 95% CI, 3.8% to 6.3%) died from definite (n = 17) or possible PE (n = 41), whereas other deaths were caused by cancer (1.9%; 22 of 1,142 patients), infection (1.5%; 17 of 1,142 patients), major bleeding (0.6%; 7 of 1,142 patients), other diseases (0.7%; 8 of 1,142 patients), and unknown causes (0.3%; 3 of 1,142 patients). Time to death according to etiologies (PE vs. others) is shown in the Figure.

Conclusions: During the first month of follow-up, non-PE-related mortality is as frequent as PE-related mortality in patients with acute symptomatic PE. Cardiac biomarkers and imaging testings might be less useful for identifying low-risk patients for outpatient therapy.

Figure: Etiologies and time to death in 1,142 consecutive patients with PE.
RISK OF ACUTE DEEP VEIN THROMBOSIS IN HIGH RISK SOUTH ASIAN POPULATION: RESULTS OF A PROSPECTIVE RANDOMIZED CONTROL STUDY

U. Ballehanina, A. Murugesan, S. Chamber, D.N. Srivastava, A. Srivastava, A. Dhar

1 Department of Surgical Disciplines, All India Institute of Medical Sciences [AIIMS], New Delhi, INDIA; 2 Department of Radiodiagnosis, All India Institute of Medical Sciences [AIIMS], New Delhi, INDIA

Keywords: DVT, LMWH’s, venography

Background: Deep vein thrombosis (DVT) is one of the most common complications in post-operative patients. It is associated with considerable morbidity and mortality. Majority of patients with postoperative DVT are asymptomatic. The pulmonary embolism, which is seen in 10% of the cases with DVT, may be a fatal complication. Thus, it becomes imperative to prevent DVT rather than to diagnose and treat.

Context: Deep vein thrombosis in high risk South Asian patients undergoing major operations.

Aims: To document the risk of DVT following major operations and to evaluate the effectiveness of Nadroparin therapy in preventing postoperative DVT.

Materials and methods: Prospective randomised control study comparing effectiveness of Nadroparin therapy in preventing DVT in a cohort of 65 patients undergoing major abdominal operations.

Sixty-five patients were randomised preoperatively; Group-I received Nadroparin prophylaxis and Group-II no prophylaxis. The primary outcome DVT was assessed, seven to ten days after operation using bilateral lower limb venogram. Secondary parameters like adverse effects, intraoperative blood loss, operating time, postoperative platelet count, intraoperative blood transfusion requirements and the total duration of postoperative bed rest were also compared.

Statistical analysis used: The relative risk of DVT and secondary outcome measures among the patients receiving Nadroparin was compared to those in control group, using t-test, Chi-square test and paired samples test.

Results: There was no evidence of DVT in both the groups as documented using posterior platelet limb venogram, also there was no statistical difference among both groups in secondary parameters.

Conclusions: The incidence of DVT is very low or absent even among high risk South Asian population. More studies needed to find the physiologic basis of South Asian population. The LMWH’s did not increase the relative high immunity to DVT in this population. The LMWH’s did not increase the DVT incidence.

Table 1:

<table>
<thead>
<tr>
<th>Group</th>
<th>Moderate risk</th>
<th>High risk</th>
<th>Very high risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (N)</td>
<td>14</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Group II (C)</td>
<td>13</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>29</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2: Types of operations performed

<table>
<thead>
<tr>
<th>operation</th>
<th>Group-I (N)</th>
<th>Group-II (C)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystectomy</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>CBD exploration</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Rectal Operation</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Exploratory laparotomy</td>
<td>14</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Oesophagectomy</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ilio-Iguinal lymph node dissection</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>31</td>
<td>65</td>
</tr>
</tbody>
</table>

ADAPTATION OF THROMBOEMBOLIC DISEASE PROPHYLAXIS IN MEDICAL PATIENTS

A. A. Villagrasa Vilella, E. Ferrer Cobo, A. Acal Arias, B. Borjabad Gonzalez, L. Canas Alejandara

Department of Internal Medicine, Hospital de Granollers, Granollers, Barcelona, Spain

Keywords: thromboprophylaxis in medical patients

Background/Aims: The thromboembolic disease (TED) is a term that includes deep venous thrombosis (DVT) and pulmonary embolism (PE), since PE is considered a complication of DVT. PE is the third cause of death in hospitals. TED prophylaxis is aimed at prevention of DVT to reduce mortality as DVT itself as the risk of PE and mortality. Prophylactic measures include physical measures and pharmacological treatment (usually low-molecular-weight heparin, LMWH).

Granollers’ Hospital is a county hospital of 300 beds for acute cases, the Internal Medicine Service includes internal medicine and other medical specialities, and has 70 beds. The LMWH used in our hospital is enoxaparin.

Material and methods: We reviewed the medical history and prescribed treatment of patients admitted in the Internal Medicine Unit in 3 non-consecutive dates. We have collected demographic variables and the circumstances relating to current and previous thromboembolic disease. It has been calculated as an indication of thromboprophylaxis using PRETEMED index.

Results: We reviewed 64 patients, 53.8% men, mean age 68.7 ± 15.5. 13 (20%) were chronic anticoagulation at admission (69.2% for atrial fibrillation (AF), 15.4% by previous TED, 15.4% for having prosthetic valve).

In 3 (4.6%) the reason for admission involved the indication for anticoagulation (1 AF debut, 1 acute ischemic heart disease, 1 chronic AF previously not anticoagulated). 3 (4.7%) had contraindications for pharmacological prophylaxis (2 disorders of the coagulation for liver disease, 1 High Digestive Hemorrhage).

Of the 37 patients with indication for prophylaxis with LMWH, 25 (73.5%) of those with no contraindication to receiving enoxaparin dosage, 3 (8.8%), receiving enoxaparin at insufficient doses and 6 (17.6%), received no pharmacological prophylaxis of the MTE. Of the 12 patients with no indication of drug prophylaxis, 4 (33.3%) were treated with enoxaparin.

Conclusions: Thromboprophylaxis in medical patients in our hospital is suboptimal.

Table 1: PRETEMED index

<table>
<thead>
<tr>
<th>Adjusted Risk</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Physical measures</td>
</tr>
<tr>
<td>4</td>
<td>Prophylaxis with LMWH is suggested</td>
</tr>
<tr>
<td>&gt;4</td>
<td>Prophylaxis with LMWH is recommended</td>
</tr>
</tbody>
</table>

Table 2: PRETEMED prophylaxis recommendation

<table>
<thead>
<tr>
<th>PRECIPITATING PROCESS</th>
<th>ADJUSTED WEIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pregnancy/postruminal Air travel &gt;6 hours</td>
<td>Active inflammatory intestinal disease Serious acute infection Malignancy Cardiac Failure NIHY III</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Active inflammatory intestinal disease Serious acute infection Malignancy Cardiac Failure NIHY III</td>
<td>CVV with cogen limbs paralysis CPOO serious decompensation AMI Heart Failure IV Lower limbs traumatism with no surgery</td>
</tr>
</tbody>
</table>

ASSOCIATED PROCESS

<table>
<thead>
<tr>
<th>ASSOCIATED PROCESS</th>
<th>PRECIPITATING PROCESS</th>
<th>ADJUSTED WEIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Mellitus</td>
<td>Hyperosmolarity</td>
<td>Nephrotic syndrome Thrombophylia Previous DVT Vasculitis (Bleotc/Wegener)</td>
</tr>
<tr>
<td>HIV infection</td>
<td>Lower limbs paralysis</td>
<td>Previous DVT Vasculitis (Bleotc/Wegener)</td>
</tr>
<tr>
<td>Previous DVT</td>
<td></td>
<td>Previous DVT Vasculitis (Bleotc/Wegener)</td>
</tr>
</tbody>
</table>

DRUGS

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>ADJUSTED WEIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulants</td>
<td>Anticoagulants inhibitors</td>
</tr>
<tr>
<td>Anticoagulants inhibitors</td>
<td>Anticoagulants inhibitors</td>
</tr>
<tr>
<td>Anticoagulants inhibitors</td>
<td>Anticoagulants inhibitors</td>
</tr>
</tbody>
</table>

OTHERS

<table>
<thead>
<tr>
<th>OTHERS</th>
<th>ADJUSTED WEIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central venous catheter</td>
<td>Bed rest &gt; 4 days</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>Bed rest &gt; 4 days</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>Bed rest &gt; 4 days</td>
</tr>
</tbody>
</table>

Corresponding Author: Umashankar Ballehanina, All India Institute of Medical Sciences [AIIMS], New Delhi, India, umashankarkb@gmail.com

Corresponding Author: Ares Aurora Villagrasa Vilella, Hospital de Granollers, Av. Francesc Ribas s/n, Granollers, Spain,aremosv@gmail.com
**P463**

**THROMBOSIS AND CELIAC DISEASE: REVIEW OF 5 CASES**

**D. Hakem, S. Médaoud, A. Boudjelida, H. Lafer, D. Bensalah, D. Meslouh, L. Stof, N. Ouadahi, A. Berrah**

Department of Internal Medicine, Dr Mohammad-Lamine Debagueh Hospital, Bab-El-Oued University Hospital Centre, Algiers, Algeria

**Keywords:** celiac disease, Budd-Chiari, cerebral venous thrombosis

**Background:** Celiac disease (CD) is an autoimmune enteropathy and is part of a panel of disorders recognized as being thrombogenic.

**Aims:** To review some case reports of venous thrombosis (VT) of unusual sites associated with a CD.

**Patients and methods:** Retrospective study of singular sites of VT occurred in CD and collected in internal medicine practice from January 2000 to December 2009 (individual recruitment).

**Results:** 5 patients are studied, 5 women, average age is 44 years (21- 65). VT is localized in abdomen (4) realized Budd-Chiari Syndrome (2) portal cavernous (2) and in brain (1). VT revealed CD in 4 times (abdominal VT). The last case of CD is localized in abdomen (5) realized Budd-Chiari Syndrome (2) portal cavernous (2) and in brain (1). VT revealed CD in 4 times (abdominal VT). The last case of CD is localized in abdomen (5) realized Budd-Chiari Syndrome (2) portal cavernous (2) and in brain (1). VT revealed CD in 4 times (abdominal VT). The last case of CD is localized in abdomen (5) realized Budd-Chiari Syndrome (2) portal cavernous (2) and in brain (1). VT revealed CD in 4 times (abdominal VT).

**Discussion:** Cerebral venous thrombosis and abdominal thrombosis are unusual mode of revelation of CD complicated to malignant degeneration, an acquired thrombophilia, an hepatocellular insufficiency and a severe malabsorption (Vit B12 deficiency).

**Conclusions:** Unusual sites of thrombosis in CD are rare. Maghrebine literature report series of VT in CD by many authors so it is necessary to considered this affection as an etiological cause of VT, particularly in these areas.

**Corresponding Author:** Djanette Hakem, Dr Mohammad-Lamine Debagueh Hospital, Bab-El-Oued University Hospital Centre, Bd Said Touati, Bab-El-Oued, Algiers City; Algeria, hakem-dj@yahoo.fr

---

**P142**

**TIME-TRENDS IN TREATMENT AND CARDIOVASCULAR EVENTS IN PATIENTS WITH HEART FAILURE: A PHARMACOSURVEILLANCE STUDY**


1 Academic Medical Center, Department of Vascular Medicine, Amsterdam, Netherlands; 2 City Hospital Birmingham, UK; 3 Utrecht University, Utrecht, Netherlands;

**Keywords:** epidemiology, heart failure, pharmacotherapy, prognosis

**Aims:** Prognosis for patients with a first hospitalisation for heart failure (HF) may have improved, but data beyond 2003 are lacking. We assessed the temporal relationship of cardiovascular events and treatment in patients with a first hospitalisation for HF between 1998-2007.

**Methods:** Data were obtained from the PHARMO Record Linkage System, a Dutch population-based registry of pharmacy records linked with hospital discharge records. Patients were selected based on a first hospital discharge of documented HF. Two time-periods were compared: 1998-2002 and 2003-2007. We analyzed all prescribed cardiovascular medication and the occurrence of events within the first year after hospitalisation for HF. Cardiovascular events were defined as rehospitalisation for HF, myocardial infarction or stroke; ischemic events as myocardial infarction or stroke. Logistic and Cox regression analyses was performed to calculate odds ratios (OR), hazard ratios (HR), and 95% confidence intervals (CI) for each of the two time-periods.

**Results:** We identified 17,921 patients (8374 between 1998-2002, 9,547 between 2003-2007). Mean age was 75±11 and 76±11 years, respectively. There was an increase in almost all prescriptions in the second period, particularly beta-blockers (Table). In the first year after hospitalisation there was no clear reduction in the risk for any cardiovascular event between the two time-periods. The incidence of ischemic events was reduced in the second time-period compared to the first.

**Conclusion:** This large study shows that prescription of cardiovascular medication in patients with a first hospitalisation for HF increased in recent years, while the incidence of ischemic events decreased.

**Table**

<table>
<thead>
<tr>
<th>Prescriptions</th>
<th>1998-2002 (%)</th>
<th>2003-2007 (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAAS-inhibitors</td>
<td>45.29 (64.1)</td>
<td>54.45 (57.0)</td>
<td>1.13 (1.08-1.20)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>5052 (36.4)</td>
<td>5533 (55.9)</td>
<td>2.21 (2.08-2.34)</td>
</tr>
<tr>
<td>Vitamin K antagonists</td>
<td>2456 (29.3)</td>
<td>3203 (33.5)</td>
<td>1.22 (1.14-1.30)</td>
</tr>
<tr>
<td>Antiplatelet agents</td>
<td>2379 (28.4)</td>
<td>3651 (32.0)</td>
<td>1.18 (1.11-1.26)</td>
</tr>
<tr>
<td>Statins</td>
<td>1397 (15.6)</td>
<td>2719 (28.5)</td>
<td>2.15 (2.00-2.32)</td>
</tr>
</tbody>
</table>

**Events in the first year**

<table>
<thead>
<tr>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cardiovascular events (including rehospitalisation HF)</td>
</tr>
<tr>
<td>Ischemic events only (myocardial infarction or stroke)</td>
</tr>
<tr>
<td>Pulmonary embolisms</td>
</tr>
</tbody>
</table>

**Corresponding Author:** Olan Universities, Amsterdam, the Netherlands; o.r.depeuter@amc.uva.nl
CHEST PHYSICIANS’ KNOWLEDGE OF APPROPRIATE THROMBOPROPHYLAXIS: FINDINGS FROM THE PROMOTE STUDY

B. Bikdeli 1,2, B. Sharif-Kashani 1, S. Raeizi Roodhashi 1, P. Shahabi 1, S. Ehteshami-Afsar 1, M. Masjedi 1

1 National Research Institute of Tuberculosis and Lung Disease, Masih-Daneshvari Hospital, Shahid Beheshti University MC, Tehran, Iran; 2Cardiovascular Research Center, Shahid Beheshti University MC, Tehran, Iran

Keywords: VTE prophylaxis, knowledge, questionnaire

Introduction: Venous thromboembolism (VTE) is a major cause of morbidity and in-hospital mortality. Several guidelines recommend thromboprophylaxis for at-risk patients, however, guideline adherence is missing worldwide. The PROMOTE (Prophylaxis-fOrMOnetoProphylaxis-assessment) questionnaire was designed to evaluate the knowledge of chest physicians regarding VTE prophylaxis.

Methods: The questionnaire was developed using a hierarchic method to encompass the most important issues regarding thromboprophylaxis and contained five background questions and thirteen clinical scenarios each covering one or more aspects of VTE prophylaxis. During the 4th International Congress on Pulmonary Disease, Intensive Care and Tuberculosis, the questionnaire was distributed to the chest physicians (pneumologists, thoracic surgeons, intensive-care specialists, cardiologists and internists). The 8th edition of the American College of Chest Physicians (ACCP) guidelines on antithrombotic and thrombolytic therapy were used to evaluate thromboprophylaxis appropriateness.

Results: 83 completed questionnaires were received (response rate: 37.1%). The most commonly cited VTE risk factors were: bedridden state (80%), surgery (68%), cancer (61.2%), obesity (52.5%), hypercoagulability (47.5%), old age (41.2%), VTE history (40%), hygrostatic state (36.2%), heart failure (30%), stroke (22.5%), and COPD (22.5%). Overall appropriate response rate to the questions was 67.7% (95%CI: 64.5%-71%). Cardiologists, and surgeons had the most and the least appropriate responses (77.1%, and 62.7%, respectively). The most striking knowledge gaps were about improper low-molecular-weight heparin dosing (failure rates of 66.2% and 58.1% for two different clinical scenarios), inadequate use of non-pharmacological prophylaxis for those with contraindications to anticoagulants (failure rates of 56.9%, and 39.2% in two separate scenarios), and inadequate prophylactic measures for young patients undergoing major surgical procedures (failure rate: 52.6%).

Conclusions: Lack of proper knowledge could partly justify the huge gap between the guidelines recommendations and the current VTE prophylaxis practice. PROMOTE is the first systematically-developed questionnaire to address the VTE prophylaxis knowledge assessment amongst the chest physicians and might be a useful tool to improve VTE prophylaxis state.

Corresponding Author: Behnood Bikdeli, National Research Institute of Tuberculosis and Lung Disease, Masih-Daneshvari Hospital, Shahid Beheshti University MC and Cardiovascular Research Center, Shahid Beheshti University MC, Tehran, Iran, bikdeli@sbhm.ac.ir

PREVENTING VENOUS THROMBOEMBOLISM – POLICIES, PROGRAMS AND PROGRESS IN AUSTRALIA

D. MacLellan 1, J.P. Fletcher 2

1 Health Services Performance Improvement Branch, New South Wales Department of Health, Sydney, NSW, Australia; 2Department of Surgery, University of Sydney, New South Wales Department of Health, Sydney, NSW, Australia

Keywords: VTE, prevention, policies

Background: While extensive evidence exists to guide the proper prophylaxis and treatment of venous thromboembolism (VTE), several studies show that the evidence is not being followed. In a large multinational cross sectional survey of hospitalised patients identified at risk for VTE, only approximately 50% of these at risk patients were receiving appropriate prophylaxis. The burden of VTE is considerable and a recent study in Australia estimated the total cost of VTE per patient per annum including medical costs, lost productivity etc was AS 475,150 (US$ 334,000). Appropriate VTE prophylaxis and prevention delivers significant cost benefits.

Aim: To compare the policies available and progress in ongoing VTE prevention in Australian States and their progress in VTE prevention in hospitalised patients.

Methods: The questionnaire was developed using a hierarchic method to encompass the most important issues regarding thromboprophylaxis and contained five background questions and thirteen clinical scenarios each covering one or more aspects of VTE prophylaxis. During the 4th International Congress on Pulmonary Disease, Intensive Care and Tuberculosis, the questionnaire was distributed to the chest physicians (pneumologists, thoracic surgeons, intensive-care specialists, cardiologists and internists). The 8th edition of the American College of Chest Physicians (ACCP) guidelines on antithrombotic and thrombolytic therapy were used to evaluate thromboprophylaxis appropriateness.

Results: 83 completed questionnaires were received (response rate: 37.1%). The most commonly cited VTE risk factors were: bedridden state (80%), surgery (68%), cancer (61.2%), obesity (52.5%), hypercoagulability (47.5%), old age (41.2%), VTE history (40%), hygrostatic state (36.2%), heart failure (30%), stroke (22.5%), and COPD (22.5%). Overall appropriate response rate to the questions was 67.7% (95%CI: 64.5%-71%). Cardiologists, and surgeons had the most and the least appropriate responses (77.1%, and 62.7%, respectively). The most striking knowledge gaps were about improper low-molecular-weight heparin dosing (failure rates of 66.2% and 58.1% for two different clinical scenarios), inadequate use of non-pharmacological prophylaxis for those with contraindications to anticoagulants (failure rates of 56.9%, and 39.2% in two separate scenarios), and inadequate prophylactic measures for young patients undergoing major surgical procedures (failure rate: 52.6%).

Conclusions: Lack of proper knowledge could partly justify the huge gap between the guidelines recommendations and the current VTE prophylaxis practice. PROMOTE is the first systematically-developed questionnaire to address the VTE prophylaxis knowledge assessment amongst the chest physicians and might be a useful tool to improve VTE prophylaxis state.

Corresponding Author: Behnood Bikdeli, National Research Institute of Tuberculosis and Lung Disease, Masih-Daneshvari Hospital, Shahid Beheshti University MC and Cardiovascular Research Center, Shahid Beheshti University MC, Tehran, Iran, bikdeli@sbhm.ac.ir

IMPROVEMENT IN VENOUS THROMBOEMBOLISM PROPHYLAXIS RATES WILL BE SLOW OR NON-EXISTENT. IN MANY RESPECTS, VTE PREVENTION IS NOT BEING FOLLOWED. IN A LARGE MULTINATIONAL CROSS SECTONAL SURVEY OF HOSPITALISED PATIENTS IDENTIFIED AT RISK FOR VTE, ONLY APPROXIMATELY 50% OF THESE AT RISK PATIENTS WERE RECEIVING APPROPRIATE PROPHYLAXIS. THE BURDEN OF VTE IS CONSIDERABLE AND A RECENT STUDY IN AUSTRALIA ESTIMATED THE TOTAL COST OF VTE PER PATIENT PER ANNUM INCLUDING MEDICAL COSTS, LOST PRODUCTIVITY ETC WAS AS 475,150 (US$ 334,000). APPROPRIATE VTE PROPHYLAXIS AND PREVENTION DELIVERS SIGNIFICANT COST BENEFITS.
AETIOLOGIES OF JUGULAR THROMBOSIS OBSERVED IN INTERNAL MEDICINE PRACTICE

D. Hakem, N. Hamzaoui, N. Ouadahi, D. Zemmour, M. Boucelma, M. Ibrir-Khati, A. Berrah

Dr Mohammad-Lamine Debaghine, Bab El Oued universitary Hospital Centre, Algiers City, Algeria

Keywords: jugular thrombosis, lemiriere syndrome

Background: Deep vein thrombosis is frequently observed in internal medicine practice but the jugular localizations are rare and this imposes to determine imperatively their aetiology

Aims: To review the main aetiology of jugular thrombosis observed in our practice.

Patients and methods: Retrospective and descriptive study from January 2000 to December 2009 in an internal medicine center. The studied items relate to history, clinical presentation, biological and morphological additional investigations, therapeutic modalities and the following up.

Results: We brought together 13 cases, 7 women and 6 men. The average age was 49 years. These patients showed in the majority of the cases a stereotypical suggestive symptomatology made by chelodolynias with oedema filling the bottom of bag known as clavicular. The found causes were infectious pathology in 3 cases (Lemierre syndrome in two cases), neoplasia in 2 cases (lung, colon), thrombophila in 2 cases, systemic erythemic lupus associated with a syndrome of antiphospholipids (2) and with a nephritic syndrome is identified (1), Behcet’s Disease (3) and finally 1 case staged of thrombophilia in 1 year of follow-up. All the patients were treated by anticoagulant treatment associated with the treatment of the identified cause (antibiotic therapy, corticosteroids, antimalarial drugs, antineoplastic chemotherapy, immuno-suppressives drugs, etc.). The evolution was favorable (on the general and vascular plan) in all patients except for patients with neoplasia.

Conclusion: Diagnosis of the jugular thrombosis can be strongly suspected by clinical signs and confirmed secondarily by the additional examinations mostly limited to an echo vascular doppler method and angio IRM (neck, brain). The prognosis depends strictly on the aetiology and the precocity of the care. Even if the ‘good-hearted’ (‘benignant’) causes (in particular those of infectious origin) are curable, regrettable the thromboses which accompany neoplasias or chronic inflammatory diseases constitute events which come to darken even more the prognosis of these diseases (recurrence, extensive and propagating thrombosis, etc.).

Corresponding Author: Djianeete Hakem, Dr Mohammad-Lamine Debaghine, Bab El Oued universitary Hospital Centre, Said Touati Street, Algiers City, Algeria, hakem_dj@yahoo.fr

P434

THROMBIN GENERATION AND OTHER COAGULATION MARKERS IN PATIENTS WITH LIVER CIRRHOSIS

E. Papakonstantinou 1, E. Yfantis 1, A. Theofani 1, T. Loukas 1, I. Rapti 2, E. Dimou 2, C. Mantì 1, S. Hadziyannis 2

1 Hematology Department, Triassion General Hospital, Athens, Greece; 2 Hepatology Department, Henry Dunant Hospital, Athens, Greece

Keywords: thrombin generation, cirrhosis

Background: The role played by coagulation defects in the occurrence of bleeding in cirrhosis is unclear. Conventional coagulation tests (PT/INR, aPTT) seem unable to predict the severity of bleeding problems in patients with liver cirrhosis, possibly because they do not adequately reflect the balance between procoagulant and anticoagulant clotting factors. Recently a test has become available to routinely measure the endogenous thrombin generation potential (ETP) by Dade Behring (Germany).

Aims: The comparison of ETP values and other coagulation markers between controls and patients with liver cirrhosis.

Methods: 56 samples of consecutive patients with histologically confirmed liver cirrhosis, and 30 samples of controls were investigated for PT/INR, fibrinogen, D-dimers and ETP parameters. We used the chromogenic method on the fully automated Behring Coagulation System (BSC) for the measurement of thrombin generation parameters.

Results: 6 patients had alcoholic cirrhosis, 22 HCV, 5 PBC, 7HBV, 1HBV and HDV and 13cirrhosis of unknown origin.

<table>
<thead>
<tr>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Ttage</td>
<td>19.3</td>
</tr>
<tr>
<td>Max sep</td>
<td>54.3</td>
</tr>
<tr>
<td>CazmaAlain</td>
<td>123.5</td>
</tr>
<tr>
<td>ETPmU</td>
<td>394.7</td>
</tr>
<tr>
<td>INR</td>
<td>0.8</td>
</tr>
<tr>
<td>FIBmg/l</td>
<td>403.9</td>
</tr>
<tr>
<td>DDmg/l</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The automated ETP test can play an important role in the evaluation of haemostatic liver function in patients with cirrhosis. A potential clinical implication of these findings is that the laboratory investigation of the coagulation function, presently performed with the PT and APTT, may be inadequate to assess the true risk of bleeding when patients with cirrhosis undergo invasive procedures such as liver biopsy and transplant surgery. Perhaps the measurement of thrombin generation might be more suitable to evaluate the hemorrhagic risk. Although plausible, this hypothesis needs to be sustained clinically by a prospective study

Corresponding Author: Eumorfia Papakonstantinou, Hematology Department Triassion General Hospital, Athens Greece. leshi@iol.gr

P629

PERSISTENCE OF RESIDUAL THROMBUS IN PATIENTS WITH IDIOPATHIC VENOUS THROMBOSIS AND ITS RELATIONSHIP WITH OTHER RISK FACTORS FOR RECURRENTNESS

D. Piñar Cabezos 1, E. Caro 1, M.D. Jover 1, J. Cama 1, J.F. Almazán 1, M. Waez 1, P. Marco 1, R. Sanchez 1

1 Internal Medicine Department, Alicante General University Hospital, Alicante, Spain; 2Hematology Department, Alicante General University Hospital, Alicante, Spain

Keywords: thrombus residual, relapse thrombosis

Background/Aims: The persistence of venous thrombosis after a first episode of unprovoked venous thrombosis has been associated with the risk of recurrent event. Several recent articles have examined the relationship between residual venous obstruction and D-dimer levels. The aim of this study is to analyze the relationship between the persistence of venous thrombus and the presence of thrombophilia and postphlebitic syndrome.

Methods: Prospective study: we included all patients with a first episode of idiopathic venous thrombosis, diagnosed between January 2005 and January 2008. Six months after the first episode all patients underwent compression ultrasonography, a thrombophilia study, a d-dimer level and a clinical re-evaluation searching for postphlebitic syndrome.

Results: 84 patients were analysed, with a minimum follow-up time of six months and a maximum of two years, median of 17 months (12-24). 53.6% were men. The average age was 61.7 ± 18.4 years (range 19-91 years). After the acute phase, all patients were treated with oral anticoagulants or low-molecular-weight heparin. An echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively. Echo Doppler was performed to 58 patients, 25 (29.8%) had residual thrombus and 33 (39.3%) were normal. 70% of patients with abnormalities in Doppler and postphlebitic syndrome were aimed at 32.1% and 3.7% respectively.

Conclusion: The persistence of venous thrombosis after six months of treatment in patients with a first episode of idiopathic venous thromboembolic disease is associated significantly with development of postphlebitic syndrome. In this study there was no association with the presence of thrombophilia and relapse.

Corresponding Author: Diana Piñar Cabezos, Internal Medicine Dept, Alicante General University Hospital, Maestro Alonso S/n, PC 03012, Alicante, Spain, sancrez_rosmar@gva.es
P612

VENOUS THROMBEMBOLISM AFTER ORTHOPAEDIC MAJOR SURGERY IN PATIENTS WITH CORRECT THROMBOPROPHYLAXIS


Hospital Povisa, Vigo, Spain

Keywords: thrombembolism, orthopaedic major surgery, thromboprophylaxis

Background: Venous thromboembolism (VTE) prophylaxis is routinely administered during the in-hospital period and after discharge in patients who undergo orthopedic major surgery (OMS). However, VTE risk may persist and the standard duration of thromboprophylaxis may not provide adequate protection.

Aims: To describe the clinical characteristics, risk factors, complications and evolution of VTE after OMS in patients with appropriate prophylaxis.

Materials and methods: A retrospective, descriptive study was carried out of the histories of patients diagnosed of VTE between January 2000 and December 2008. In all patients the thromboprophylaxis regimen was continued for 30 days and in 39 patients occurred within 3 months after surgery.

Results: 41 patients were diagnosed of VTE (63% female; mean age 67.8 years; average stay 16.4 days). Type of surgery: hip fracture: 12; hip replacement: 10; knee replacement: 6; ankle fracture: 6; femur fracture: 3; other fractures 4. Time from surgery 32.3 days. During in-hospital period 13 episodes occurred and 28 occurred after discharge (the prophylaxis period had finished in 17). Risk factors are present in 15 patients: previous VTE (5), venous insufficiency (4), cancer (3), stroke (4), COPD (4), BMI >30 (4), smoking (3), chronic heart failure (2), and thrombophilia (1). Screening for thrombophilia was carried out in 10 patients and was positive in 9: hyperhomocysteinemia (4), heterozygote/homozygote carrier of C677 MTHF (3/2), protein C deficiency (2) and homozygous carrier of prothrombin G202010A (1). All patients received anticoagulant treatment for at least 3 months and was permanent in 12 cases (10 with atrial fibrillation and 2 for recurrent VTE). Two patients developed major bleeding, one died for sepsis and recurrent VTE occurred in 2 cases.

Conclusions: Despite an appropriate prophylaxis some patients develop VTE after OMS because the risk is present for more time than prophylaxis is usually recommended. Screening for thrombophilia may detect unsuspected thrombotic defects.

Corresponding Author: Laura Gonzalez Vazquez, Hospital Povisa, Calle Salamanca Nª 5, Vigo, Spain, 25116Vigo.com

P410

BIOCHIP ARRAY PROFILING OF THE MEDIATORS OF INFLAMMATION, D-DIMER AND THROMBOMODULIN IN END STAGE RENAL DISEASE (ESRD)

V. Bansal, R. Davis, E. Litinas, I. Thethi, D. Hoppensteadt, J. Fareed, Loyola University Chicago, Maywood, IL, USA

Keywords: inflammation, end stage renal disease, biochip array

The purpose of this study is to profile several inflammatory mediators in order to better understand their role in the underlying mechanism of vascular changes in ESRD. Plasma samples from 49 patients with ESRD were collected prior to maintenance hemodialysis sessions. A group of 56 normal individuals, both male and female, was included as control. Cerebral Artery II chips were used in the RandomX® system to simultaneously measure Neuron Specific Enolase (NSE), Neutrophil Gelatinase-associated Lipocalin (NGAL), Soluble Tumor Necrosis Factor Receptor 1 (TNFRI), D-Dimer, Thrombomodulin (TM), and C-reactive protein (CRP). As compared to the normal individual, all of the markers studied showed an upregulation in patients with ESRD. Most notably, TNFRI showed a 19.8 fold increase in patients with ESRD (mean 7.8 ± 2.8 ng/ml, range 0.8 to 13.7) compared to the control (mean 0.4 ± 0.2, range 0.1 to 1.0). TM was increased 5.2 fold (mean 6.5 ± 2.6, range 0.7 to 14.1) compared to control (mean 1.2 ± 0.6, range 0.6 to 2.3). Similarly, NGAL showed a 4.6 fold increase (mean 1390 ± 257, range 406 to 1729), compared to control (mean 299 ± 99, range 115 to 603), and CRP a 4.2 fold increase (mean 5.7 ± 4.2 ug/ml, range 0.6 to 13.2) compared to control (mean 1.4 ± 1.7, range 0.2 to 11.4). DD and NSE were also increased 3.0 and 1.8 fold respectively. These studies show that some newer markers such as TNFRI, NGAL and NSE are upregulated in ESRD. The marked increase in TM is highly suggestive of endothelial damage. Similarly, the increase in TNFRI supports a state of increased cellular damage. The elevations in NGAL and CRP imply a state of increased inflammation and indicate a polypathologic process which may predispose ESRD patients to both cardiovascular and cerebrovascular thromboembolic events.

Corresponding Author: Vinod Bansal, Loyola University Chicago, 2160 S. First Avenue, Maywood, IL, USA, dbhopen@lumc.edu

P452

ULTRASOUND MONITORING OF LONG TERM CENTRAL VENOUS CATHETERS, OUR EXPERIENCE IN THE LAST TWO YEARS

M. Boddi, M. Bernetti, S. Berardino, A. Berni, B. Chellini, F. De Antoniis, R. Abbate

Laboratory of Internistics and Vascular Ultrasound, Department of Medical and Surgical Critical Care, University of Florence, Florence, Italy

Keywords: central venous catheter, (CVC), CVC complications, deep vein thrombosis

Background: The widespread use of CVC has exponentially increased the request for echocolorDoppler examination to optimize the management of CVC complications, mainly deep vein thrombosis (DVT). However, the ultrasound “surveillance” of CVC is currently being carried out according to different protocols, shared, since guide-lines are not available. We reviewed, retrospectively, the prevalence of CVC related DVT and/ or fibrin-sheet detected by echocolorDoppler in patients observed in the last two years.

Materials and methods: Between December 2007 and December 2009, 353 echocolorDoppler of subclavian-jugular vein were performed in oncologic patients endowed with long-term CVC, 134 men, aged 18-85 years (mean age 55±14). We considered as positive the US tests that showed the presence of intraluminal thrombotic material involving the venous wall (sub-occlusive or occlusive DVT), or fibrin-sieve without wall involvement. US exams were performed in 173 patients without clinical suspicion of complications (control), to assess the diagnostic efficacy of US test alone. We repeated US exams in the remaining 54 for clinical suspected CVC complications (inflammation and thrombosis). The remaining 126 US exams were performed before CVC removing.

Results: 75/353 examinations (21.2%) were positive for CVC-related apposition or DVT. And 19% of the 126 US exams performed before removing were positive. Compared to US exams for former control, the frequency of positive Doppler was significantly higher in the group of clinically suspected CVC complications (p=0.039) and the probability of US test positivity doubled when CVC complications were clinically suspected (OR 2.12, 95% CI 1.07-4.19, p=0.03). On the contrary, in the absence of clinical suspect, the probability of US test positivity halved (OR 0.47, 95% CI 2.24-0.93, p=0.029).

Conclusions: In the ultrasound monitoring of CVC-related complications, the pretest probability based on clinical suspect of complications was significantly related to the presence of thrombosis. According to our data ultrasound CVC surveillance in absence of clinical suspected complications is questioned.

Corresponding Author: Maria Boddi, University of Florence, Via Morgagni 85, Florence, Italy, m.boddi@dac.unifi.it
EVALUATION OF A NEW AUTOMATED PANEL OF ASSAYS FOR THE DETECTION OF ANTI-PF4/HEPARIN ANTIBODIES IN PATIENTS SUSPECTED OF HAVING HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

C. Legnani, M. Cini, C. Pili, O. Boggian, M. Frascaro, G. Palareti

Department of Angiology and Blood Coagulation “Marino Golinelli”, University Hospital S. Orsola-Malpighi, Bologna, Italy

Keywords: HIT, diagnosis, immunassay

Background/Aims: HIT is a life-threatening complication of heparin treatment; the prognosis depends on early and accurate diagnosis, and prompt start of alternative anticoagulants. Because of high sensitivity, the commercially available immunological assays are widely used, though not suited to be run on single samples and with a turnaround time of 2-3 hours. We evaluated two new, rapid, automated, quantitative chemiluminescent immunassays in HIT suspected patients: HemosIL AcuStar HIT-IgG(PF4-H) (specific for IgG anti-PF4/heparin antibodies) and HemosIL AcuStar HIT-Ab(PF4-H) (detecting IgG, IgM and IgA anti-PF4/heparin antibodies) (Instrumentation Laboratory).

Methods and results: HIT confirmation/exclusion was based on the flow chart proposed by Pouplard et al. (J Thromb Haemost 2007), which combines the results of the HIT pretest probability (PTP), estimated by the “4Ts” clinical score, and of the ID-Heparin PF4 PaGIA, a rapid immunoenzymatic. In patients with positive ID-Heparin PF4 PaGIA test and in those with negative ID-Heparin PF4 PaGIA but with high PTP, a platelet aggregation assay was also performed. 102 patients with suspected HIT were included; HIT was diagnosed in 17 (16.7%). No false negative cases were observed using either the HemosIL AcuStar HIT-IgG(PF4-H) or the HIT-Ab(PF4-H) assay (sensitivity and negative predictive values = 100%; negative likelihood ratios <0.01). The specificity was higher for the HemosIL AcuStar HIT-IgG(PF4-H) in comparison with that of the HemosIL AcuStar HIT-Ab(PF4-H) (96.5% vs 81.2%). Higher values of the HemosIL AcuStar HIT-IgG(PF4-H) were associated with increased HIT PTP. Patients with confirmed HIT and thrombocytopenic disorders had significantly higher levels of HemosIL AcuStar HIT-IgG(PF4-H) than those without thrombocytopenic complications.

Conclusions: The HemosIL AcuStar HIT-IgG(PF4-H) and HIT-Ab(PF4-H) assays showed a very high sensitivity and therefore they can reliably be used to rule out HIT in suspected patients. The diagnostic specificity was greatly increased by using the HemosIL AcuStar HIT-IgG(PF4-H). The assays are reproducible (CVs <6%), rapid (turnaround time 30 min), automated, quantitative, and can be run for single sample testing.

Corresponding Author: Cristina Legnani, University Hospital S. Orsola-Malpighi, Via Albertoni, 15, Bologna, Italy, cristina.legnani@asnp.bo.it

Keywords: lupus anticoagulant, antiphospholipid syndrome

NEW GUIDELINES FOR LA: SENSITIVITY AND SPECIFICITY OF ICA IN MIXING STUDIES AND % OF CORRECTION IN CONFIRMATORY TEST CUT-OFF VALUES OBTAINED WITH PLASMAS FROM HEALTHY CONTROL

M. Martinuzzo, G. Cerrato, M.L. Iglesias Varela, Y. Adamczuk, R.R. Forastieri

Hematology, Favaloro Foundation University Hospital, Favaloro University, Buenos Aires, Argentina

Keywords: lupus anticoagulant, antiphospholipid syndrome

Background/Aims: The updated guidelines for LA diagnosis indicate locally calculate the index of circulating anticoagulant (ICA) for mixing studies and % of correction (%C) or normalized ratio (NR) for confirmatory tests. Our aim was to calculate the index of circulating anticoagulant (%C) or normalized ratio (NR) for mixing studies. The cut-off values for %C confirm or normalized ratio (NR) confirm APTT show good sensitivity and high specificity, but for dRVVT show low sensitivity and high specificity. The cut-off values for %C confirm APTT has high sensitivity but low specificity, whereas for dRVVT show lower sensitivity and high specificity. The cut-off values demonstrate that ICA-APTT has high sensitivity but low specificity, whereas for dRVVT show lower sensitivity and high specificity. The cut-off values for %C confirm APTT has high sensitivity but low specificity, whereas for dRVVT show lower sensitivity and high specificity.

Results:

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>SEN (%)</th>
<th>SEN (%)</th>
<th>SPC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT</td>
<td>ICA</td>
<td>LA/OA</td>
<td></td>
</tr>
<tr>
<td>%C conf</td>
<td>17%</td>
<td>81.5</td>
<td>81.4</td>
</tr>
<tr>
<td>NR conf</td>
<td>1.20</td>
<td>82</td>
<td>81.2</td>
</tr>
<tr>
<td>dRVVT</td>
<td>ICA</td>
<td>12.8</td>
<td>67.4</td>
</tr>
<tr>
<td>%C conf</td>
<td>14%</td>
<td>79</td>
<td>63.5</td>
</tr>
<tr>
<td>NR conf</td>
<td>1.16</td>
<td>80</td>
<td>63.3</td>
</tr>
</tbody>
</table>

Conclusions: The combination of mixing studies and confirmatory tests for APTT and dRVVT interpreted according the new guidelines can clearly differentiate the presence of LA from other coagulopathies.
DIFFERENT VARIANTS OF THE THROMBIN GENERATION TEST (TGT) SHOW DIFFERENCES IN SENSITIVITY TO MICROPARTICLES AND THE CONTACT SYSTEM: ANALYSIS OF ECAT SURVEYS

C. Kluft, P. Meijer, R. Kret

ECAT Foundation, Leiden, the Netherlands;
Good Biomarker Sciences, Leiden, the Netherlands

Keywords: thrombin generation, microparticles, factor XII.

Background: ECAT-surveys on TGTs showed three categories of tests when ordered by time-to-peak (TTP). Rapid tests: Innovin ETP (Siemens) and in-TDT (Pentapharm) (50-60 sec); intermediate tests: CAT 5 and 1 pM (Thrombinscope) (300-550 sec), and slow tests: RCH and RCL (Technoelone) (1200-1600 sec), with a 30-fold difference in TTP.

Methods: The survey included pooled plasma, microparticle-depleted plasma and a factor XII-deficient patient plasma. Between 4-11 laboratories participated per test. Analysed were time (TTT) and quantity variable (AUC).

Results: MP-depleted plasma showed no difference in TTP, but a progressive increase in the other tests by decreasing factor from 11-14% in the CAT and 19-29% in the RCH (p<0.0002). The same was found for the AUC with the largest decrease of 35% for RCL (p<0.0005). Re-addition of MPs restored the original situation. The factor XII deficient plasma showed no effect on TTP for ETP and CAT 1 and 5 pM; for RCH and RCL, TTP increased to 238-327% (p<0.028). The AUC for the ETP was unaffected, with a decline in: CAT 5 pM, 20% (p<0.001); CAT 1 pM, 34% (p<0.002); RCH -8% (p=0.008) and RCL -92% (p=0.003). The effects of factor XII deficiency were not mimicked by addition of CTI, showing only small to moderate effects depending upon the plasma source, but could be confirmed by inhibiting factor Xla.

Conclusions: The different sensitivities of TGTs to MPs and contact activation predicts them to associate differently with clinical situations in which those aspects are important. TTP is apparently insensitive to both; RCL is very sensitive to contact factor activation and MPs. Future ECAT-surveys should include samples with variation in MPs and contact activation to match with features of the TGT variants.

Corresponding Author: Cornelis Kluft, ECAT Foundation, Zernikedreef 9, Leiden, the Netherlands, kluft@euronet.nl

IMPROVEMENT OF THE POSITIVE PREDICTIVE VALUE OF A COMBINATION OF D-DIMER WITH CONCOMITANT DISEASES FOR DIAGNOSIS OF DEEP VEIN THROMBOSIS AND PULMONARY EMBOLISM

J. Harenberg, L. Goldammer, S. Marx, C. Weiss

Clinical Pharmacology, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Mannheim, Germany; Institute of Biomedical Statistics Ruprecht-Karls-University Heidelberg, Mannheim, Germany

Keywords: thrombosis, pulmonary embolism, positive predictive value.

Background/Aims: Patients with suspicion of deep vein thrombosis (DVT) or pulmonary embolism (PE) undergo D-dimer testing or objective methods for identification of the thrombotic event. We hypothesized, that the absence or presence of frequent concomitant diseases may improve the pretest clinical probability for DVT/PE.

Patients and methods: Patients were admitted to the emergency room with clinical suspicion of DVT or PE. The biographic data, the additional diagnosis, thrombophilia status, D-dimer and compression ultrasound (DVT-patients) or spiral CT (PE-patients) were documented. Patients with objectively documented DVT or PE were compared with those in whom the suspicion was not confirmed.

Results: DVT was confirmed in 77/132 patients and PE in 53/135 patients. Patients with DVT more frequently had a thrombophilic disorder (p=0.0002) and less frequently an erosipiel (p=0.002) compared to those in whom DVT was not confirmed. Patients with a positive D-dimer had a lower area under the receiver operating curve (ROC-area 0.8045) than those with the additional characteristics area: 0.7541 (p=0.0471). Patients with PE had more frequently a history of DVT/PE (p=0.0003) and less frequently chronic obstructive lung disease (p=0.01), atrial fibrillation without anticoagulation (p=0.02) and coronary heart disease (p=0.006).

Conclusions: Prospective studies are warranted to validate the findings compared to published pretest probability scores.

Corresponding Author: Job Harenberg, Clinical Pharmacology, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Maybachstrasse 14, Mannheim, Germany, job.harenberg@med.uni-heidelberg.de

AUTOANTIBODIES AGAINST PROTHROMBIN AND PROTHROMBIN-PHOSPHATIDYLserIN COMPLEX: A DIAGNOSTIC TOOL FOR THE ANTIPHOSPHOLIPID SYNDROME?

K.M.J. Devreese, K. Peerlinck, M. Hoylaerts

1 Coagulation Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Gent, Belgium; 2 Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

Keywords: antiphospholipid syndrome, antiprothrombin, thrombosis.

Background: The antiphospholipid syndrome (APS) is an autoimmune disease characterized by thromboembolic complications (TEC) and the presence of antiphospholipid antibodies (aPL). The laboratory criteria include lupus anticoagulant (LAC), anticardiolipin and anti-β2glycoprotein I antibodies IgG or IgM. Many other aPL are described in APS, such as anti-prothrombin antibodies (aPT) or antibodies against the prothrombin-phosphatidylserin complex (aPT/PS).

Materials and methods: Plasmas were selected from confirmed LAC-positive patients (n=55). 36 patients suffered from thrombosis (arterial and/or venous thrombosis) and 19 patients were free of TEC. LAC assays were performed according to the recommendations of the ISTH using screening, mixing and confirmation tests in the aPTT and DRVVT test system, applying the updated guidelines. aPT and aPT/PS IgG antibodies were measured through enzyme-linked immunosorbent assays (ELISA) (AESKULA, AESKU Diagnostics, Germany). The upper limit of the reference range was calculated using 50 healthy individuals, with a positive titer being defined as higher than the 99th percentile of the normal population. Odds ratios (95% confidence interval) were calculated and sensitivity, specificity, positive and negative predictive values (PPV, NPV) were calculated against the clinical background of the patients.

Results: Sensitivity, specificity, PPV and NPV were 19.4%, 68.4%, 53.9% and 30.95%, respectively, for aPT IgG and 41.7%, 79.0%, 70.0% and 41.7%, respectively, for aPT/PS IgG. Odds ratios were 0.523 (0.147-1.865) and 2.679 (0.740-9.698) for aPT IgG and aPT/PS IgG, respectively.

Conclusions: Our data show an association between aPT/PS IgG antibodies in APS patients and thrombosis. No association is found with aPT IgG antibodies.

Does high sensitivity troponin measurement aid in the diagnosis of venous thromboembolism?

K.E. Hogg, S. Haslam, E. Hinchliffe, F. Lecky

University of Manchester, Royal Preston Hospital, Greater Manchester, UK

Keywords: diagnosis, venous thromboembolism.

Background/Aims: Diagnosing venous thromboembolism (VTE) can be a multistep, time-consuming process, which is often left to the most junior doctors to co-ordinate. Errors in the diagnostic process are not uncommon. The THREAD study aimed to assess the potential diagnostic role of new biomarker assays, in attempt to simplify diagnosis. This study reports the results from a new troponin T assay, with a significantly lower detection limit, in the diagnosis of pulmonary embolism (PE) and deep vein thrombosis (DVT).

Materials and Methods: The prospective diagnostic study was conducted at a single general hospital in the UK, between September 2008 and June 2009. Outpatients investigated for DVT and all patients investigated for PE were eligible. Exclusions were age <16, lack of capacity and refusal. All patients underwent an evidence based protocol to diagnose or exclude VTE, along with a three month clinical follow up. The patients’ serum underwent blinded analysis for troponin T using the Roche high sensitivity troponin assay with a lower limit of detection of 0.005ng/mL. Potential for diagnostic cut-off was assessed by constructing receiver operating characteristic curves for all patients, for patients assessed for DVT alone, for those assessed for PE, and outpatients.

Results: 919/926 patients investigated for VTE were approached for consent and 806 patients were enrolled to the study. DVT was diagnosed in 84/452 (18.6%) patients and PE in 68/354 (19.2%) patients. The mean age was 57, 60% female, 13% inpatients and mean time since symptom onset 13 days. The area under the ROC curve (AUC) for all VTE was 0.57 (95%CI 0.52-0.62), for DVT 0.51 (95%CI 0.44-0.58) and for PE 0.64 (95%CI 0.57 – 0.70). The AUC for PE was 0.71 (95%CI 0.63-0.78) in new patients presenting to the emergency department.

Conclusions: High-sensitivity troponin T cannot be used alone to diagnosed VTE.

Corresponding Author: Kerstin E Hogg, University of Manchester, 3rd Floor, TARN corridor, Clinical Sciences Building, Salford Royal Hospital, Salford, Greater Manchester, UK, kerstin.hogg@manchester.ac.uk
P212
CAN ISCHEMIA MODIFIED ALBUMIN BE USED TO TEST FOR VENOUS THROMBOEMBOLISM?
K.E. Hogg, S. Haslam, E. Hinchliffe, F. Lecky
University of Manchester, Royal Preston Hospital, Greater Manchester, UK

Keywords: diagnosis, venous thromboembolism

Background/Aims: Venous thromboembolism (VTE) remains a significant cause of death. Improved diagnostics have not reduced mortality rates. The process of diagnosing VTE remains multi-factorial. The THREAD study assessed novel biomarkers for the diagnosis of VTE, in attempt to identify a future simple test. The aim of this study was to assess the role of ischemia modified albumin (IMA) testing in the diagnosis of VTE. This was a prospective diagnostic study. Patients age > 16 investigated for PE or DVT at a single hospital were eligible for consent. Exclusion criteria were lack of capacity and refusal. The first blood sample drawn was analysed for IMA. Each participant underwent a reference standard investigation to exclude or diagnose PE or DVT and was followed clinically for three months. Receiver operating characteristic (ROC) curves were constructed for IMA and IMA:albumin in the diagnosis of all VTE, PE, DVT and predefined subgroups. Financial constraints lead to an interim analysis after 380 patients to establish whether further IMA assessment was warranted.

Results: Between September 2008 and June 2009, 354 patients were consented and investigated for PE, and 452 patients for DVT (806 in total). All 354 patients investigated for PE had blinded IMA testing as did the first 199 DVT patients. Interim analysis demonstrated further IMA testing for DVT futile. The prevalence of VTE was 19.7%. The IMA:albumin ratio performed consistently better than IMA alone. The AUC for IMA:albumin in all VTE was 0.60 (95%CI 0.54 – 0.66), in DVT 0.56 (95%CI 0.46 – 0.65) and in PE 0.63 (95%CI 0.56 – 0.71). In patients presenting to emergency department with symptoms of PE, the AUC for IMA:albumin was 0.69 (95%CI 0.60 – 0.78).

Conclusions: IMA testing cannot be used alone to diagnose DVT or PE, although there is a moderate association with PE in emergency department patients.

Corresponding Author: Kerstin E. Hogg, University of Manchester; 3rd Floor; TARN corridor; Clinical Sciences Building, Salford Royal Hospital, Salford, Greater Manchester, UK; kerstin.hogg@manchester.ac.uk

P214
CRP TO AID THE DIAGNOSIS OF PULMONARY EMBOLISM
K.E. Hogg, F. Lecky
University of Manchester, Greater Manchester, UK

Keywords: diagnosis, venous thromboembolism

Background/Aims: Diagnosing pulmonary embolism (PE) can be a difficult process for junior doctors, because it relies on clinical probability scoring and knowledge of how to apply and interpret D-dimer, VQ and CT scanning. The THREAD study aimed to assess the potential diagnostic role of novel biomarkers, in attempt to identify a future, more simple test. The aim of this analysis is to assess the potential role of CRP in the diagnosis of PE.

The prospective diagnostic study was conducted at a single general hospital in the UK, between September 2008 and June 2009. All patients investigated for PE were eligible. Exclusions were age <16, lack of capacity and refusal. All patients underwent an evidence based protocol to diagnose or exclude PE, along with a three month clinical follow up period. CRP was not conducted as a blinded research test, instead, the study documented the initial CRP result when the investigating physician ordered the test. Potential for diagnostic use was assessed by constructing receiver operating characteristic (ROC) curves for all patients investigated for PE and emergency department patients investigated for PE.

Results: 411/414 patients investigated for PE were approached for consent and 354 patients investigated for PE were enrolled to the study. PE was diagnosed in 19.2% patients. Of this cohort, 269 patients had CRP testing ordered by their physician. The area under the ROC curve (AUC) for CRP in the diagnosis of PE was 0.72 (95%CI 0.65-0.78). The AUC for PE was 0.77 (95%CI 0.69-0.84) in patients presenting to the emergency department (N=199).

Discussion: This is a limited exploratory analysis. Results will be available for the full cohort of patients (N=354) by the conference.

Conclusions: CRP has a moderate association with PE in emergency department patients, however could not diagnose PE alone.

Corresponding Author: Kerstin E. Hogg, University of Manchester; 3rd Floor; TARN corridor; Clinical Sciences Building, Salford Royal Hospital, Salford, Greater Manchester, UK; kerstin.hogg@manchester.ac.uk

P292
REFERENCE VALUES FOR THROMBOELOASTOMETRY (ROTEM®) IN CYCLOMOLS MONKEYS (MACACA FASCICULARIS)
L. Spiezia 1, D. Bertini 1, M. Baldorin 2,3, S. Gavasso 1, C.M. Radu 1, C. Bulato 1, P. Dabbrioli 1, E. Cozzi 1, P. Simioni 1
1 Dept of Cardiologic, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School; 2 CORIT (Consorzio Ricerca sul Trapianto Organi); 3 Veterinary Pathology and Hygiene Inst. Padua, Italy

Background/Aims: The imbalance in clotting homeostasis, tending towards hypercoagulation, is recognized as the real barrier to the long-term survival of porcine xenografts in this species combination. The present study aimed to validate in primate blood the applicability of whole blood thromboelastometry, performed by ROTEM®, an interesting tool to study the qualitative characteristics of clot formation as far as strength, firmness and swiftness are concerned.

Methods: ROTEM® (Pentapharm GmbH, Munich, Germany) was used to investigate native coagulation (NATEM®), the intrinsic (INTEM®) and extrinsic (EXTEM®) pathways, the function of fibrinogen (FIBTEM®), and the presence of fibrinolysis in 10 hypercoagulable monkeys. Using classic validation approaches, the normal thromboelastographic profile was defined and the influence of haematoctit (Hct, %), platelet count (x10^9/L), fibrinogen (mg/dl), and factor VIII (FVIII, %) was evaluated.

Results: In all four (NATEM®, INTEM®, EXTEM®, FIBTEM®) assays considered, Clotting Time (CT, sec) and Clot Formation Time (CFT, sec) were shorter in primate than human. Moreover, α-angle (°), Maximum Clot Firmness (MCF, mm), and Area Under the velocity Curve (AUC, mm x100) were higher in primate than human. No substantial difference was observed as for Hct and platelet count between the two species. On the contrary FVIII was higher in primate than human and, interestingly enough, fibrinogen was lower in monkeys than human.

Conclusions: ROTEM® depicts a hypercoagulable profile in primate as compared to human. Together these data suggest that, with regard to coagulation, xenotransplantation in primates represent a much more difficult situation than xenotransplantation in humans.

Corresponding Author: Luca Spiezia, luca.spiezia@unipd.it
LABORATORY DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA BY THROMBIN GENERATION ASSAY: COMPARISON OF INDIVIDUAL PATIENT AND AGGREGATE DATA META-ANALYSIS

M. Marcucci 1, J.D. Douketis 2, A. Tosetto 3, C. Tudur-Smith 4, T. Baglin 5, M. Cushman 6, S. Eichinger 7, G. Palareti 8, D. Poli 9, R. Campbell Tait 9, A. Iorio 1

1 Dept. of Medicine, University of Perugia, Perugia, Italy; 2 Dept. of Medicine, McMaster University, Hamilton, ON, Canada; 3 Dept. of Hematology, S. Bortolo Hospital, Vicenza, Italy; 4 Centre for Medical Statistics and Health Evaluation, University of Liverpool, Liverpool, UK; 5 Dept. of Haematology, Addenbrookes Hospital, Cambridge, UK; 6 Dept. of Medicine, University of Vermont, Colchester, USA; 7 Dept. of Medicine, University of Vienna, Vienna, Austria; 8 Dept. of Medicine, University of Bologna, Bologna, Italy; 9 Centro di Riferimento Regionale per la Trombosi, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italy; 10 Dept. of Haematology, Royal Infirmary, Glasgow, UK

Keywords: meta-analysis, D-dimer, prognosis

Background: Individual patient data (IPD) meta-analysis, even if more resource demanding, as compared to aggregate data (AD) meta-analysis, can more rigorously elaborate time-to-event data and investigate sources of heterogeneity.

Methods: We compared the performance of 2 meta-analyses pooling the same set of studies on D-dimer to stratify the risk of thrombosis recurrence after anticoagulation stopping in patients with a first unprovoked venous thromboembolism (VTE). AD meta-analysis provided annualized recurrence rates and a pooled risk ratio for positive versus negative D-dimer patients by a mixed-effects Poisson model. In addition to annualized rates, in the IPD meta-analysis a Kaplan-Mayer survival analysis was performed to obtain cumulative hazard for recurrence 1, 3 and 5 years after stopping anticoagulation according to D-dimer status, either as defined in each source study or basing on pre-specified cut-off points (250 and 500 ng/mL), also for age and D-dimer test timing subgroups. IPD-based study-stratified multivariable Cox regression was compared to meta-regression based on AD.

Results: Overlapping annualized VTE recurrence rates were found by the two approaches (8.8-8.9 for positive, 3.5-3.7 for negative D-dimer patients). IPD-based cumulative hazard after 3 years was 25.4 (95% confidence interval [CI] 21.3-30.4) for positive and 9.3 (95% CI 7.1-12.1) for negative D-dimer patients. AD-based pooled risk ratio and IPD-based hazard ratio suggested a 2.2-2.5-fold higher recurrence risk for positive versus negative D-dimer patients. Subgroup analysis and Cox regression showed that none of the hypothetical confounders (age, BMI, sex, hormonal therapy, genetic thrombophilia, timing of D-dimer testing, qualitative/quantitative definition of D-dimer status) affected the D-dimer prognostic efficacy. Meta-regression was not able to demonstrate it.

Conclusions: The AD and IPD meta-analyses on D-dimer yielded comparable findings but only IPD was able to explore the trend over time of recurrence risk and the effect of patient-level confounders on the prognostic value of D-dimer.

Corresponding Author: Alfonso Iorio, Dept. of Medicine, University of Perugia, Perugia, Italy. 1iorio@sump.it

P539

D-DIMER TO DETERMINE RISK FOR DISEASE RECURRENCE AFTER UNPROVOKED VENOUS THROMBOEMBOLISM: ADDRESSING UNANSWERED QUESTIONS WITH A LARGE INDIVIDUAL PATIENT META-ANALYSIS

J. Douketis 1, A. Tosetto 2, M. Marcucci 1, T. Baglin 3, M. Cushman 4, S. Eichinger 7, G. Palareti 8, D. Poli 9, R. Campbell Tait 9, A. Iorio 1

1 Dept. of Medicine, McMaster University, Hamilton, ON, Canada; 2 Dept. of Hematology, S. Bortolo Hospital, Vicenza, Italy; 3 Dept. of Medicine, University of Vermont, Colchester, VT, USA; 4 Dept. of Medicine, University of Vienna, Vienna, Austria; 5 Dept. of Medicine, University of Bologna, Bologna, Italy; 6 Dept. of Medicine, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; 7 Dept. of Haematology, Royal Infirmary, Glasgow, UK

Keywords: venous thrombosis, D-dimer, oral anticoagulants

Background: In patients with a first unprovoked venous thromboembolism (VTE), an elevated D-dimer after anticoagulant therapy stopping is a risk factor for recurrent VTE. Questions remain about the effect of the timing of D-dimer testing, patient age and the D-dimer cut-point on the ability of D-dimer to distinguish risk for recurrent disease.

Methods: We did a patient-level meta-analysis of prospective studies in patients with a first unprovoked VTE who had D-dimer testing after anticoagulation stopping and were followed for recurrent VTE. Kaplan-Meier analysis was used to determine the cumulative incidence of recurrent VTE in patients with a negative or positive D-dimer according to timing of D-dimer testing (<3 weeks, 3-5 weeks, or >5 weeks post-anticoagulation) and patient age (<65 years, >65 years, or >75 years). We compared risk for recurrence first according to D-dimer status as defined in the source studies then using a pre-specified cut-point (500 µg/mL). We used the log-rank test to compare the risk for recurrent VTE according to D-dimer status (negative or positive) and the Cox regression analysis to adjust for potential confounders.

Results: We identified a total of 5 studies with a total of 889 patients with a first unprovoked VTE who had follow-up for a mean (standard deviation [SD]) of 26.9 (19.1) months. After 3 years, the cumulative incidence of recurrent VTE was significantly higher after a positive D-dimer (25.4%; 95% confidence interval [CI] 21.3-30.4) than after a negative D-dimer (9.3%; 95% CI 7.1-12.1; hazard ratio, 2.5; 95% CI 1.9-3.3), irrespective of timing of post-anticoagulation D-dimer testing. Patient age and D-dimer cut-point.

Conclusions: In patients with a first unprovoked VTE who have D-dimer measured after stopping anticoagulation testing, the timing of the D-dimer testing, patient age and the D-dimer assay cut-point used do not affect the ability of D-dimer to distinguish patients at higher or lower risk for recurrent VTE.

Corresponding Author: Maurizio Marcucci, Medicina Interna Vascolare, Università degli Studi di Perugia, Ospedale Santa Maria della Misericordia, via Dottori, loc. S.Andrea delle Fratte, Perugia, Italy. marcucci.maurizio@gmail.com

P575

LABORATORY DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA BY THROMBIN GENERATION ASSAY: EFFECTS OF THROMBOMODULIN AND FONDAPARINUX

A. D’Angelo, G. Pavani, P. Della Valle, A. Fattorini, L. Crippa

Coagulation Service & Thrombosis Research Unit, Scientific Institute San Raffaele, Milan, Italy

Keywords: HIT, HIT antibodies, thrombin generation test, thrombomodulin, fondaparinux

Background: Type II heparin-induced thrombocytopenia (HIT) is diagnosed on the basis of clinical and laboratory criteria. HIT antibodies (HIT-Abs) may be detected by immunnochemistry or by functional methods. ELISAsays are highly sensitive, but functional methods are more specific for the diagnosis of HIT and have a major role in avoiding prolonged anticoagulation in non-HIT patients. Recently, a thrombin generation assay (TGA) for the functional detection of HIT-Abs has been described (Tardy-Poncet et al, JTH 2009).

Materials and Methods: We have tested for HIT-Abs citrated plasma from 46 consecutive patients with the clinical suspicion of HIT in a modified TGA. Mixture of donor platelets (180-200 x 10^11/L, f.c.) and patients’ plasma are incubated with tissue factor (0.5 pnmol f.c., Thrombinoscope bv, Maastricht, the Netherlands) in presence of unfractionated heparin (UFH 0.2 and 1 IU/mL, f.c.) or with or without the combined addition of thrombomodulin (TM, 5 nmol f.c.) and fondaparinux (200 ng/ml, f.c.).

Results: Of the 46 patients, 20 had no HIT-Abs by ELISA (HPIA Asserachrom, Stago, Asnieres sur Siere, France) and only 6 had HIT-Abs by both ELISA and the functional method described by Greinacher et al (HPIA, TH 1991). The table shows the median ETP (nmol of thrombin) and Peak (mmol/min) values observed in the different groups of patients. In the absence of UFH, +/-+ patients showed increased Peak values both with and without TM and Fondaparinux, and increased ETP values only with the combination of TM and Fondaparinux. Under any assay conditions, the addition of UFH (0.2 IU/mL) reduced by more than 50% ETP and Peak values in +/- and +/-+ patients (p <0.001), but did not change significantly either parameter in +/+ patients (p>0.58). Interestingly, plasma from two NIHPA negative patients was strongly positive for HIT-Abs in the thrombin generation assay. The TGA is a promising tool for the detection of HIT-Abs.

Patients n HPIA TM/Fondaparinux AD IPD

<table>
<thead>
<tr>
<th>ETP</th>
<th>UFH 0.2 IU</th>
<th>UFH 1 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>Peak</td>
<td>Peak</td>
</tr>
<tr>
<td>+/-</td>
<td>20</td>
<td>11.4</td>
</tr>
<tr>
<td>+/-+</td>
<td>16</td>
<td>101.4</td>
</tr>
<tr>
<td>+/+</td>
<td>6</td>
<td>143.5</td>
</tr>
<tr>
<td>p</td>
<td>0.009</td>
<td>0.39</td>
</tr>
<tr>
<td>+/+</td>
<td>2</td>
<td>192</td>
</tr>
</tbody>
</table>

# by HPIA % of positive control OD/HPIA/TGA

Corresponding Author: Armando D’Angelo, Coagulation Service & Thrombosis Research Unit, Scientific Institute San Raffaele, Via Olgettina 60, 20132 Milano, Milan, Italy. armando.dangelo@hsr.it
COAGULATION ASSESSMENT BY ROTATIONAL THROMBOELASTOMETRY (ROTEM) ANALYSIS IN PATIENTS WITH SPLENIC VEIN THROMBOSIS WITH AND WITHOUT HEPATIC CIRRHOSIS.

V. Rossetto 1, M. Senzolo 2, M.T. Sartori 1, D. Bertini 1, M. Fadin 1, F. Zerbinati 1, L. Spiezia 1, P. Simioni 1
1 Department of Cardiology, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy; 2 Department of Surgical and Gastroenterological Sciences, Gastroenterology Unit, University of Padua Medical School, Padua, Italy

Background: Splanchnic vein thrombosis (SpVT) developed in the presence of normal liver rather than hepatic cirrhosis have deep differences in etiologic risk factors. The coagulation pattern and the determinants of thrombosis in patients with and without cirrhosis have not been defined yet.

Patients and Methods: The following four different groups were enrollee: a) and b) subjects with objectively diagnosis of SpVT with and without hepatic cirrhosis; c) hepatic cirrhosis subjects without SpVT; d) healthy donors. Blood was drawn by each subject and both ROTEM assays (INTEM, EXTEM, FIBTEM and NATEM) and thromboelaphila screening were performed.

Results: In INTEM, EXTEM, NATEM and FIBTEM assays, there were no differences in any of measured parameters (MCF, AUC, α-angle) among cirrhotic patients, both with and without splanchnic vein thrombosis. Patients with SpVT and healthy liver, had higher MCF, AUC, and α-angle in INTEM (p=0.004, 0.003, 0.004, respectively), EXTEM (p=0.01, 0.02, 0.01, respectively) and FIBTEM (p=0.05, 0.01, respectively) than patients with SpVT and hepatic cirrhosis. ROTEM® parameters correlate with platelets count, FIX, FXI, Fibrogenin, AT, PC, PS plasma levels that were significantly lower in cirrhotic patients and with FVIII plasma levels that were significantly higher in cirrhotic patients than subjects with healthy liver (180 ± 75 vs 163 ± 79%, p=0.02). Moreover, MCF and AUC in INTEM and α-angle in EXTEM, correlated with the reduction of PT.

Conclusions: Correlation between ROTEM parameters and lower plasma levels of natural inhibitors of coagulation and with impaired PT levels, suggests sensitivity of rotation thromboelastrometry to liver failure. Differences in ROTEM parameters have been found between patients with and without cirrhosis but not between those with and without splanchnic vein thrombosis.

Corresponding Author: Valeria Rossetto, Dept of Cardiology, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy, valeria.rosetto@ospedale.it

A GLOBAL ASSAY SENSITIVE TO ACTIVATED PROTEIN C ABNORMALITIES IS PREDICTIVE FOR CHEMOTHERAPY-ASSOCIATED VENOUS THROMBOEMBOLISM

P. Ferroni 1, F. Martini 1, I. Portarena 2, I. Grenga 3, F. La Farina 1, G. Massimini 3, A. Laudisi 1, M. Roselli 2, F. Guadagni 1
1 Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele Pisana, Rome, Italy; 2 Medical Oncology, Department of Internal Medicine, Tor Vergata Clinical Center, University of Rome Tor Vergata, Rome, Italy; 3 IRCCS San Raffaele Pisana, Rome, Italy

Keywords: Chemotherapy, venous thromboembolism, thrombophrophylaix

Background/Aims: Consensus guidelines by multiple cancer organizations do not recommend routine prophylaxis for the primary prevention of venous thromboembolism (VTE) for out-patients receiving chemotherapy. Nonetheless, identifying patients with cancer who are most at risk for VTE is essential to improve time-delivery chemotherapy and quality of life. Thus, the need for the identification of novel candidate biomarker(s) to be used as predictors for VTE in cancer out-patients.

Patients and Methods: This study was designed to investigate the adequacy of a global assay designed to evaluate the functionality of the activated protein C (APC) system to predict VTE in cancer patients undergoing chemotherapy. Analysis was performed on citrated plasma samples of 208 out-patients prior to and before starting the second cycle of a new chemotherapy regimen.

Results: Patients' were classified as low, intermediate or high-risk according to a risk assessment model recently validated by Khorana and colleagues. Analysis of samples obtained during chemotherapy showed an impairment of the APC system in patients who developed VTE compared to those who did not (p<0.0001). Cox proportional hazards regression analysis for event-free survival of patients stratified on the basis of steady vs. impaired APC function demonstrated in the latter a worst cumulative event-free survival (56%) compared to patients with stable values (90%, p<0.0001) with a 0.21 HR (CI 0.05-0.30). This assay fully retained its predictive value in a Cox proportional hazards survival regression analysis even after risk-set stratification of patients according to the Khorana's class of risk (p<0.0001) with a 0.18 HR for steady vs. impaired APC function (p<0.0001) in the intermediate risk group.

Conclusions: Use of this novel global assay sensitive to APC abnormalities in out-patients on active chemotherapy, especially in combination with Khorana's risk assessment model, may help identifying a population of cancer patients at risk for VTE that might benefit from thrombophrophylaix.

Corresponding Author: Patrizia Ferroni, Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele Pisana, Via della Pisana, 235, Rome, Italy, patrizia.ferroni@sanraffaele.it

PREVALENCE OF ANTI-HEPARIN PLATELET FACTOR 4 ANTIBODIES WITH DISSEMINATED INTRAVASCULAR COAGULATION

Loyola University Medical Center, Maywood, IL, USA

Keywords: HIT, heparin antibodies, DIC

Disseminated intravascular coagulation (DIC) represents a complex syndrome with multiple pathophysiologic components. Most patients with DIC exhibit thrombogenic responses due to endogenous consumption of platelets. A systematic study on the prevalence on anti-heparin platelet factor 4 (AHPF4) antibodies and HIT syndrome in DIC patients has not been presented. To determine the prevalence of AHPF4 antibodies in patients with suspected DIC syndrome a total of 25 plasma samples were retrospectively analyzed utilizing the two commercially available methods (GTI, Brookfield, WI and Hyphen Biomedical, Paris, France). Out of 25 patients, 24 samples were positive for the AHPF4 antibody in the GTI method (OD>0.400), whereas only 16 were positive in the Hyphen Biomedical assay (OD>0.500). Interestingly, only 9 samples were positive in both of these assays. None of the positive samples in either the GTI or the Hyphen assay exhibited a positive 14C serotonin response. Additional analysis of these samples revealed the presence of platelet activation products such as platelet factor 4 (PF4), selectin and p-selectin. These studies suggest that circulating AHPF4 antibodies are non-functional and do not produce any thrombogenic responses. The elevated circulating PF4 levels and other cytokines may be contributory to the generation of these antibodies in the DIC patients.

Corresponding Author: Jawed Fareed, Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL, USA, dfoppen@lumc.edu

ANALYTICAL PERFORMANCES OF A NEW LIQUID ANTI-XA ASSAY FOR UFH/LMWH AND FONDAPARINUX

J. Beltran 1, C. Legros 1, V. Sigueret 1, I. Gouin-Thibault 1, F. Nicham 1, B. Woodhams
1 Enzymology Research and Development department, STAGO, Genneviéres, France; 2 Department of Hematology, Charles Fox hospital, Ivy sur Seine, France; 3 Scientific direction, STAGO, Genneviéres, France

Keywords: antiocoagulation, assay

Aims: We evaluated the analytical performances of the new ready-to-use chromogenic assay, STA® Liquid Anti-Xa, for the automated determination of anti-Xa activity in plasma from patients treated with UFH, LMWH and fondaparinux.

Materials and methods: Specific calorifier sets for fondaparinux or for UFH/LMWH were used. UFH/LMWH calibration set allows determination of both UFH and LMWH anti-Xa activities using either dedicated and hybrid calibration.

Stability, detection limit, linearity and precision were evaluated on three different lots of reagents on STA® R analyser. Manufactured lyophilised controls and normal pool plasma spiked with fondaparinux, UFH/LMWH International Standards or marketed LMWH preparations (enoxaparin, nadroparin, dalteparin) were used. Anti-Xa activity in plasma from 149 patients treated with either UFH or LMWH was assessed using STA® Liquid Anti-Xa and commercial STA® Rotachrom Heparin. Results expressed with dedicated and hybrid calibrations for both reagents were compared.

Results: The three reagent lots were stable up to 7 days at STA® R and 3 months at 24C. Detection limits were 0.14g/mL and 0.1 anti-Xa IU/mL for fondaparinux and LMWH/UHFX, respectively. Linearities were up to 2.04g/mL, 2.0 anti-Xa IU/mL and 1.1 IU/mL for fondaparinux, LMWH and UFH, respectively. A good agreement was shown between anti-Xa activities measured in plasma spiked with LMWH International Standards or LMWH preparations (minimum variation < 5% at 2.0 anti-Xa IU/mL). Inter/intra assay coefficients of variation for UFH/LMWH and fondaparinux levels ranged from 20.0 to 7.0%. Good correlations of plasma anti-Xa activity levels from patients treated with either UFH or LMWH were observed using the two reagents (R2>0.90), with either dedicated or hybrid calibration.

Conclusions: STA® Liquid Anti-Xa, without prior reconstitution, is suitable for measuring a wide range of anti-Xa values, including supra-therapeutic ones. It provides a reliable tool for monitoring and clinical investigations for UFH/LMWH and fondaparinux.

Corresponding Author: Jérôme Beltran, Enzymology Research and Development department, STAGO, 125, avenue Louis Roche, Genneviéres, France, jbeltran@stago.fr
VALIDATION OF A NEW POINT-OF-CARE INR ANALYZER

B. Jørgensen, D. Scholer

Department of Clinical Biochemistry, Division of Haemostasis & Point-of-Care testing, Viborg Regional Hospital, Viborg, Denmark

Keywords: POCT, INR, self management

Background/Aims: A point-of-care hand-held analyzer (INRatio2, Inverness Medical) utilizing in strip electrical impedance clot detection technology in combination with recombinant tissue factor activation in whole-blood was recently launched in Denmark. We aimed to validate this device using preset national quality specifications for intra-assay variation (CVa) and analytical bias of point-of-care INR analyzers.

Materials and methods: 36 unselected patients on warfarin, and in for scheduled INR measurement at our out-patient clinic had blood drawn by standard antecubital venipuncture. Citrated samples were prepared for routine automated INR measurements, whereas unstabilized whole-blood was immediate applied onto two analyzers for parallel duplicate measurement. The preset quality specifications were 5% for CVa, and 6% for bias. Bias was further evaluated after adjusting the routine method’s raw values to perfectly fitting the mean calibration of all Danish laboratories (N=82) participating in a regular national external QC programme.

Results: The analyzer INR’s averaged 2.6 (range 1.6-4.3). CVa of the 36 duplicates was 4.7%. Analyzer variation averaged 0.025 INR-points. Bias against the routine method was 5.4% over the measured range and slightly higher (7.1%, 0.14 INR-points) at 2 INR than at 4 INR (2.9%, 0.12 INR-points). Bias against the contemporary Danish INR calibration was 2.2%, and slightly higher (4.2%, 0.08 INR-points) at 2 INR than at 4 INR (0%, 0 INR-points).

Conclusions: The INRatio2 qualified for use in general practice and patient self monitoring of oral anticoagulant therapy in Denmark.

Corresponding Author: Bo Jørgensen, Department of Clinical Biochemistry, Division of Haemostasis & Point-of-Care testing, Viborg Regional Hospital, Heibergs Alle 4, Viborg, Denmark, boj@post3.tele.dk

DIFFERENT D-DIMER CUT OFF VALUES IN HIP INJURY PATIENTS WITH HIGH VTE RISK

A. Bronic, D. Car, M. Pavic

University Hospital of Traumatology, Zagreb, Croatia

Keywords: D-dimers, hip injuries

Patients undergoing lower-extremity orthopaedic procedures are in the highest-risk category for developing VTE. Interpretation of increased D-dimer (Dd) values and objective testing to exclude VTE could be hard due to trauma, increased age and significant comorbidity. Likewise, false results also can be expected in case of use of inappropriate cut off values. Our aim was to generate separate receiver operating characteristic curve to determine whether different Dd cut-off values on admission and after surgery could be more informative in patients admitted during one year to our hospital due to hip injuries. Data from 57 patients (median age 76 y) were collected retrospectively. D-dimer values were obtained from plasma samples by immunofiltration method (Nycop card Reader II, Axsylid, Norway, cut off value 0.3 mg/L). According to comprehensive physician report, VTE was suspected in 19 patients (33%) during hospitalization but confirmed only in 9 patients (16%) by doppler ultrasonography. Obtained cut off value with a NPV of 90% on admission day was 1.0 mg/L (sensitivity 56% and specificity 77%). After the surgery obtained cut off value of 1.4 mg/L have the same NPV (90%) with improved sensitivity of 78% and concomitant decrease in specificity to 38%. By the using increased cut off levels limited additional information could be provided. Further investigation on this issue is necessary and apart from surgery, subgroups of patients with coexisting conditions that could influence on D-dimer levels should be considered.

Corresponding Author: Ana Bronic, University Hospital of Traumatology, Draskoviceva 19, Zagreb, Croatia, anabronic@yahoo.com

SPATIALLY HETEROGENEOUS EXPERIMENTAL MODEL OF BLOOD COAGULATION: FROM BASIC RESEARCH TO DIAGNOSTICS OF PROTHROMBOTIC AND BLEEDING TENDENCIES

M.A. Panteleev 1, A.N. Balandina 1, V.M. Emelianenko 2, O.A. Fadeeva 1, G.M. Galstian 2, S.S. Karamzin 2, E.N. Lipets 2, N.P. Soshitova 2, I.D. Tarandovskii 2, O.A. Ataullakhanov 2

1 Center for Theoretical Problems of Physicochemical Pharmacology, Moscow, Russian Federation; 2 National Research Center of Hematology, Moscow, Russian Federation

Keywords: experimental models of coagulation, in vitro, diagnostics

Background/Aims: An important but often underestimated aspect of blood coagulation in vivo is its spatial heterogeneity, i.e. the fact that coagulation proteins and reactions are non-uniformly distributed in space, both in plasma and on the membranes of blood cells. Over the last decade, several groups developed spatially heterogeneous in vitro systems to investigate basic mechanisms of blood clotting regulation. However, currently available diagnostic assays are homogeneous and cannot correctly mimic this aspect of blood coagulation functioning. The purpose of the present study was to evaluate diagnostic potential of the spatially heterogeneous experimental models.

Materials and methods: Blood was collected from patients with several types of coagulation disorders, including prothrombotic defects due to septic shock, cardiovascular events and oncological diseases, as well as bleeding defects due to hemophilia A and B of varying severity. Fibrin clot formation in a thin layer of non-stirred platelet free plasma was initiated by a surface with immobilized tissue factor and monitored by videomicroscopy. As controls, established clotting assays such as thrombin generation assay and prothrombin time test were performed.

Results: The spatial velocity of fibrin clot propagation in the experimental system was decreased in hemophilia and increased in cases of prothrombotic risk in good correlation with the results of patient examination, reports on hemorrhage events frequency or pro-thrombotic risk markers. Pro-thrombotic changes in plasma caused spontaneous clotting. The assay was also able to monitor coagulation correction caused by replacement and bypassing therapy in hemophilia, and by heparin therapy in prothrombotic disorders.

Conclusions: The data indicate that these spatially heterogeneous experimental approaches have a good potential for coagulation diagnostics.

Corresponding Author: Mikhail A. Panteleev; Center for Theoretical Problems of Physicochemical Pharmacology, 4 Kozygina str., Moscow, Russian Federation, mapanteleev@yandex.ru
A NEW AND SPECIFIC RAPID CHROMOGENIC “ANTI-XA” ASSAY FOR TESTING RIVAROXaban IN PLASMA

M.M. Samama 1, J. Amiral 2, G. Céline 3, E. Perzborn 1, F. Depasse 3

1 Hotel Dieu University Hospital, Paris, France; 2 HYPHEN BioMed Research, Neuville sur Oise, France; 3 Biomnis Laboratories R&D, Research, Ivry sur Seine, France; 4 Bayer HealthCare, Wuppertal, Germany

Keywords: Anti-Xa chromogenic assay, rivaroxaban specific, new anticoagulants

Background: Drug measurement in patients receiving the direct oral anti-factor Xa inhibitor, rivaroxaban, is useful, especially when it is used for curative indications, in presence of decreased clearance or of overdosage. Current Anti-Xa heparin assays are not appropriate as they are designed for catalytic indirect factor Xa inhibitors such as heparin-like molecules, sodium danaparoid or fondaparinux. Direct factor Xa inhibitors interact in a molar to molar model, and inhibition kinetics are different.

Aims: To develop a specific assay, insensitive to heparin-like molecules, in order to measure specifically rivaroxaban concentration in plasma.

Methods: New two stage chromogenic assay, based on the inhibition of human factor Xa in presence of a chaotropic buffer, in which the AT dependent heparin activity is ineffective whilst all the rivaroxaban activity is preserved: in a first step human factor Xa in a constant and in excess concentration is incubated with the sample; the residual factor Xa is measured in a second step using a specific factor Xa substrate. The assay offers a dynamic range from 0.000 to 0.025 µg/ml of rivaroxaban in the assayed dilution. For the expected therapeutic concentrations, plasmas are assayed diluted 1:20, with an assay range from 0.00 to 0.50 µg/ml. Rivaroxaban recovery is identical whether spiked in assay buffer or in plasma. The method is highly robust, has an inverse and linear dose response curve (r²>0.999), is highly reproducible identical whether spiked in assay buffer or in plasma. The method is highly robust, has an inverse and linear dose response curve (r²>0.999), is highly reproducible

Conclusions: This new simple assay, insensitive to heparins, is fully automatable. It offers an original and reliable laboratory method for measuring anti-Xa activity induced by rivaroxaban.

Figure: Dose response curves of rivaroxaban and fondaparinux with the new assay.

M. Mitic 1, M. Kovac 2, R. Lazic 1, D.J. Jurisic 1, M. Seekic 1, I. Mitic 3

1 Thrombosis and Hemostasis department, Institute of Laboratory Medicine, Clinical Centre of Voivodina, Novi Sad, Serbia; 2 National Blood Transfusion Institute, Belgrade, Serbia; 3 Clinic of Nephrology and Clinical Immunology, Clinical Centre of Voivodina, Novi Sad, Serbia

Keywords: antiphospholipid antibodies, thrombosis

Background: Antiphospholipid syndrome (APS) is a clinical entity characterized by the presence of antiphospholipid antibodies (APA) along with clinical manifestation: arterial (AT) or venous thrombosis (VTE) or obstetric complications (OC).

Aims: To investigate the clinical course of APS patients (pts) with different numbers of positive tests for APA and to determine whether the risk of thrombosis recurrence is higher in pts with more than one positive test in Serbian population.

Materials and methods: The data on 175 pts with APS have been analyzed, 102 with primary and 73 with secondary APS, 106 females and 69 males, age range 14-67, av. 38.9y. Lupus anticoagulant (LA) was identified by coagulation tests according to the ISTH SSC recommendations, using Instrumentation Laboratory (IL) reagents and coagulometer ACL 9000 (IL, Milan, Italy). ACL IgG and IgM and antitheta2GPI IgG and IgM were measured using commercial kits.

Results: One, two and three positive tests were found in 124 patients, 37 patients and 14 patients respectively. Clinical manifestation were VTE – in 84 patients, AT in 44 patients, in 9 patients both AT and VTE, in 35 OC and in 3 both VTE and OC. Among thrombotic episodes 13 occurred at unusual sites. In the single test positive group there have been 100 episodes of thrombotic events and 15 recurrences, in the double test positive group 30 thrombotic episodes occurred, with 14 recurrences and in the triple test positive group, out of 10 thrombotic events 8 recurrences occurred, two patients had both AT and VTE recurrences. The difference between the number of positive tests and the rate of recurrences is highly statistically significant, Chi square 13.5, p=0.001. Most episodes of unusual site thromboses (12 of 13) occurred in single positive test group with LA positivity.

Conclusions: Multiple test positivity for APA predicts high rate of thrombotic recurrences.

Corresponding Author: Gorana Mitic, Thrombosis and Hemostasis department, Institute of Laboratory Medicine, Clinical Centre of Voivodina, Hajduk Veljka 1, Novi Sad, Serbia, miticgn@yahoo.com

EVALUATION OF A FAST ELISA ASSAY FOR MEASURING HIT IGG ANTIBODIES

H. Vett, S. Geiter, M. Graf

Technoclone GmbH, Vienna, Austria

Keywords: HIT, heparin, antibody

Heparin-induced thrombocytopenia (HIT) is caused by development of antibodies to platelet-heparin complexes during therapy with unfractionated heparin. As a first step in laboratory diagnosis a fast but sensitive and specific test for the presence of the reactive antibodies is required. The aim of this study is to evaluate the performance of the new TECHNOZYM® HIT IgG ELISA with a total assay time of 70 minutes. Sample material is citrated plasma or serum from normal plasma donors and from HIT positive patients. Samples were tested in the TECHNOZYM® HIT IgG ELISA and in the Zymutest HIA IgG (Hyphen). In the TECHNOZYM® assay, plates are coated with purified hPF4 and polyvinyl-sulfonate (PVS). Assay time is reduced by co-incubating diluted sample and anti-IgG conjugate. The TECHNOZYM® ELISA provides good differentiation between positive samples (n=17, mean OD 2.2) and normal samples (n=16, mean OD 0.14). A preliminary study for normal range (n=45) resulted in a mean value of OD 0.073 and a maximum value (1 of 45 samples) of 0.288. Studies for defining a cut-off value are ongoing.

There was no difference between serum and plasma samples from the same patient. In positive samples > 90% of IgG binding could be inhibited by addition of heparin, confirming specific detection of heparin-associated antibodies. Specificity of the assay was also confirmed by normal results in samples from patients with other autoimmune disorders (Lupus, TTP). Neither triglycerides up to 240 mg/dL nor hemoglobin up to 17 mg/dL interfered with the assay. Reproducibility is good, with an intra-assay variation of 4.3% (n=40, OD 3.3) and an inter-assay variation (n=10, OD=2.7) of 5.8%. Results obtained for patient samples (n=36) and normals (n=9) correlated well with those obtained in the Zymutest assay (R2 = 0.6).

The new TECHNOZYM® HIT IgG ELISA provides faster results than a classical two step ELISA. The co-incubation of sample and conjugate does not influence reproducibility and specificity.

Corresponding Author: Helga Vett, Technoclone GmbH, Brunner Straße 59/5, Vienna, Austria, sales@technoclone.com
IMAGING OF LIVING PLATELETS IN NORM AND PATHOLOGY BY QUANTITATIVE PHASE MICROSCOPY

L. Vasilenko, V. Metelin, S. Gasparjan, V. Tychinsky, T. Vishenskaya

1 Russian gerontological scientific clinical center, Moscow, Russian Federation; 2 State Classic Academy “Maimonida”; Russian Federation; 3 State Institute for Radioengineering, Electronics and Automation, Russian Federation

Keywords: Platelet, morphology, phase-interference microscopy

Background: Quantitative platelet disorders are always associated with qualitative platelet alterations. The circulating platelet multiplicity reflects cell distinctions in size, density, metabolic, functional, biochemical features and the level of megakaryocyte polymorphism. Quantifying the optical phase delays associated with living cells provides access to information about morphology and dynamics at the nanometer scale.

Materials and Methods: Morpho-functional status of peripheral blood platelet was determined by express-method of vital computer morphometry using computer phase-interference microscope Cytoscan (Russia). The microscope is a modification of a Linnik interferometer with a He-Ne laser (1=633 nm) as a source of coherent light. The microscope is equipped with the disector image tube to register the interference signal, and an electronic unit for computer-assisted cell imaging. Measurements of optical phase difference (OPD) were performed sequentially at each point of the image. To register the interference signal and to convert it into local phase values, it linear periodical modulation of the reference. The complex algorithm of morphometry included definition of optic and geometrical characteristics of unfixed and unstained living platelets, statistical analysis of data and creation of medical documents. We have analyzed the computer platelet images (three dimensions of whole cells and their parts, different cross-sections and histogram), the optic-geometrical parameters of each isolated platelet and the distribution of platelets by sizes to detect the heterogeneity of cell population. It allowed to identify four platelet forms that have different morphological features and different parameters of size distribution (Pic).

Conclusions: The technology is now available to investigate single living platelet, its morphology and function, together with analyzing heterogeneity of all circulating platelet population. Moreover morphometric parameters of living platelets can be predictors of possible following hemostasiological disorders. The proposed method opens additional perspectives for analyzing the heterogeneity of platelet populations without sophisticated experimental techniques.

Figure: Scheme of phase-interference images of living platelets.

1- resting form; 2 - platelet with low activation level; 3 - platelets with high activation level; 4 - degenerate functionally incomplete platelet

Corresponding Author: Irina Vasilenko, Russian gerontological scientific clinical center, 1-st Leonova street, 16, Moscow, Russian Federation, hadik2@rol.ru

P294

INFLUENCE OF WARFARIN ON MULTIPLE ELECTRODE PLATELET AGGREGOMETRY (MULTIPLEPLATE).

C. Bulato, S. Carraro, P. Simioni

Department of Cardiologic, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy, michelangelo.sartori@aosp.bo.it

Background: The newly developed point-of-care instrument, the multiple electrode aggregometry using computer phase-interference microscope Cytoscan (Russia). The microscope is a modification of a Linnik interferometer with a He-Ne laser (1=633 nm) as a source of coherent light. The microscope is equipped with the disector image tube to register the interference signal, and an electronic unit for computer-assisted cell imaging. Measurements of optical phase difference (OPD) were performed sequentially at each point of the image. To register the interference signal and to convert it into local phase values, it linear periodical modulation of the reference. The complex algorithm of morphometry included definition of optic and geometrical characteristics of unfixed and unstained living platelets, statistical analysis of data and creation of medical documents. We have analyzed the computer platelet images (three dimensions of whole cells and their parts, different cross-sections and histogram), the optic-geometrical parameters of each isolated platelet and the distribution of platelets by sizes to detect the heterogeneity of cell population. It allowed to identify four platelet forms that have different morphological features and different parameters of size distribution (Pic).

Conclusions: The technology is now available to investigate single living platelet, its morphology and function, together with analyzing heterogeneity of all circulating platelet population. Moreover morphometric parameters of living platelets can be predictors of possible following hemostasiological disorders. The proposed method opens additional perspectives for analyzing the heterogeneity of platelet populations without sophisticated experimental techniques.

Figure: Scheme of phase-interference images of living platelets.

1- resting form; 2 - platelet with low activation level; 3 - platelets with high activation level; 4 - degenerate functionally incomplete platelet

Corresponding Author: Irina Vasilenko, Russian gerontological scientific clinical center, 1-st Leonova street, 16, Moscow, Russian Federation, hadik2@rol.ru

P314

WELLS RULE AND D-DIMER FOR THE DIAGNOSIS OF ISOLATED DISTAL DEEP VEIN THROMBOSIS

M. Sartori, B. Cosmi, C. Legnani, E. Conti, L. Valdré, G. Palareti

Angiology and Haemostasis Unit, University Hospital, Bologna, Italy

Keywords: isolated distal deep venous thrombosis, diagnosis, D-dimer, clinical probability

Background: Wells and colleagues developed a diagnostic rule to estimate the probability of the presence of proximal symptomatic deep venous thrombosis (DVT). The accuracy of the Wells rule has not been validated for use in primary care patients in whom symptomatic isolated distal deep venous thrombosis (IDDVT) is suspected.

Aims: To validate the diagnostic accuracy of the Wells rule and D-dimer testing for IDDVT.

Patients and methods: Cross-sectional study with data collection from 1 September 2009 to 15 February 2010, including 190 consecutive outpatients who were referred by the emergency department or by a primary care physician to our ultrasound laboratories. All patients underwent history-taking and physical examination to calculate the Wells rule score, D-dimer testing, and a comprehensive real-time B-mode and colour Doppler ultrasonography examination of both legs by a vascular medicine physician. The proximal deep veins were examined first, then, only in patients with normal proximal findings, the calf veins were evaluated, including the axial (peroneal and posterior tibial) and the muscular veins.

Results: The prevalence of IDDVT was 11%. 8 patients in the low-risk group according to Wells rule had IDDVT, whereas 10% of patients in the high-risk group had IDDVT. The Wells rule had a sensitivity of 47%, a specificity of 59% with a positive predictive value of 91%. D-dimer was higher in patients with IDDVT as compared to those without IDDVT. Two patients with negative results on a D-dimer test (<500 ng/mL) had IDDVT. Sensitivity and specificity of D-dimer were 87% and 55%, with a predictive negative value of 98%.

Conclusions: The Wells rule does not guarantee estimation of risk in patients in whom IDDVT is suspected. D-dimer <500 ng/mL does not exclude the presence of IDDVT.

Corresponding Author: Michelangelo Sartori, Azienda Ospedaliera di Bologna, Policlinico Sant’Orsola Malpighi, Pad. 2, Via Alberroni, 15, Bologna, Italy, michelangelo.sartori@aosp.bo.it
CORRELATION BETWEEN D-DIMER AND THE PERSISTENCE OF RESIDUAL THROMBOSIS ON ULTRASOUND DOPPLER AT THE END OF ANTICOAGULANT TREATMENT IN DEEP VEIN THROMBOSIS

A. Riera-Mestre 1, J. Jordán 1, A. Romera 1, E. Pina 1, R. Pujol 1

1 Internal Medicine Department, Hospital Universitari de Bellvitge - IDIBELL, L’Hospitalet de Llobregat, Spain; 2 Angiology and Vascular Surgery, Hospital Universitari de Bellvitge - IDIBELL, L’Hospitalet de Llobregat, Spain; 3 Hemostasis Unit, Hospital Universitari de Bellvitge - IDIBELL, L’Hospitalet de Llobregat, Spain

Keywords: D-dimer, residual thrombosis, deep vein thrombosis

Introduction: The performance of Doppler ultrasound (DU) is laborious and involves a care burden. There is evidence of the prognostic value of both D-dimer (DD) or the persistence of residual thrombus (RT) and recurrent deep venous thrombosis (DVT).

Aims: To find the correlation between DD levels and the persistence of RT on DU in DVT of lower limbs followed one month after oral anticoagulant therapy withdrawal.

Materials and methods: This a prospective study including consecutively patients with DVT who have completed anticoagulant therapy after a minimum of three months and show no exclusion criteria: patients under 18 years, coexistence of pulmonary embolism, bilateral DVT, neoplasia, indication of persistent anticoagulation or needing for early withdrawal anticoagulant treatment. A lower limb DU and a determination of DD (IL Test) were performed one month following oral anticoagulant therapy withdrawal, approximately.

Results: 32 patients have been included, 59% were male, with a mean age of 59.8 ± 19.8 years. Regarding the location, 3% were originated in the cava vein, 16% at iliac, 41% at the femoral and 37% in the popliteal veins. All patients received low-molecular-weight heparin (LMWH) as initial therapy at a minimum of 5 days. Treatment was followed with warfarin (72%), acenocoumarol and LMWH (6%) as long-term therapy, with a mean duration of 12 ±6 months. A determination of DD and a lower limb DU at 35 ±15 and 35 ± 17 days of anticoagulant treatment withdrawal, respectively, were made. Determination of DD was positive in 19% of cases and 47% showed RT. Among patients with positive DD levels, all 83% with RT, while 61% of patients with a negative determination of DD, showed vein recanalization.

Conclusions: There is a relationship between a positive determination of DD and RT, and between negative values of DD and vein recanalization one month after oral anticoagulant therapy withdrawal in DVT of lower limbs.

Corresponding Author: Antoni Riera-Mestre, Internal Medicine Department, Hospital Universitari de Bellvitge - IDIBELL, Feixa Llarga s/n, L’Hospitalet de Llobregat, Spain, arierar@bellvitgehospital.cat

EVALUATION OF HIT SUSPECTED PATIENTS WITH POLYSPESIFIC ANTIGEN (IGG/A/M) AND MONOSPECIFIC (IGG) EIA ASSAY

A.J. Gafou 1,2, A. Skepetari 1, M. Bellia 1, V. Tsevrenis 1, K. Maragos 1, E. Nomikou 1, V. Papadopoulou 1, A. Maris 2, G. Theodosis 2

1 1St Regional Transfusion And Haemophilia Center, Hippocratio Hospital, Athens, Greece; 2 Haematology Department, Hippocratio Hospital, Athens, Greece

Keywords: HIT

Background: In suspected heparin-induced thrombocytopenia (HIT), the ELISA polyspecific antigen assays (IgG/A/M) recognize PF4/heparin-reactive antibodies, most of which are not platelet activating. Those clinical insignificant antibodies mostly are of IgM/ A classes. When activation assays are not available the clinical decision of replacing heparin with an alternative anticoagulant, is difficult as these drugs have increased bleeding risk. The use of the IgG-specific ELISA assays may enhance the diagnostic specificity of the ELISA assays.

Aims: Evaluation of HIT suspected patients with polyspecific antigen (IgG/A/M) and monospecific (IgG) ELISA assay.

Patients and methods: Samples of patients suspected to have HIT were collected between November 2008 and February 2009 and tested with both assays (IgG ELISA, M/A -GTI Inc. and ELISA IgG-GTI Inc.). According to the manufacturer a positive result necessitates > 0,400 OD value and >50% decrease of this value in the confirmatory step using high heparin concentration. In patients the pretest probability (4Ts score) and the therapeutic modality were recorded.

Results: 11 consecutive patients were tested for heparin-reactive antibodies. 6 patients had a clinical score >3. 6 patients had positive result with ELISA IgG/M/A and only 1 patient has positive result with ELISA IgG. The last patient had the highest 4T’s score (score 6, development of new thrombosis under heparin treatment) and was also the only patient who was treated with a direct thrombin inhibitor. All the patients with a negative ELISA IgG/M/A result were also ELISA IgG negative.

Conclusions: The use of ELISA IgG assay decreased the positive results from 54.5% to 9.1%. In those laboratories where the activation assays are not available the incorporation in the diagnostic algorithm of a ELISA IgG assay confers to a better specificity in HIT suspected cases.

Figure 1: ELISA IgG/M/A and IgG OD values

THROMBIN GENERATION ASSESSMENT IN CYNOMOLGUS MONKEY USING CAT METHOD.

P. Dabrilli, V. Rossetto 1, A. Radu 1, C. Bulato 1, S. Gavasso 1, M. Boldrin 2, E. Cozzi 2, P. Simioni 2

1 Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Padua, Italy; 2 Department of Surgical and Gastrointestinal sciences, University of Padua, CORIT, Padua, Italy.

Background/Aims: Microvascular thrombosis due to activation of clotting cascade is a key feature of the rejection process of pig organs transplanted into primates. Such coagulopathy is a barrier to the long-term survival of porcine xenografts in this species combination. Little is known about coagulation assessment in primates.

Aim of the study: to evaluate thrombin generation (TG) in cynomolgus monkey; to compare TG between cynomolgus and human.

Materials and methods: We evaluated TG profiles in cynomolgus monkeys and in healthy human controls. TG assay was performed in poor platelet plasma (PPP) using the calibrated automated thrombogram (CAT, Thrombinoscope BV) method. TG was triggered using the PPP reagent (Thrombinoscope BV) and parameters considered were: ETP (endogenous thrombin potential, nM*min), Cmax (maximum thrombin concentration, nM) and lag time (time to clot, min).

Results: We evaluated TG in 22 cynomolgus monkeys and 50 human controls. Mean ETP measured (nmol*m*min ± SD) was 1971.79 ± 276.88 in monkey and 1089.32 ± 292.79 in human (p<.000); mean Cmax (nmol ± SD) was 401.64 ± 60.38 in monkey and 288.04 ± 63.73 in human (p<.000); mean Lag Time (min ± SD) was 1.35 ± 0.19 in monkeys and 1.76 ± 0.85 in human (p<.002).

Conclusions: Thrombin plays in vivo a pivotal role in the coagulation system being involved in clot formation, platelet activation and PC anticoagulant pathway. Our method (CAT) revealed significantly lower lag time in monkey than in human while ETP and Cmax values were higher in monkey than in human. This data are consistent with a higher prothrombotic profile in cynomolgus as compared to humans suggesting that, with regard to coagulation, xenotransplantation in cynomolgus may represent a much more difficult situation than in humans.

Corresponding Author: Paolo Dabrilli, Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua, Padua, Italy, paolo.dabrilli@gmail.com
ROLE OF MULTIDETECTOR CT PULMONARY ANGIOGRAPHY IN THE DIAGNOSTIC ALGORITHM OF PULMONARY HYPERTENSION

M.I. Torres 1, M.C. Fernández Capitán 1, J.J. Ríos 1, S. Alcolea 1, M. Fernández Veililla 2, M.A. Rodríguez Dávila 2, J. Camacho 2, C. Navarro 2, A. Lorenzo Hernández 2

1 Radiology Department, Hospital Universitary La Paz, Madrid, Spain; 2Internal Medicine Department, Hospital Universitary La Paz, Madrid, Spain; 3 Pneumology Department, Hospital Universitary La Paz, Madrid, Spain

Keywords: pulmonary hypertension, CT Angiography, diagnosis

Aims: describe the prevalence of right ventricular dysfunction signs in patients suffering from pulmonary hypertension. To establish the usefulness of MDCT angiography versus ventilation/perfusion (V/Q) scintigraphy in the diagnostic algorithm of pulmonary hypertension.

Materials and methods: MDCT angiographies of 76 patients were recorded between November 2006 and January 2010. The inclusion criterion was a threshold of systolic main pulmonary artery (MPA) pressure of 40 mmHg or higher at rest. In 44 patients (56.5%) the V/Q scintigraphy was available.

MDCT pulmonary angiographies were evaluated for right ventricle (RV) dysfunction signs (RV/LV ratio > 1), left bowing of interventricular septum) and vascular prognostic ratios. Patients were divided into two groups (A pulmonary artery < 29 mm and B ≥ 29 mmHg) for the statistical analysis.

Results: The mean and standard deviations of MPA and RV were recorded, being significantly higher on group B (p > 0.001). Twenty patients (45%) presented a correct match between the MDCT angiography and the V/Q scintigraphy. Four negative V/Q scintigraphy presented signs of chronic thromboembolism in the MDCT angiography. MDCT also contributed to diagnosis of interstitial lung disease.

Conclusions: MDCT angiography is an accurate tool depicting RV dysfunction signs. It is more sensitive for detection of chronic thromboembolism as it shows thromboembolic signs in patients with negative V/Q scintigraphy and can assess coexisting parenchymal lung disease. Hence, it may be used as a first-line-tool in the diagnostic approach of PH.

Corresponding Author: Alicia Lorenzo Hernández, Hospital Universitary La Paz, Paseo de la Castellana 281, Madrid, Spain, alicia.lh@sser.es

STANDARDIZATION OF HEPARIN THERAPY MONITORING

A.L. Berkovski 1, E. Sergeeva 2, A. Suvorov 2, L. Mondove 3, M. Kabardina 3, L. Birjukova 3

1 Hematology Science Center, Moscow, Russian Federation; 2Laboratory for Standardization of Control Methods; 3Department of Hemodialysis, Moscow, Russian Federation

Keywords: APTT, heparin

Background: Activated partial thromboplastin time (APTT) is the most widely used method for laboratory monitoring of heparin therapy. APTT reagents have different heparin sensitivity that results in a variance in the amount of heparin administered to the patients. Standardization of this monitoring was recommended by ISTH/ISCH. Aims: To determine by APTT in-vitro and ex-vivo heparin therapeutic range using the particular heparin-APTT reagent-instrument system.

Materials and methods: The index APTT dependence on unfractionated heparin was studied by automatic coagulometer ACL Elite Pro (IL) and six APTT reagents (Coagulotest, APTT-reagent, Actin FS, Actin FSL, APTT SP and Daptin). 43 hemodialysis patients with heparin support were included to this study.

Results: In-vitro APTT values both in plasma with and without heparin (APTTp and APTTnp) and APTT indexes=A/PTTp/APTTp measurements depended on used reagents. These differences were due to heparin sensitivity of the reagent with 1.5-1.8 variations at therapeutic range (0.2-0.4IE/ml). Ex-vivo (heparin therapy) APTT values also depended on used reagents. For example, APTT indexes for Actin FSL and Daptin are equal to 1.58 – 3.66 and 1.24 – 2.66 respectively. APTT of patients plasma before heparin administrations was different from APTT of normal plasma. So we used APTT index=APTTp/APTTp (o – plasma samples before anticoagulant therapy) as more correct index. APTT indexes and heparin concentrations were graphically compared and APTT/heparin therapeutics ranges were determined for each reagent.

Conclusions: APTT indexes correspond to heparin therapeutic range to the hemodialysis patients were established for each used APTT reagents. It’s seems necessary to standardize APTT monitoring of heparin therapy for applicable APTT reagents.

Corresponding Author: Aron L. Berkovski, Hematology Science Center, Noviy Zhkovsky prospeeg, 4, Moscow, Russian Federation, aron@blood.ru

USEFULNESS OF INNOVIN DILUTED PROTHROMBIN TIME FOR THE DETECTION OF LUPUS ANTICOAGULANT

R. Loreth, G. Klose, F.W. Albert

Department of Clinical Haemostaseology, Medical Clinic III, West Palatinate Clinical Center, Kaiserslautern, Germany

Keywords: dPT, diluted prothrombin time, lupus anticoagulant

Aims: Lupus anticoagulants (LA) are antibodies which inhibit in vitro phospholipid-dependent tests of coagulation. No single screening test can detect all LA-positive patients, so the SCC Subcommittee for the Standardization of LA recommends at least two independent tests for LA screening. Commonly used screening tests are based on the Kaolin Clotting Time (KCT), a LA-sensitive aPTT or on the dilute Russell’s Viper Venom Time (dPTT). Dilute prothrombin time (dPT) has also reported as a sensitive test for LA-screening. Therefore, we evaluated the usefulness of a homemade dPT in comparison to different commercial available tests.

Methods: All tests were performed on the BCS analyser (Siemens Healthcare Diagnostics, Germany). In a first step quality and performance of the homemade dPT using recombinant thromboplastin Innovin (Siemens Healthcare Diagnostics, Germany) in a 1/200 dilution was evaluated. Subsequently, we estimated in 22 patients, who were previously tested positive for LA, dPT, KCT (Kaolit, Life Diagnostics, USA), dRVVT (LA1, LA2, Siemens Healthcare Diagnostics, Germany) and MixConLA (Instrumentation Laboratories, Germany).

Results: Intra-assay coefficient of variation (CV) for dPT was 1% and inter-assay CV 5.6%. Normal values assigned in 50 healthy individuals ranges from 35 to 51 sec. 51 sec were chosen as the cut-off value. We obtained negative results in 2 patients with dPT, in 1 patient with MixConLA, in 8 patients with KCT and in 3 patients with dRVVT.

Corresponding Author: Ralph Loreth, Department of Clinical Haemostaseology, Medical Clinic III, West Palatinate Clinical Center Kaiserslautern, Hellmut-Hartert-Straße 1, Kaiserslautern, Germany; rloreth@westpfalz-klinikum.de

RESEARCH OF LUPUS ANTICOAGULANT - LABORATORY PROFILE OF PATIENTS OF HOSPITAL OF SCHOOL MEDICINE OF UNIVERSITY OF SÃO PAULO, BRAZIL

M. Colombini, R. Mizuta

Central Laboratory Division, Central Institute Clinics Hospital (ICHC), University of São Paulo Medical School Clinics Hospital, São Paulo, Brazil

Keywords: lupus anticoagulant, laboratory

The antiphospholipid antibodies, lupus anticoagulant (LA) is one of the markers of antiphospholipid syndrome, systemic acquired condition and may be primary or secondary, and is characterized by recurrent thromboses in the arterial, venous, or both, recurrent fetal loss and thrombocytopenia. These antibodies may be present in normal, autoimune diseases; neural diseases, use of medications, viral infections and some parasites. Guidelines have been published, recommending criteria for laboratory diagnosis, and suggest to perform a screening that is the use of at least two functional tests, based on the presence of phospholipids in order to improve identification of the antibody. The main options for testing include the time activated partial thromboplastin time and viper venom Russell diluted. Our objective was to determine the positivity for lupus anticoagulant in patients who are investigated by the Central Laboratory of Coagulation, Clinical Hospital, of both sexes, different ages, from various clinics of medical specialties, and from various detention centers, so as, the interference of the use of anticoagulation in this investigation. We performed laboratory research to LA in 504 patients. Blood samples were collected in tubes with sodium citrate 3.2%, followed by double centrifugation to obtain platelet-poor plasma. We also performed the PTTA and dVVRT as tests triadore; and dVVRT with excess phospholipid as the confirmatory test, using reagents Dade Behring ®, and processed in the BCS System analysis company. Of the 504 patients, 67.8% were female, 11.9% were male, and 20.3% were children, ranging from 02 months to 18 years age among both sexes. The average age among women was 49.5 years of age, while for men the average was 54 years. In 94% (474/504) of patients the search results were negative. Of the remainder, 6.0% of cases was positive, 70% in women, 16.7% in men and 13.3% in children. 12.5% of samples were considered positive when at least one of the screening tests was corrected by the mix test. Negative samples were those that were not corrected by the mix. In these cases, the use of oral anticoagulants or heparin was proven. Reagent dVVRT showed sensitivity of 100% compared to PTTA [73.3% (22/30)]. The presence of another inhibitor was suggested in 03 cases, two of them in the male group, and the other in pediatric patients. Our results show consistency with the literature data. There was a predominance of research in female patients and positivity in this group. In patients in the pediatric group, the results were as expected. There was no prejudice to the investigation in most anticoagulated patients and dVVRT was the most sensitive of screening tests.

Corresponding Author: Marjorie Colombini, University of São Paulo Medical School Clinics Hospital, 235 Avenue. Cons measured: São Paulo, Brazil, marjorie.colombini@hcnet.usp.br
IMPORTANCE OF PREOPERATIVE AND INTRAOPERATIVE DUPLEX SONOGRAPHY IN PATIENTS WITH PROGESSION OF SUPERFICIAL VENOUS THROMBOPHLEBITIS TO DEEP VEIN THROMBOSIS

I.P. Lozev 1, I. Topalov 2, I. Losev 2, K. Girov 2

1 General Surgery, Medical Institute, Ministry of Interior, Sofia, Bulgaria; 2 Bulgarian Academy of Science, Sofia, Bulgaria

Aims: We have evaluated the progression of isolated superficial venous thrombophlebitis to deep-vein thrombosis in patients with no initial deep venous involvement using duplex ultrasonography.

Methods: Patients with thrombosis isolated to the superficial veins, with no evidence of deep venous involvement by duplex ultrasound examination, were evaluated by follow-up duplex ultrasonography to determine the incidence of disease progression into the deep veins of the lower extremities. Initial and follow-up duplex scans evaluated the femoropopliteal and deep calf veins in their entirety. Follow-up studies were done at an average of 6.3 days. In patients with embolicogenic ascending thrombophlebitis in the area of the SFJ, SPJ or thrombophlebitis of perforating veins depending on sonography finding we perform retrograde venous thrombectomy (RVT), assisted by sonography or only crossectomy. In ligation of thrombosed perforating veins (PV), its junction with adjacent magistral deep vein is marked using intraoperative sonography.

Results: From January 2002 to January 2009, 286 patients were identified with isolated superficial venous thrombosis. Forty (14%) patients had documented progression to deep vein involvement. The most common site of deep-vein involvement was progression of disease from the greater saphenous vein into the common femoral vein (19 patients, 47.5%), with 11 of these, extensions noted to be nonocclusive, and 8 having a free-floating component. Five patients had extended above-knee saphenous vein thrombi through thigh perforators to occlude the femoral vein in the thigh. Nine patients had extended below-knee saphenous disease into the popliteal vein, and 7 patients had extended below-knee thrombi into the tibioperoneal veins with calf perforators.

Conclusions: Superficial thrombophlebitis is not always benign and self-limiting disease. Affecting of SFJ, SPJ or thrombophlebitis of perforating veins, can cause complications as a DVT, which requires careful evaluation, active conservative therapy, often ultrasonography follow up and if necessary timely and adequate operation.

Corresponding Author: Ilia P. Lozev; General Surgery, Medical Institute ,Ministry of Interior, 79 Schoelev Blvd., Sofia, Bulgaria, ilia.lozev@vbg.bg

P38

INFLUENCE OF STREAM ON TENSILE STRENGTH OF NONTHROMBOCYTE PLASMA CLOTS

A. Vasilievitch Savushkin

Chair of Human Physiology, Chita State Medicine Academy, Chita, Russian Federation

Keywords: plasma, clotting, tensile strength

Laboratory coagulation tests are being carried out in constant standard conditions, but clotting in an organism realize in another ones, which are significantly different. Such important factors as pressure and stream are not reproduced at all. Therefore test information could be inadequate to origin process.

The aim of our study was to compare tensile strength of nonthrombocyte plasma clots formed in unmoving and in stream conditions. Samples of blood were obtained from 30 volunteers. After centrifugation nonthrombocyte plasma was divided on several parts. All of them were put into special stretching equipment where liquid could flow under hydraulic pressure for some period of time and then formed the clot. After that, it was stretched before rupture, and the rupture forces were registered. Pressure values were 0, 245, 343, 441, 540, 638, and 736 (N/m2). The next values of overage shear rates were 0, 15+9, 26+9.5, 34+13, 44+17, 54+21, 63+25, 73+30, and 80+40 (sec). These parameters are correspondent to the vein part of vascular system.

The rupture forces were 209+73 (control, 100%), 213+72 (102%), 219+73 (105%), 215+83 (103%), 194+78 (93%), and 178+76 (85%) (mN). There is tendency to decrease in the clot strength with the increase of velocity in the highest values. The last magnitude was statistically different from the control that was obtained by the pair comparison method (Student’s authentic coefficient was 2.07, and confidence value was 0.95).

So, it was shown that the tensile strength of nonthrombocyte clots formed in condition with high flow velocity was less then for unmoving ones.

Corresponding Author: Alexandr Vasylievich Savushkin. Chair of human physiology, Chita State Medicine Academy, Gorkogo str., blld. 39, A, Chita, Russian Federation, savushkin_al@mail.ru

P658

THROMBOSIS COMMON BIOMARKERS IN ATHEROSCLEROSIS AND RHEUMATIC DISEASES

N. Kakauridze, M. Jebashvili

Tbilisi Medical University, National Center of Therapy, Tbilisi, Georgia

Keywords: thrombosis, biomarkers, APLA

Aims: The goal of this work is to determine the thrombosis risk factors (fibrinogen, CRP, anti cardiolipin antibodies (CLA) and its connection with lipid spectrum in patients with atherosclerosis and rheumatic diseases.

Materials and methods: 35 patients (mean age 41.5 +/- 13.5 years) were divided into 3 groups: I group -13 patients with positive CLA-IgG and rheumatic disease (systemic lupus erythematosus, rheumatoid arthritis, rheumatic heart disease); II group - 12 patients with positive CLA and verified diagnosis of atherosclerosis; III group - control group included negative CLA 14 persons with atherosclerosis.

Blood samples taken after 12 hours fasting were examined. Lipid spectrum: total cholesterol (TC), triglycerides(TG), cholesterol of high density lipoproteins (HDL-C) and cholesterol of low density lipoproteins (LDL-C) were determined by spectrophotometer ‘Janway- England’ with enzyme method by means of reagent BILALO, France. Fibrinogen concentration after Rutberg methods. Serum CRP levels were measured by high-sensitive immunonephelometric technique. IgG and IgM CLA were studied by solid-phase immunoenzyme assay.

There was a good correlation between Clauss fibrinogen and fibrinogen antigen (r2=0.77). Although in the 50 samples from orally anticoagulated patients with an INR >4.5 were also included. Clauss fibrinogen was measured on the BNA analyser (Behring, Marburg) with human fibrinogen antibodies (Siemens, Marburg). Fibrinogen antigen was measured on the BNA analyser (Siemens, Marburg) and Multifibren U (Siemens, Marburg). Derived fibrinogen was calibrated against Clauss fibrinogen. Fibrinogen antigen was measured on the BNA analyser (Siemens, Marburg) with human fibrinogen antibodies (Siemens, Marburg).

Thus, CRP and CLA are indicators of thrombosis events in atherosclerotic CLA patients and their correction highly depended on dyslipidemia.

Corresponding Author: Nona Kakauridze, Tbilisi Medical University, National Center of Therapy, Chachava 83, Tbilisi, Georgia, nnakauridze@tsmu.edu

P59

ABILITY OF DERIVED FIBRINOGEN TO CLINICAL PRACTICE: COMPARISON WITH CLAUS FIBRINOGEN AND FIBRINOGEN ANTIGEN

R.M. Loreth, G. Klose, F.W. Albert

Department of Clinical Haemostaseology, Medical Clinic III West Palatinate Clinical Center, Kaiserslautern, Germany

Keywords: derived fibrinogen, Clauss fibrinogen, fibrinogen antigen

Background: There is no international agreement about the right method for fibrinogen measurement. The PT-derived fibrinogen is commonly used for routinely determination of plasma fibrinogen. Some studies show low agreement between derived fibrinogen and Clauss fibrinogen, especially in oral anticoagulated patients with elevated INR. To study the suitability of derived fibrinogen for our laboratory we compared derived fibrinogen with Clauss fibrinogen and fibrinogen antigen in 350 routine samples.

Materials and methods: In 350 consecutive samples we measured derived fibrinogen and Clauss fibrinogen on the BCS analyser (Siemens, Marburg) with Innovin (Siemens, Marburg) and Multifibren U (Siemens, Marburg). Derived fibrinogen was calibrated against Clauss fibrinogen. Fibrinogen antigen was measured on the BNA analyser (Behring, Marburg) with human fibrinogen antibodies (Siemens, Marburg).

There were positive correlation between TG and ACLA and negative correlation between HDLC and CLA. But relation between ACLA and CRP was revealed only in II group. It is interesting that CRP level was significantly higher in II group, than in I and III group.

In patients with no initial deep venous thrombosis to deep-vein thrombosis in patients with no initial deep venous thrombosis.

Conclusions: On the background of missing standardisation for fibrinogen determination derived fibrinogen can be used for routinely laboratory measurement.

Corresponding Author: Ralph M. Loreth, Department of Clinical Haemostaseology, Medical Clinic III West Palatinate Clinical Center, Kaiserslautern, Westpfalz-Klinikum GmbH, Klinische Hämostaseologie Medizinische Klinik III, Hellmut-Hartert-Straße 1, Kaiserslautern, Germany, rloethe@westpfalz-klinikum.de
PERFORMANCE OF COAGULOMETRIC FUNCTIONAL METHODOLOGY BASED ON THE DELUTE RUSSELL’S VIPER VENOM TIME ON DETERMINATION FROM ACTIVATED PROTEIN C RESISTANCE SECONDARY THE PRESENCE OF LEIDEN MUTATION

M.P. Colombini, R. Mizuta, I. Baptista

Clinical Hospital Of School Medicine of University of São Paulo, Brazil

Keywords: Leiden mutation, protein C resistance

Our objective was to assess the sensitivity of dRVVT test, a new test, to analyze the functional activated protein C resistance by comparing our results with those obtained in molecular research for the presence of Leiden mutation.

We selected 108 patients from Vascular Surgery Department that were reported at the same time to the Coagulation Laboratory to perform the functional APCR test, and to the Heart Institute of University School of Sao Paulo (INCOR) for to investigate the presence of Leiden mutation. The functional test based on dRVVT was analyzed using coagulometric methodology, while the presence of mutation was investigated by polymerase chain reaction, a molecular assay. Of all patients, the women group represented the majority, 60.2% (65/108) (Figure 1). 98.1% of the samples (106/108) showed concordant results between methodologies, 87.9% (95/108) negative results (no mutation) and 10.2% (11/108) the presence mutation in its heterozygous form (Figure 2). Positive samples showed values below cut-off. Two cases were discordant, one of them a false positive result, and the other, a false negative result. Both cases were repeated with the maintenance of the results. In the situation of false positive result we made the dRVVT with the sample diluted in negative result. Both cases were repeated with the maintenance of the results.

Figure 1: Sex distribution and positivity by group

Figure 2: Comparison between both methodologies

Corresponding Author: Marjorie Paris Colombini, Clinical Hospital Of School Medicine of University of São Paulo, Dr. Eneas de Carvalho Aguiar, 255 Avenue. Cerqueira César - São Paulo, Brazil, marjorie.colombini@hcnet.usp.br

MEAN PLATELET VOLUME IS NOT A PREDICTOR OF VASCULAR ACCESS FAILURE IN PATIENTS ON HEMODIALYSIS

M. Simkovic 1, S. Dusilova-Sulkova 2, J. Maly 1

1 2nd Department of Internal Medicine, Department of Clinical Hematology, University Hospital and Medical School, Hradec Kralove, Czech Republic; 2 Division of Nephrology, Department of Gerontology and Metabolism, University Hospital and Medical School, Hradec Kralove, Czech Republic

Keywords: mean platelet volume, thrombosis, vascular access

Background: Increased mean platelet volume (MPV) has been identified as an independent risk factor of acute coronary syndromes, stroke and venous thromboembolism. End-stage renal disease is associated with high prevalence cardiovascular disease. Maintaining vascular access for regular hemodialysis is a serious issue due to frequent thrombotic occlusion. However, data concerning relevance of MPV as a predictor of vascular access thrombosis are not available.

Aims and methods: In order to assess the role of MPV in vascular access thrombosis, we performed a prospective analysis of all patients on regular hemodialysis at the University Hospital Hradec Kralove from October 2007 till February 2010.

Results: The study included 93 patients with median age 65 years (range 36 - 89). Vascular access thrombosis was observed in 33 (35%) patients during the follow-up time. The mean platelet volume was not significantly different in patients with or without vascular access thrombosis (10.4 +/- 1.6 vs. 10.3 +/- 1.1 fL). MPV was equal in diabetic and non-diabetic patients and remained unchanged after 2 months from the thrombotic event.

Conclusions: Our results indicate that mean platelet volume is not a marker of vascular access thrombosis in hemodialysis patients.

Acknowledgment: This work was supported by the grant of Czech Ministry of Health (MZO 0017906).

Corresponding Author: Martin Simkovic, 2nd Department of Internal Medicine, Department of Clinical Hematology, University Hospital and Medical School, Sokolska 58, Hradec Kralove, Czech Republic, simkovicm@gmail.com

IS VENOUS COLOR FLOW DUPLEX SCAN AN APPROPRIATE INITIAL SCREENING TEST FOR GERIATRIC INPATIENTS WITH SUSPECTED PULMONARY EMBOLISM ?

R.L. Kreidy 1, M. Waked 2, E. Stephan 2

1 Department of Surgery, 2Department of Medicine, University of Balamand, Beirut, Lebanon

Keywords: venous thrombosis, pulmonary embolism, duplex scan

Background/Aims: The contribution of lower extremity venous color flow duplex ultrasonic to the diagnostic strategy for pulmonary embolism has been demonstrated. However, the prevalence of this test in clinically suspected pulmonary embolism is not very high (10% to 18%). Thromboembolic risk increases with advanced age (1.9 per 10 years increase) and in hospitalized patients (average 1.5 risk factor per patient). No published studies have provided adequate information about the value of venous duplex scan in the diagnosis of pulmonary embolism in geriatric inpatients. The aim of this study is to determine the performance of lower extremity venous duplex scan in hospitalized elderly patients with clinically suspected pulmonary emboli.

Patients and results: From January 2008 to January 2010, 100 consecutive geriatric inpatients (45 males and 55 females) with clinically suspected pulmonary embolism were assessed in an academic tertiary care center with complete bilateral lower extremity venous duplex scan. Age varied between 60 to 96 years (mean 77 years). Eighty-two percent of the patients were above 70 years, 41 % above 80 years and 8% above 90 years. Thirty-one patients were diagnosed for recent venous thrombosis (13 proximal and 18 distal). Venous thrombosis was localized on the right side in 9 patients, on the left side in 14 patients and on both sides in 8 patients. Twenty-two of the 31 patients had associated risk factors for thromboembolism (16 had 1, 5 had 2 and 1 had 4). The most common risk factors observed were history of venous thromboembolism (9), obesity (5), immobilization (3), malignancy (2), severe heart failure (2), fracture and recent surgery (2).

Conclusions: Venous color flow duplex scan appears to be a useful adjunct or even a reasonable initial screening test in the diagnostic algorithm of hospitalized geriatric patients with clinically suspected pulmonary embolism. This is particularly true in patients with associated risk factors for thromboembolism and in uremic patients not candidate for helical spiral lung computed tomography.

Corresponding Author: Raghid Louis Kreidy, Department of Surgery, University of Balamand, Youssel Starsock Street, Achrafieh, Beirut, Lebanon, Docrkdyl@Jncco.Com.Lb
THE FACTOR V HR2 HAPLOTYPE (FY A4070G) AMONG WOMEN WITH VENOUS THROMBOEMBOLISM

E. Rossi, T. Za, A. Cinimillo, S. Betti, A. D’Orazio, G. Leone, V. De Stefano
Institute of Hematology, Catholic University, Rome, Italy

Keywords: factor V HR2, venous thromboembolism, risk factor

Background: The HR2 haplotype in the factor V (FV) gene produces a mild increase of factor V-dependent antithrombin (AT) resistance. It is doubtful if it is a risk factor for venous thromboembolism (VTE) and if it increases the risk conferred by FV Leiden (FVL). It is unknown whether FV HR2 could be relevant in some situations leading to acquired APC resistance such as pregnancy or use of oral contraceptives.

Aims: To investigate the prevalence of FV HR2 among women with VTE due to different provoking factors.

Patients and Methods: We investigated 393 women with deep venous thrombosis of the legs in 348 cases (in 87 of them with pulmonary embolism, PE) and isolated PE in 45 cases. The first thrombosis occurred at a median age of 33 years (range 14-82), and was provoked in 303 patients (pregnancy or puerperium n=101; oral contraceptive n=84; surgery or other transient risk factors n=118). A group of 204 healthy women (median age 37, range 19-61) were the controls. All women were tested for inherited thrombophilia; the prevalence of FV HR2 was checked by a PCR assay for the A4070G polymorphism in the FV gene.

Results: Inherited thrombophilia was found in 141 patients (35.8%) (deficiency of natural anticoagulants n=22, FVL n=71, PTG20210A n=33, multiple abnormalities n=15) and 19 controls (9.3%) (FVL n=8, PTG20210A n=11). The FV A4070G was found in 59 patients (15.0%) and in 18 controls (2.9%) (p=0.005) and 1.95% (95% CI 1.5-2.4), and 1.95% (95% CI 1.5-2.4) respectively. There was a positive association between LE-DVT and/or upper extremities+PE, of whom 98.1% were LE-DVT+PE. 30% presented PE, and 53% DVT. Of the latter, 94.2% had LE-DVT. The risk of suffering VTE, LE-DVT+PE, or LE-DVT was calculated according to thrombophilia factors which, following adjustment for chronic lung diseases, chronic heart failure, abnormal creatinine levels, post-operative, immobility, cancer, prior VTE, journeys longer than 6 hours over three preceding weeks, oestrogen therapy during two previous months, leg varicosities, and idiopathic VTE, maintained the positive association between PTG20210A and DVT and the 95% CI 1.3-2.4). There was a positive association between LE-DVT and EVL (OR: 1.91; CI 95% 1.3-2.4) and antiphospholipid syndrome (APS) (OR: 1.2; CI 95% 1.1-1.4) and APC-R (OR: 2; CI 95% 1.5-2.8).

Conclusions: FV A4070G is a mild risk factor for VTE; its prevalence is uniform among the patients, independently either of the circumstances of first VTE or the presence of other inherited thrombophilic traits.

Corresponding Author: Elena Rossi, elena2309@yahoo.it

SELECTION CRITERIA OF PATIENTS WITH VENOUS THROMBOEMBOLISM FOR LABORATORY INVESTIGATION OF INHERITED THROMBOPHILIA

E. Rossi, T. Za, A. Cinimillo, S. Betti, G. Leone, V. De Stefano
Institute of Hematology, Catholic University, Rome, Italy

Keywords: inherited thrombophilia, venous thromboembolism, laboratory screening

Background: Laboratory screening for inherited thrombophilia is warranted in young patients, especially those with severe venous thromboembolism (VTE) occurred spontaneously or recurrently. Laboratory screening in older patients is discouraged, especially in the case of mild clinical manifestations or provoked events. Such policy could miss a number of carriers, leaving undiagnosed their kindreds.

Aims: To investigate whether clinical parameters are predictive of the presence of inherited thrombophilia in VTE patients.

Patients and Methods: We analyzed the files of 1,835 patients referred to our Thrombosis Center between 1996 and 2009. The median age at the first thrombosis was 37 years (range 0-89); 736 were males (40.1%). Patients were stratified according to family history of VTE, age of first VTE (< 45 years), the type of VTE (defined severe in the case of proximal DVT and/or pulmonary embolism and mild in the case of distal DVT or superficial vein thrombosis), the circumstances of the first VTE (unprovoked or provoked), the presence of recurrent events. Multiple regression was carried out labeling as dependent variable overall thrombophilia or alternatively severe thrombophilia.

Results: Severe thrombophilia (AT, PC, PS deficiency, multiple defects) was detected in 211 patients (11.4%) and mild thrombophilia (heterozygous factor V Leiden or PTG20210A) in 415 (22.6%). The prevalence of overall thrombophilia or severe thrombophilia were significantly associated to family history of VTE (p=0.005 and p=0.02, respectively) and recurrent events (p=0.0001 and p=0.04), but not to age. Notably, 28% of patients with severe thrombophilia and 30% of patients with mild thrombophilia had clinical onset after 45 years of age. A severe VTE or a first unprovoked event were associated to overall thrombophilia (p=0.008) or to severe thrombophilia (p=0.015), respectively. After exclusion of the patients with severe thrombophilia, clinical severity of VTE (p=0.03) and recurrent events (p=0.0001) kept associated to mild thrombophilia, whereas family history had a borderline significance (p=0.05).

Conclusions: Family history of VTE, clinical severity, and recurrent VTE are predictors of inherited thrombophilia. Selection of the patients according to their age or to the circumstances of VTE could lead to miss diagnosis in a relevant number of cases.

Corresponding Author: Elena Rossi, Largo Gemelli 8, Rome, Italy, elena2309@yahoo.it

HEREDITARY DEFICIENCY OF NATURAL INHIBITORS OF COAGULATION (ANTITHROMBIN, PROTEIN C OR PROTEIN S) CONFERS INCREASED RISK OF ARTERIAL THROMBOEMBOLIC EVENTS. RESULTS FROM A PROSPECTIVE FAMILY COHORT STUDY

D. Tormene, V. Ferri, S. Carraro, M. Facchin, P. Simioni
Department of Cardiologic, Thoracic and vascular Sciences, University of Padua; Padua, Italy

Keywords: thrombophilia, arterial thrombosis

Background: Whether hereditary antithrombin (AT), protein C (PC) or protein S (PS) deficiency is associated with arterial thromboembolic events is controversial.

Methods and results: The objective of this study was to prospectively assess the incidence of arterial thrombotic events in subjects with a deficiency of natural inhibitors of coagulation. We conducted a prospective cohort study in asymptomatic family members of unselected patients who presented with a venous thromboembolic event and who were found to have a deficiency of antithrombin, protein C, or protein S. All arterial thrombotic events were diagnosed by objective diagnostic tests. A total of 640 consecutive subjects belonging to 86 families with hereditary deficiency of AT, PC or PS with a mean age (at the baseline) of 38 years (range, 15 to 79) in the carrier and in the non carrier group were enrolled in the study. A total of 4240 and 3810 patient observation years was obtained respectively in the two groups. Atherosclerosis risk factors were similar in both the two groups. Nineteen arterial thrombotic events occurred in the carrier group (5.6%), compared with seven events in the non carrier group (2.3%) [p=0.07]. The hazard ratio (multivariable analysis) was 4.9 (95% CI 1.5 to 16.3).

Conclusions: Compared with nondeficient family members, subjects with antithrombin, protein C or protein S deficiency have a higher risk for arterial thrombotic events.
Is associated with enhanced in vivo lipid peroxidation and platelet activation that are reversible, at least in part, following folic acid supplementation.

Corresponding Author: Francesca Santilli, Chieti, Italy, f.santilli@unich.it

P263

THE DOWNSTREAM SEQUENCE ELEMENT (DSE) IN 3'-END OF PROTHROMBIN GENE IS NECESSARY FOR GAIN OF FUNCTION EFFECT OF PROTHROMBIN G20210A POLYMORPHISM

V. Djordjevic 1, L. Pruner 1, L. Rakicovic 3, M. Kovac 2, P. Miljic 2, N. Antonijevic 3, S. Kojic 1, D. Radojkovic 1

1 Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia; 2 Hemostasis Department and Hemophilia Center, National Blood Transfusion Institute, Serbia; 3 Institute of Hematology, University Clinical Center of Serbia; Serbia. 4 Institute of Cardiovascular Diseases, University Clinical Center of Serbia, Serbia

Keywords: prothrombin G20210a, gene expression

Background/Aims: The G to A substitution at position 20210 of the 3'-untranslated region of (UTR) the FII gene is associated with increased prothrombin level and enhanced blood coagulation. Although the mechanism of increase in prothrombin levels remains unknown, it has been explained in mRNA at the post-transcriptional level. For more efficient translation, it is still the subject of debate. The aim of this study was to investigate the effect of G20210A polymorphism on prothrombin mRNA expression, within context of different 3'-end lengths.

Materials and methods: Cos-7 cell line were stable transfected with pCMV mammalian expression vectors carrying wild-type (Pt-wt) and 20210A mutant (Pt-20210a) full-length prothrombin cDNA with 3'UTR (1), as well as, wild-type (PDSE-wt) and 20210a mutant (PDSE-20210A) cDNA with 3'UTR extended for 50bp downstream sequence including DSE regulation region (2). The mRNA expression were determined using real-time PCR, on PDSE-20210A construct 7500 Sequence Detection System. PCR was performed using Taq Man Gene Expression Assays.

Results: Relative quantification of mRNA, surprisingly indicated that both Pt-wt (relative quantification value-RQ=1) and mutant Pt-20210A constructs have similar expression level (RQ=0.98, 95%CI 0.95-0.99). However, in the presence of 50bp downstream sequence containing DSE regulation region, PDSE-20210A construct has 1.65-fold (95%CI 1.55-1.76, p<0.005) increased expression level in comparison with wild-type PDSE-wt construct.

Conclusions: In conclusion, our results have provided novel insights into mechanism of prothrombin expression regulation, pointing out the potential importance of downstream sequence in 3'-end of prothrombin gene.

References

Corresponding Author: Valentina Djordjevic, Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, Vodeye Stepe 4444, PO Box 23, Belgrade, Serbia, pg20210a@eunet.rs
FACTOR XIII ACTIVATION AND FIBRIN CROSS-LINKING IN INDIVIDUALS WITH FACTOR V LEIDEN MUTATION: A NOVEL PROTEIN CLEAVAGE MECHANISM THAT MIGHT CONTRIBUTE TO THE INCREASED RISK OF THROMBOSIS

Z. Koncz 1, Z. Bagoly 1, G. Haramura 1, Z. A. Mezei 1, L. Muszbek 1,2

1 Clinical Research Center, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary; 2 Thrombosis, Haemostasis and Vascular Biology Research Group of the Hungarian Academy of Sciences, Debrecen, Hungary

Background: Factor V Leiden mutation (FVLeiden) is associated with impaired down-regulation of procoagulant activity and loss of FV anticoagulant function that result in an increased risk of venous thromboembolism. As downstream effects of FV Leiden on the final phases of clot formation are less well studied, we investigated its effect on factor XIII (FXIII) activation, on the cross-linking of fibrin and α2 plasmin inhibitor (a2PI).

Methods: Clotting was initiated in plasma samples of fifteen healthy individuals with known FV Leiden genotypes and wild type for FXIIIA A subunit (FXIIIA-A) Val34Leu polymorphism by recombinant human tissue factor and phospholipids with or without recombinant human thrombomodulin (rHM). Clots were recovered and analyzed by SDS-PAGE and Western blotting for FXIII-A and a2PI. The extent of FXIII activation, the cross-linking of fibrin γ-chains and the incorporation of a2PI into the clot was evaluated by quantitative densitometry.

Results: The presence of rHM significantly slowed down the activation rate of FXIII in the plasma of wild type individuals as compared to FV Leiden carriers. Time required for half maximal FXIII activation was approximately 1.5-fold prolonged in wild types (mean±SEM: 629±75.3 sec) in the presence of rHM as compared to carriers of FV Leiden (mean±SEM: 437±63.6 sec). The delay of FXIII activation caused by rHM in wild type individuals was more than 4-fold reduced in heterozygotes and more than 8-fold in homozygotes. The inefficiency of rHM on delaying FXIII activation in FVLeiden carriers resulted in earlier cross-linking of fibrin γ-chain and a2PI to fibrin.

Conclusion: Earlier FXIII activation and, as a consequence, earlier cross-linking resulting in fibrin cross-linking might represent a novel mechanism contributing to the increased thrombosis risk in FV Leiden carriers.

Corresponding Author: Zsuzsa Koncz, Clinical Research Center, University of Debrecen, Medical and Health Science Center, Nagyerdi krt. 98, Debrecen, Hungary, koncz_zsuzsa@yahoo.com

HEPARIN RESISTANCE AND ANTITHROMBIN DEFICIENCY

E.L. Coath, P.C. Cooper, K.P. Hickey, M. Makris
Sheffield Haemophilia and Thrombosis Centre, Sheffield, UK

Keywords: antithrombin, thrombin generation

Background: Antithrombin deficiency is associated with an increased risk of venous thrombosis and is reported to show heparin resistance, which is clearly important as affected patients are not usually treated with unfractionated (UFH) or low-molecular-weight (LMWH) heparin. We were struck by how rare this phenomenon was in our population of >150 individuals with this deficiency and decided to study it initially in vitro.

Materials and methods: We investigated the effect of UFH (5th International Standard) at concentrations of 0.6–0.9 U/ml and LMWH (1st International Standard) at concentrations of 0.5 U/ml on plasma immunodepleted of antithrombin. We supplemented the immunodepleted plasma with pooled normal plasma to obtain antithrombin concentrations of 0, 5, 10, 15, 20, 30, 50, 75 and 100% and studied the effects of the multiple UFH and LMWH concentrations on each. We measured a standard APTT (Synthasol) and thrombin generation (TG) in a platelet poor assay using SM tissue factor and 4U/ml phospholipids on a Fluoroskan Ascent.

Results: In the absence of heparin, there was an inverse relationship between TG (ETP and peak thrombin) and antithrombin concentration, but no effect was seen with the APTT. When APTT was used as the endpoint, heparin resistance was only observed at antithrombin concentrations of >30%. Almost complete suppression of TG was observed with 0.5U/ml heparin at antithrombin concentrations of >30% for UFH and >50% for LMWH. Increasing the UFH concentration was able to suppress TG almost completely even for the sample with 10% AT, when 3U/ml UFH was required. For LMWH a dose of 3ml/ml suppressed TG completely for the 30% AT sample. It was not possible to obtain complete TG suppression with AT samples of 5% or less with UFH or 20% or less for LMWH.

Conclusions: We conclude that whilst heparin resistance is easily observed with the TG assays and can largely be overcome with increasing doses of UFH or LMWH, it cannot be demonstrated with the APTT for samples with AT of 30% or more.

Corresponding Author: Fiona L. Coath, Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Glossop Road, Sheffield, UK, macleay73@sheffield.ac.uk

CARRIERS OF THE PROTHROMBIN MUTATION G20210A ASSOCIATED WITH THROMBOSIS HAVE HIGHER THROMBIN GENERATION THAN ASYMPTOMATIC CARRIERS

C. Brocal, J. Bernabéu, P. Beneit, E. López, J. Lucas, P. Marco
General Hospital of the University, Alicante, Spain

Keywords: thrombin generation, prothrombin 20210A mutation, thrombophilia, thrombosis

Background/aims: Prothrombin mutation G20210A is associated with thrombophilia, nevertheless an important percentage of carriers does not manifest in clinical thrombosis. Our objective was to analyse the parameters that contribute to the generation of thrombin and its relationship with the risk for the thromboembolic vein disease in heterozygous patients with the allelic variant of PT G20210A.

Materials and methods: Three groups were included in this study: group 1, carriers of the PT mutation without previous thrombotic events (n=22); group 2, carriers of the mutation and previous thrombotic events (n=17); the control group, subjects without thrombophilia or family history of thrombosis (n=22). The generation of thrombin test was determined, using fluorometric method (Thrombinscope, Synapse BV, Maastricht, the Netherlands). The results were expressed as medians and the percentiles 25 and 75. The statistical analysis were carried out using SPSS (SPSS Inc, Chicago, IL, USA) version 17.0, using the U de Mann Whitney test and with Spearman’s correlation test. All significant differences were defined as p<0.05.

Results: The variables the peak of thrombin and the ETP were significantly higher in carriers of the PT G20210A mutation, with respect to the control group. In carriers of PT G20210A mutation, the variables of the peak of thrombin and ETP were significantly higher in those that had previously an episode of thrombosis. In addition, a positive correlation was observed (p<0.01) between the D-dimer and ETP (r = 0.392) and the Start tail (r = 0.353).

Conclusions: On the basis of our results, we suggest that the determination of ETP could be considered to identify asymptomatic carriers of PT G20210A with higher risk of venous thrombosis.

Table: Thrombin generation. Median, percentiles 25th and 75th.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control (n=22)</th>
<th>Mut, Sin thromb (n=17)</th>
<th>Mut con thromb (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (min)</td>
<td>2.7 (2.5–3.5)</td>
<td>2.5 (2.3–3.7)</td>
<td>4.17 (3.2–4.5)</td>
</tr>
<tr>
<td>ETP (nM/min)</td>
<td>2097 (1985.8–2241.5)</td>
<td>240 (2158.5–2719.1)</td>
<td>3313.5 (3055–3598.9)</td>
</tr>
<tr>
<td>Peak (nM)</td>
<td>421.8 (341.4–456.5)</td>
<td>474.2 (419.3–571.3)</td>
<td>546.3 (490.6–579.9)</td>
</tr>
<tr>
<td>Peak TT (min)</td>
<td>4.7 (4.5–6.1)</td>
<td>4.8 (4.6–6.1)</td>
<td>6.7 (5.4–7.8)</td>
</tr>
<tr>
<td>Start tail (min)</td>
<td>22 (19.9–24)</td>
<td>21 (19–23)</td>
<td>28 (24.5–31)</td>
</tr>
</tbody>
</table>

Corresponding Author: Concepcion Brocal, General Hospital of the University of Alicante, Alicante, Spain, conchibmx@hotmail.com

MARKERS OF ACTIVATED COAGULATION IN PATIENTS WITH HEREDITARY DEFICIENCY OF ANTITHROMBIN, PROTEIN C OR PROTEIN S

P.S. Miljic, I. Elezovic, M. Colovic
Institute of Haematology, Clinical Center of Serbia, Belgrade, Serbia

Keywords: thrombophilia, activation markers

Background/Aims: Hereditary deficiencies of antithrombin, protein C or protein S create a state of blood hypercoagulability because of increased thrombin generation. Theoretically, measurement of blood hypercoagulability may enable identification of individuals with high risk of thrombosis, but the results of several studies are contradictory and inconclusive in that regard.

Patients and methods: In this study we investigated the levels of thrombin-antithrombin complex (TAT), prothrombin fragment F1+2 and D-dimer in 117 patients with hereditary deficiency of antithrombin (n=60), protein C (n=30), and protein S (n=27). Seventy-nine patients experienced clinical manifestations of thrombophilia as follows: venous thrombosis (n=63), arterial thrombosis (n=11) or recurrent pregnancy loss (n=5), while 38 individuals were asymptomatic carriers of thrombophilia. Results were compared to those obtained in 71 healthy persons with normal thrombophilia mutations.

Results: The mean values of F1+2, TAT and D-dimer were significantly higher in symptomatic thrombophilia carriers than in asymptomatic carriers or healthy persons, but with broad overlaps between these three groups. In carriers who experienced venous thrombosis the mean levels of F1+2, TAT and D-dimer were significantly higher than in those who experienced arterial thrombosis. Significantly higher mean value of F1+2 was observed in carriers who experienced spontaneous venous thrombosis than in carriers with provoked thrombosis. Interestingly, this difference was not observed when TAT and D-dimer were compared between these two groups. The levels of activation markers were not different between carriers with recurrent and carriers with single episode of venous thrombosis.

Conclusions: Measurement of activation markers is not reliable tool for prediction of thrombotic risk in thrombophilia carriers because of broad overlap between symptomatic and asymptomatic individuals. Increased thrombin generation seems to play important role in onset of spontaneous venous thrombosis but not in occurrence of arterial thrombosis in thrombophilia carriers.

Corresponding Author: Predrag S. Miljic, Institute of Haematology, Clinical Center of Serbia, Koste Todorovic No 2, Belgrade, Serbia, predrag@beotel.rs
EVOLUTION OF ANTIPHOSPHOLIPID ANTIBODY TITERS AND CLINICAL EVENTS IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME

E. Caro Martínez, M.D. Jover Ríos, D. Pilar Cabezos, M. Pérez, A. Ramírez López, J. Cama Barbieri, R. Sánchez Martínez

Internal Medicine Service, Alicante General University Hospital (HGUA), Alicante, Spain.

Keywords: antiphospholipid syndrome, antiphospholipid antibodies

Introduction: Antiphospholipid syndrome (APS) is characterized by venous or arterial thrombosis, repeated abortions, thrombocytopenia and antiphospholipid antibodies (aPLs). After a thrombotic event, current treatment guidelines advised on oral anticoagulation indefinitely. Knowledge of its evolution over time could help to change the long-term treatment.

Aims: To assess the development of aPLs titers in patients with APS and describe the clinical events associated with APS.


Results: 34 patients, median age 45 with thromboembolic disease (62%), APTT elongation (12%) and events associated with arterial disease (26%). Most patients had varices (27%), hyperlipidemia (21%) and hypertension (18%); 18 patients (65%) had final APS as the international consensus criteria of 2006; 15 patients (45%) probable APS; 64% APA positive (55% IgG, 27% IgM, 18% both) and the confirmation 27.39 GPL U / ml IgG, 11.4 GPL U / ml IgM; 24% positive B2GP titers (50% IgG, 25% IgM, 25% each) mean titer of 23.56 U / ml IgG, 17.19 U / ml IgM. 20% positive AL.

At 9 months, 26% showed negative result for APA titers. No patient developed new arterial or venous thrombotic events. Treatment given at diagnosis of APS (oral anticoagulants or aspirin) not was changed.

Conclusions:
- A significant percentage of patients have negative result for APA titers after 9 months of follow-up and no new onset of symptoms following appropriate treatment;
- We recommend the study and monitoring of SAF in patients with thrombotic events in determining the risk of recurrence after a first episode;
- We also recommend an interhospital study in patients with negative antibodies to determine the therapy and the safety after suspending treatment in low risk patients.

Figure: Titers of antiphospholipid antibodies at diagnosis of APS

THE PREVALENCE OF THE MOST COMMON CAUSES FOR PRIMARY THROMBOPHILIA AMONG SAUDI PATIENTS ATTENDING THE ANTICOAGULANT CLINIC

S.K. Al Jaouni

Hematology Department, King Abdul Aziz University Hospital, Faculty of Medicine, Jeddah, Kingdom of Saudi Arabia

Keywords: inherited thrombosis, DVT, pulmonary embolism

Aims: The purpose of our study was to determine whether the activated protein C resistance (APC resistance), factor V Leiden and prothrombin mutation are the most common inherited risk factors for venous thrombosis among Saudi patients attending an anticoagulant clinic.

Methods: The study describes the results of screening all patients attending our anticoagulant clinic with a history of proven recurrent venous thromboembolism (VTE), pulmonary embolism, the first spontaneous life threatening thrombosis or at an unusual site, and patients with unexplained repeated abortion.

The tests done were antithrombin (AT), protein C (PC), protein S and activated protein C resistance (APC). Molecular testing to detect the mutation of factor V Leiden (FVL) and prothrombin G20210A and MTHFR C677T mutation.

A total of 3,875 patients were referred. 580 patients have been fulfilled the criteria of the study, between October 1998 to November 2008, age (14-51 years old) and 9 patients were neonates. This study was conducted at King Abdul Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia.

Results: Summarized in the Table.

Conclusions: Factor V Leiden is the most common cause of inherited thrombosis among Saudi patients followed by protein S. Homozygous protein S & C deficiencies are a serious cause of extensive thrombosis with high mortality during neonatal period, an affected neonate is a marker for a group at a high genetic risk. High prevalence MTHFR mutation among repeated abortion which need a further clinical studies.

Reference

Table: The causes of inherited thrombosis among Saudi population in Jeddah, with DVT/PE and recurrent abortion.

![Figure: The age distribution of patients treated at outpatient anticoagulant clinic, Jeddah, Kingdom of Saudi Arabia]
GENETIC VARIABILITY OF THE G58A POLYMORPHISM ON FIBRINOGEN A-CHAIN GENE IN ADVANCED Atherosclerosis: Effects on fibrinogen, D-dimers and plasminogen levels


Hematology Department Hippokration Hospital, Athens, Greece and 1st Cardiology Unit, Athens Medical School, Hippokration Hospital, Athens, Greece

Background: Evidence suggests that the G58A polymorphism on fibrinogen α-chain gene is associated with increased fibrinogen levels in healthy individuals. However, it is still unclear whether this polymorphism is associated with coagulation or thrombosis in patients with coronary artery disease (CAD). In the present study we examined the impact of this polymorphism on fibrinogen levels, D-dimers levels and plasminogen levels.

Methods: The study population consisted of 395 subjects, 246 of whom were patients with CAD. The G58A polymorphism was determined by polymerase chain reaction (PCR) and appropriate restriction enzymes. Fibrinogen levels were measured by immunonephelometry, while plasminogen and D-dimers levels were measured by standard coagulometry techniques.

Results: The genotype distribution was GG: 37.8%, GA: 43.4% and AA: 22.8% for patients with CAD, while GG: 33.5%, GA: 44.3% and AA: 22.2% for controls. Patients with CAD had significantly higher fibrinogen levels (mg/dL) than controls (434.7±132.7 vs 384.7±103.7, p=0.0002). However, in patients with CAD fibrinogen levels were not significantly higher for S8A homozygotes vs S8G carriers (453.6±131.4 vs 441.1±140.6, p=NS), while similar difference occurred in controls (AA: 385.2±129.4 vs GG/GA: 392.6±103.0, p=NS). Moreover, D-dimers levels (mg/L) were significantly higher in CAD patients than controls (409.7±188.2 vs 332.8±199.4, p<0.0001). In addition, there was a significant difference for S8G carriers vs S8A homozygotes for CAD patients (506.4±418.8 vs 662.2±627.1, p<0.05), but not for controls (AA: 415.6±289.6 vs GG/GA: 551.3±671.4, p=NS). Finally, CAD patients and controls had no significant difference in plasminogen levels (n/m) (119.8±79.1 vs 113.9±22.9, p=NS). Patients with CAD had no difference in plasminogen for S8A homozygotes vs S8G carriers (110.2±20.6 vs 112.2±17.2, p=NS), while no significant difference was observed for controls (AA: 112.3±20.6 vs GG/GA: 114.3±25.5, p=NS).

Conclusions: Our findings indicate that the G58A polymorphism on fibrinogen α-chain gene affects D-dimers levels in patients with coronary artery disease. These findings provide a possible mechanism by which this polymorphism may affect thrombotic process/coagulation independently of fibrinogen levels and may have important clinical implications.

Keywords: antithrombin, conformational, thrombosis

P95

SCREENING OF MUTATIONS ASSOCIATED WITH CONFORMATIONAL ALTERATIONS OF ANTITHROMBIN


Department of Haemostasis and Thrombosis, IHJEMA, National Academy of Medicine, Buenos Aires, Argentina

Conclusions: None of the analyzed patients showed a molecular alteration that could be related to the presence of unstable antithrombin; perhaps, due to the small number of patients. Anyway, conformational defects of antithrombin should be looked for in patients with venous thrombosis and no other known thrombophilic defect.

Corresponding Author: Luis Alberto Bastos, Department of Haemostasis and Thrombosis, IHJEMA, National Academy of Medicine, Pacheco de melo 3081, Buenos Aires, Argentina, luissbastos@argentina.com

P167

CLINICAL CHARACTERISTICS OF VENOUS THROMBOEMBOLISM IN FACTOR V LEIDEN OR PROTHROMBIN G20210A CARRIERS

G. Mandrillon, L. Rugeri, A. Bestion, Y. Dargaud

Unité d’Hémostase Clinique, Hôpital E.Herriot, Lyon, France

Keywords: venous thromboembolism, factor V Leiden, prothrombin 20210A

Background/Aims: Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is a multicausal frequent disease that is associated with substantial morbidity and mortality. Factor V (FV) Leiden and prothrombin G20210A mutations are the most common genetic risk factors for VTE. Few studies have compared the clinical characteristics of the thrombotic events occurring in FV Leiden and prothrombin G20210A carriers. In order to investigate whether or not prothrombin gene mutation is responsible for a higher risk of severe VTE compared with FV Leiden, we analyzed a large prospective cohort of 403 patients referred to Lyon Clinical Haemostasis Unit from January 2000 to June 2007.

Methods: The demographic characteristics of the patients, the number of cases with proximal VTE, PE, distal VTE, superficial venous thromboses as well as the rate of recurrence were retrospectively investigated in 265 patients with FV Leiden (12 homozygous) and in 164 patients with prothrombin G20210A (5 homozygous) mutations. The presence of transient risk factors, such as immobilization, surgery, air travelling, hormone therapy and pregnancy was also recorded.

Results: No difference in the age of onset of VTE was found in the two groups. The age of the first event was 38 (range 13-77) and 39 (range 12-76) years in patients with FV and FII gene mutation respectively. The age of the first event was also similar in patients with combined abnormalities (38.5, range13-69 years), while the first thrombotic event occurred earlier in patients with FV Leiden (p<0.057). The G20210A carriers had a higher frequency of severe events (PE, proximal DVT, cerebral or mesenteric thrombosis) than the FV carriers (46% vs 38%). Among these events, isolated PE were more frequent in patients with G20210A (11% comparatively to FV carriers (5%) but the difference was not statistically significant (p=0.057). Conversely, the frequency of superficial venous thromboses (SVT) were significantly more frequent in patients with FV Leiden mutation (p=0.02). The same prevalence of unprovoked VTE events was observed in the two groups (66% and 67%).

Conclusions: In both groups the main transient risk factors observed were surgery, oral contraceptive use, pregnancy and puerpérum.

In conclusion, our results show that heterozygous FV Leiden mutation is more frequently related to superficial or distal venous thromboses while prothrombin G20210A mutation might be responsible for severe VTE.

Table: comparing the first VTE events occurring in patients with FV Leiden and those with prothrombin gene mutation

<table>
<thead>
<tr>
<th></th>
<th>FV Leiden (n=265)</th>
<th>Prothrombin G20210A (n=164)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>265</td>
<td>164</td>
</tr>
<tr>
<td>Unprovoked PE</td>
<td>45 (17)</td>
<td>23 (14)</td>
</tr>
<tr>
<td>Distal DVT</td>
<td>117 (44)</td>
<td>37 (23)</td>
</tr>
<tr>
<td>Proximal DVT</td>
<td>25 (10)</td>
<td>10 (6)</td>
</tr>
<tr>
<td>Total events</td>
<td>200 (76)</td>
<td>70 (43)</td>
</tr>
</tbody>
</table>

Corresponding Author: Lucia Rugeri, Hôpital E.Herriot, Lyon, France, lucia.rugeri@chu-lyon.fr

P668

HPA4 PLATELET POLYMORPHISM, FV LEIDEN, PROTHROMBIN G20210A MUTATIONS AND THROMBOSIS

I. Mezni 1, S. Guermazi 2, I. Sfar 2, B. Hemissi 2, T. Ben Romdhane 2, M. Makkhouf 2, C. Kalla 2, S. Jendoubi-Ayed 2, T. Ben Abdallah 2, K. Ayed 2, Y. Gorgi 2

1 Laboratoire d’Immunologie: Laboratoire de recherche LR03SP01, Charles Nicolle Hospital, Tunis, Tunisia

Keywords: HPA4, thrombosis

Some genetic risk factors play an important role in susceptibility to venous or arterial thrombosis. Factor V Leiden and G20210A prothrombin mutations are well established factors of hypercoagulability. Moreover, HPA4 platelet polymorphism is associated, in our experience, with thrombosis of arterial-venous fistulas in hemodialysis patients.

We have studied by PCR-RFLP and PCR-SSP three these genetic polymorphisms in 75 patients with a history of arterial (n=12) and venous (n=61) spontaneous thrombosis compared to 75 healthy subjects in order to seek a possible correlation between these molecular factors of thrombophilia and thrombotic events.

No difference in genotype frequencies and allelic mutations V Leiden and prothrombin G20210A were found between patients and controls perhaps because of low thrombogenicity of these mutations in our population. Nevertheless, the allele HPA4 b could be a new risk factor for arterial and/or venous thrombosis. Its potential thrombosis seems difficult to assess because it is common in the general population but still in the heterozygous state.

Corresponding Author: Imen Mezni, Hôp. C. Niccolés, Bd 9 avril 1934, 1006 Tunis, Tunisia, simi. guermazia2010@gmail.com
PREVALENCE OF THROMBOPHILIA AND HOMOCYSTEINE IN PULMONARY THROMBOEMBOLIC PATIENTS

A. Lorenzo Hernández, M.I. Torres, M.A. Rodríguez Davila, I. García Plaza, N. Iniesta, S. Caro, C. Navarro, A. Martín Quirós, M.C. Fernández Capitán

1 Internal Medicine Department, Hospital Universitario La Paz, Madrid, Spain; 2 Radiology Department, Hospital Universitario La Paz, Madrid, Spain; 3 Hematology Department, Hospital Universitario La Paz, Madrid, Spain.

Keywords: thrombophilia, homocysteine, thromboembolism.

Aims: To describe prevalence of thrombophilia and homocysteine in acute pulmonary thromboembolic patients.

Methods: Review of charts of 120 consecutive patients in a cohort of 605 patients with thromboembolic pulmonary disease (TED) to study prevalence of thrombophilia and hyperhomocysteine in patients without any other factor associated with first TED.

Results: 120 patients seen between June 2007 and October 2008 were reviewed. Age was 64 years (range 22-88), 58 were men. There were no risk common factors associated with venous thromboembolism in 70%. We studied the presence of thrombophilia in 54 patients and homocysteine in 61 patients. We found the prevalence of a thrombophilic factor (TF) in 26 patients (48% patients studied for the presence of TF) i.e.: mutation of 20210 gene of prothrombin (4 patients), protein S defect (6 patients), factor V Leiden (2 patients), 2010 and FV associated in 2 patients, Lupus anticoagulant in 3 patients and others (7 patients). Of 61 patients studied for hyperhomocysteinemia (HC), elevated homocysteine was found in 33 patients (52%): average 16.2 mg/dl, max 62.5 mg/dl, min 10.2 mg/dl. In 41 patients (67% of HC tested positive): mutation of 20210 gene of prothrombin (4 patients), protein S deficit (13 patients), MTHFR C677T (PCR technique) i.e.: mutation of 20210 gene of prothrombin (4 patients), protein S deficit (13 patients), MTHFR C677T (PCR technique) i.e.: mutation of 20210 gene of prothrombin (4 patients), protein S deficit (13 patients), MTHFR C677T (PCR technique). In 20 patients (33%) with thrombophilia factors, and hyperhomocysteine. Homocysteine is the only factor associated with venous thromboembolism in a not depreciated number of patients.

Conclusions: Current guidelines do not recommend thrombophilia study in older patients at first episode of venous thromboembolism but there is a great prevalence of thrombophilia factors and hyperhomocysteine. Homocysteine is the only factor associated with venous thromboembolism in a not depreciated number of patients. TF and HC should be considered in addition with other factors in the assessment of risk of thromboembolic disease.

Corresponding Author: Alicia Lorenzo Hernández, Hospital Universitario La Paz, Paseo de la Castellana 261, Madrid, Spain. alicia.lh@terra.es

A60
THE IMPACT OF PROTHROMBIN 20210A POLYMORPHISM AND FACTOR V LEIDEN ON DEEP VEIN THROMBOSIS IN THE SOUTH IRANIAN PATIENTS

M. Karimi, M. Yavarian, A. Afsarabi, M. Ramzi, M. Zakernia, J. Dehbozorgian, M. Haghshenas, N. Rezaei, V. Moayed

Hemostasis Unit, Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Keywords: factor V Leiden, prothrombin 20210A, deep vein thrombosis

Background/Aims: Multiple genetic variants are predisposing factors for thrombosis, but the impact of each factor on disease developing can vary in different ethnic groups. The prevalence of each hereditary factors and their combination is different in diverse ethnic groups. Factor V Leiden (FVL), and prothrombin 20210A mutation were described as risk factors for thrombosis and their role on morbidity of deep vein thrombosis (DVT) are less clear.

Patients and methods: In total 135 patients with objectively documented DVT from academic clinics were studied at the south of Iran. All patients with lower limb DVT had sonography or venography to confirm diagnosis.

Results: The Prothrombin 20210A polymorphism and FVL mutation analysis was performed on genomic DNA by using the RFLP and multiplex ARMS techniques. The allele frequency of FVL and prothrombin 20210A were 0.196 and 0.181 respectively.

Conclusions: FVL and prothrombin G20210A mutations should be taken into account for prophylaxis treatment and counseling in individuals who predispose for acquired thrombophilia and DVT in the south Iranian provinces.

Corresponding Author: Mehran Karimi, Hemostasis Unit, Hematology Research Center, Nemazee Hospital, Shiraz, Iran, karimin@sum.ac.ir

CEREBRAL VENOUS THROMBOSIS IN CHILDREN: EVALUATION OF THE ASSOCIATION WITH PROTHROMBOTIC RISK FACTORS

L. Ríca Goncalves 1, C. Monteiro 1, M. Carvalho 1, M. Manuel Campos 1, R. Vaz 1, L.M. Cunha-Ribeiro 1

1Department of Transfusion Medicine and Blood Bank, Centre of Thrombosis, Hemostasis and Vascular Biology, Porto, Portugal; 2Department of Pediatric Neurology, University S. João Hospital, Porto, Portugal; 3Department of Otorhinolaryngology, University S. João Hospital, Porto, Portugal

Background: Cerebral venous thrombosis (CVT) in childhood is a serious disease. Its pathophysiology is still poorly understood. Predisposing factors (infections, trauma, cancer, leukemia, cardiac and autoimmune diseases) should be unraveled to identify at risk and establish therapy. Multiple additional factors, including prothrombotic risk factors, contribute to the symptomatic onset of CVT. The aim of this study was to assess the role of prothrombotic risk factors in association to underlying diseases as risk factors for CVT in children.

Methods: From 1999 to 2009, 15 patients aged from 6 months to 16 years with CVT were studied. Clinical conditions were investigated. The following prothrombotic risk factors were evaluated: factor V Leiden mutation, factor II G20210A and methylenetetrahydrofolate reductase (MTHFR) (677TT) polymorphisms, homocysteine levels, antithrombin, protein C and S levels, activated protein C resistance, lupus anticoagulant and antiphospholipid antibodies (anticardiolipin and anti-beta2GPI).

Results: Underlying diseases were documented in 11 patients (73.3%): 6 had infectious diseases, 2 presented cardiac malformations, 1 had leukemia, 1 had a cranial trauma and 1 had neurological congenital malformations. Prothrombotic risk factors were detected in 4 patients (26.7%): 3 patients with no detected underlying disease (1 with MTHFR homozygosity and homocysteine > 100 mol/L, 1 with positive IgM antiphospholipid antibodies and 1 with IgG anti-beta2GPI antibodies); 1 patient had a cervical abscess and positive IgM anti-beta2GPI. All patients were treated with unfractioned heparin for at least 5 days. For secondary long-term prophylaxis, warfarin was given for 3 months minimum.

Conclusions: The present results suggest that specific clinical conditions play the most important role in the origin of CVT in children. However, additional prothrombotic risk factors should not be underestimated, as 4 patients had positive results in our tests which could explain the CVT. It can also be useful to establish therapy strategies.

Corresponding Author: Luciana Ríca Goncalves, Department of Transfusion Medicine and Blood Bank, Centre of Thrombosis, Hemostasis and Vascular Biology, University S. João Hospital, Porto, Portugal, luciana.rica@gmail.com

ANTICARDIOLIPIN ANTIBODIES IN PATIENTS WITH CHRONIC HEPATITIS VIRUS INFECTION: IMPLICATION OF HCV AS A CAUSE OF ANTI PHOSPHOLIPID SYNDROME

O. Elbash 1, G. Shiha 2

1Hematology Dept., Faculty of Medicine, Mansoura University, Mansoura, Egypt; 2Hepatology Dept., Internal Medicine Hospital, Mansoura University, Mansoura, Egypt

Keywords: HCV, aCL, APS

Background: Infectious agents have been implicated in the induction of anticardiolipin (aCL) antibodies and the development of the antiphospholipid syndrome (APS). Hepatitis C, a worldwide viral infection, is a great health problem in Egypt. The APS is usually defined by the association of clinical manifestations that comprise venous and/or arterial thrombosis and thrombocytopenia, along with the presence of anticardiolipin (aCL) antibodies. However, these antibodies are not usually associated with thrombotic events, as happens with autoimmune diseases, in which these antibodies need the presence of J2-glycoprotein I.

Aim: The aim of this research was to screen for the presence of aCL antibodies (IgM and IgG ) and J2-glycoprotein I in 184 Egyptian patients with chronic HCV infection and 40 healthy subjects as a reference group.

Methods: The levels were determined by enzyme-linked immunosorbent assay. Results: aCL antibodies (IgG)were found to be positive (> 9.8 GPl) in 11 patients (5.9%) and aCL (IgM)>9.6 MPL) in 17 patients (9.2%) in comparison to the negative results of the reference group. Seven patients with positive aCL antibodies were J2-glycoprotein I-dependent. No significant association was found between aCL antibodies and clinical manifestations of APS. Finally, no cross-reactivity between aCL antibodies and HCV antigens was observed.

Conclusions: We concluded that Egyptian individuals chronically infected with HCV present a significant production of aCL antibodies, which mainly are not associated with the clinical manifestations of APS.

Corresponding Author: Osama Elbash, Hematology Dept., Faculty of Medicine, Mansoura University, Mansoura, Egypt, osamaelbash@yahoo.com
EVALUATION OF ELEVATED FVIII IN PATIENTS WITH THROMBOPHILIA

F. Pournaghi Azar
Faculty of Medicine, Tabriz University, Tabriz, Iran

Keywords: thrombosis, factor VIII, venous thromboembolism, risk factor, thrombophilia.

Background/Aims: Thrombus formation may form enhanced coagulation or impaired fibrinolysis. An increased tendency for the blood to clot is referred to as the hypercoagulable state or thrombophilia which includes various inherited and acquired clinical disorders or mixed conditions. There are many studies suggesting that elevated factor VIII may be a common and independent risk factor for thrombotic events. We tried to assess the level of factor VIII in patients with idiopathic thrombosis.

Materials and methods: Our cases were patients with idiopathic venous thrombosis having referred for hypercoagulable studies to Coagulation Lab in Tabriz University. The inclusion criterion was the occurrence of thrombotic event confirmed by objective diagnostic methods coupled with three months of follow-up without any other disorder. Our controls were from healthy blood donors and matched with the cases on sex, ethnicity, and age. Plasma of a healthy person was used to establish the normal reference range according to which our patients are compared. Factor VIII levels were measured using a one-stage assay, the PTT based Diagnostica Stago on the STA compact automated coagulation factor analyzer. SPSS and Chi-square were finally used for data analysis.

Results: One-hundred-fifty-two cases and 130 controls enrolled. The mean factor VIII level for cases was 157.26 IU/dl (SD±53.8) with the minimum level of 66 and maximum of 364 IU/dl. For controls, the mean factor VIII level was 117.78 IU/dl (SD: 29.68) with the minimum level of 42 and the maximum of 195 IU/dl. These levels were statistically significant and higher in the case group. The elevated FVIII level was higher in females than males (35.3% vs 23.8%) and increased with age. The normal range in the control group varied within 52-171 IU/dl, which is higher than the normal level of 50-150 IU/dl.

Conclusions: There are many studies showing that increased FVIII level may be an independent risk factor for thrombosis. Our results suggested elevated FVIII level in 28.9% of the patients with thrombosis compared to 3.1% in the control group. So, factor VIII measurement is recommended to be practiced in routine thrombophilia screening programs.

Corresponding Author: Fatemeh Pournaghi Azar, Faculty of Medicine, Tabriz University, Tabriz, Iran, ghoojazadehm@hotmail.com

PROC MOLECULAR PATHOLOGY AND MODULATING INNATE AND ACQUIRED FACTORS OF THROMBOTIC RISK IN PORTUGUESE PATIENTS WITH INHERITED PROTEIN C DEFICIENCY

D. David 1, C. Ferreira 1, C. Ventura 1, I. Moreira 1, I. Freire 1, T. Gago 2

1 Departamento de Genética, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; 2 Laboratório de Patologia Clínica, Hospital de Santa Cruz, Carnaxide, Portugal

Keywords: protein C mutations, hereditary and acquired risk factors, haplotypes

Protein C (PC) is a vitamin K-dependent serine protease zymogen. Its activated form, by the thrombin-thrombomodulin complex, plays an important role in maintaining the haemostatic balance through inactivation of FVa and FVIIIa. Inherited PC deficiency, mainly characterized by an increased risk of recurrent thromboembolism at a young age, is transmitted in an autosomal dominant manner. In this study, we report the genetic defects associated to PC deficiency in Portuguese families.

In addition, the modulating effect of the FV Leiden mutation, F5 HR2 haplotype, PT 20210A, PAI-1 4G and MTHFR 677T and 1298C allelic variants, as well as of acquired factors on these patients thrombotic risk was evaluated. Total plasma homocysteine levels were also determined in a subgroup of these individuals. Three unreported genetic alterations, namely a 2bp deletion in exon 7 (g.6220_6221delGC) and amino acid substitutions G145C and R220G, were identified. Another eleven previously reported mutations were also found: 1533A>G, IVS5 2A>G, g.-1533A>G, R199X, and missense mutations, P210L, R220Q, A301T, L320R, R328H, V339M and W444C. For the latter (g.-1533A>G, R199X, R220Q and W444C) represent the genetic defect in more than 50% of the families. Each of the reported mutations most likely leads to type I deficiency through different molecular mechanisms. Although the numbers are relatively small, the frequency of inherited potential prothrombotic factors in the patients group is within the normal variation. Acquired factors are the major additional prothrombotic risk factor in this group of patients, leading to a considerable reduction in the mean age of the first thrombotic event.

Corresponding Author: Dezso David, Instituto Nacional de Saúde Dr. Ricardo Jorge, Av.Padre Cruz, Lisboa, Portugal, dezso.david@insa.min-saude.pt
THROMBOTIC RISK IN THE ASSOCIATION OF PRIMARY ANTIPHOSPHOLIPID SYNDROME WITH HYPERHOMOCYSTEINEMIA

Department of Hematology at the Clinical Hospital of the University of São Paulo, São Paulo, Brazil

Keywords: primary antiphospholip syndrome, homocysteine, hyperhomocysteinemia, antiphospholipid antibodies

Background: Antiphospholipid syndrome is an autoimmune disorder characterized by the presence of antiphospholipid antibodies and, at least, one clinical manifestation, most frequently arterial and/or venous thrombosis and recurrent fetal loss. It is classified as primary antiphospholipid syndrome (PAPS) when isolated. Moderate hyperhomocysteinemia is considered a risk factor for atherosclerosis and vascular disease. It is supposed that for the occurrence of venous thromboembolic event it is necessary the association of a constitutional condition with a trigger factor, being the intensity of this trigger factor inversely proportional to the basal condition.

Aims: To evaluate the prevalence of severe thrombotic events in patients with PAPS associated to hyperhomocysteinemia.

Materials and methods: Clinical data of patients followed with the diagnosis PAPS and PAPS associated to hyperhomocysteinemia were collected. The following thrombotic events were classified as severe: primary venous thrombosis, pulmonary embolism, deep-vein thrombosis, cerebral venous thrombosis, splenic venous thrombosis and arterial thrombosis.

Results: Clinical data of 54 patients were collected (age 13 to 74 yo; 55.6% female) Hyperhomocysteinemia was shown in 16.7% patients. When considered plasmatic level of homocysteine above the 90th percentile, the number of patients in this condition increased to 50%. The association of PAPS with hyperhomocysteinemia did not show increased prevalence of severe thrombotic events. Nevertheless, when PAPS was associated to plasmatic homocysteine levels above the 90th percentile of the normal range it was possible to demonstrate higher prevalence of primary venous events.

Conclusions: Patients with PAPS and hyperhomocysteinemia have not increased prevalence of severe thrombotic events. Nevertheless, when PAPS is associated with plasmatic values of homocysteine above the 90th percentile of the normal range there is higher prevalence of primary venous thrombosis. Further studies must be developed in to evaluate if plasmatic homocysteine levels above the 90th have a pathophysiologic role or are markers of vascular abnormality in PAPS.

Corresponding Author: Elbio Antonio D’Amico, Clinical Hospital, University o f São Paulo, Avenida Eneas de Carvalho Aguiar, 155 - 1st floor, São Paulo, Brazil, elbio_damico@uol.com.br

P625

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) MUTATION, HOMOCYSTEINE, AND VENOUS THROMBOSIS IN PEDIATRICS

M. Luciani, P. Zangari, V. Pansini, V. Coletti, M. Soldati, S. Pancotti, A. Minozzi, V. Nobili, P.G. Falappa
IRCCS-Children Hospital Bambino Gesù, Rome, Italy

Keywords: methylenetetrahydrofolate reductase (MTHFR) mutation, homocysteine, venous thrombosis, pediatrics

Background: Although thrombosis is less frequent in children than in adults, it represents a significant source of morbidity and mortality. Both congenital and acquired factors contribute to the development of thrombosis. Hyperhomocysteinemia is a risk marker for venous thrombosis and it is consistently associated with methylenetetrahydrofolate reductase enzyme (MTHFR) gene mutation.

Aim: The aim of this study is to point out the possible correlation between venous thrombosis and MTHFR mutations in children.

Patients and Methods: Between December 2002 and December 2009 fifty-four children, aged between 1-192 months, affected by deep venous thrombosis were admitted at our department

Results: The MTHFR polymorphism C677T was found in all the patients (fourteen in homozygosis and forty in heterozygosis) with no other thrombophilic risk factors. The MTHFR polymorphism C677T was found in all the patients (fourteen in homozygosis and forty in heterozygosis) with no other thrombophilic risk factors.

Conclusions: Although thrombosis is less frequent in children than in adults, it represents a significant source of morbidity and mortality. Both congenital and acquired factors contribute to the development of thrombosis. Hyperhomocysteinemia is a risk marker for venous thrombosis and it is consistently associated with methylenetetrahydrofolate reductase enzyme (MTHFR) gene mutation.

Corresponding Author: Matteo Luciani, IRCCS-Children Hospital Bambino Gesù, Piazza S.Onofrio 4, Rome, Italy, luciani@bghp.net

P615

ANTIPROTHROMBINIC ANTIBODY THROMBOEMBOLISM: A CHILD STILL LOOKING FOR A HOME

E.S. Papadakis, V. Papadopoulos, A. Mpanti, K. Loukidis, M. Topalidou, A. Kioumi, I. Korantzis
Hemostasis Unit, Papageorgiou Hospital, Thessaloniki, Greece

Keywords: antiprothrombinic, APS, thrombembolism

Thromboembolism with presence of antibodies against antithrombin (aPT) has been associated with anti-phospholipid syndrome (APS), but it has not been incorporated as an independent factor in the revised Sapporo criteria for the diagnosis of APS due to the possibility of decreased diagnostic specificity. Indeed, up to date studies investigating aPT antibody role in thromboembolic disease yielded controversial results. The role of antiprothrombin antibodies in pregnancy loss or complications is far from being elucidated. We present three cases of women with aPT-antiprothrombin like syndrome with antibodies present in at least two times more than 12 weeks apart. Case 1 had consecutive spontaneous abortions after in vitro fertilization and moderate titers of aPT without any other risk factors for thrombosis. Case 2 is a smoker with a history of central nervous system thrombosis and two spontaneous abortions without evidence of thrombophilia. Case 3 is a woman with deep venous thrombosis during third trimester of pregnancy, homoyzogote for the factor V Leiden mutation, with low titers of aPT and no other risk factor for thrombosis. We conclude that prospective studies are needed to evaluate the diagnostic specificity and sensitivity of aPT and their association with clinical features of APS.

Corresponding Author: Emmanuel S Papadakis, Hemostasis Unit, Papageorgiou Hospital, Thessaloniki, Greece, mlpapaz@hol.gr

P679

SNP ARRAY–BASED DIAGNOSIS OF HEREDITARY COAGULOPATHY IN HUMAN

H.J. Kim 1,2, D.K. Kim 3,4, H.S. Chung 1, M.J. Song 1, K.O. Lee 1, I.A. Park 1, K.Y. Yoo 5, C.W. Yoo 5, J.H. Yoo 5, S.H. Kim 1
1 Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; 2 Cardiac & Vascular Center, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; 3 Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; 4 Samsung Biomedical Research Institute, Samsung Medical Center, Seoul, South Korea; 5 Korea Hemophilia Foundation, Seoul, South Korea; 6 Department of Laboratory Medicine, Ilsan , Seoul, South Korea

Keywords: Coagulation, SNP array, copy number mutation

Background: The recent advent of genome-wide molecular analysis has facilitated our understanding of human genome and disease. In particular, the copy number (CN) aberrations revealed previously underrepresented genomic aberrations. In this study, we applied the SNP (single nucleotide polymorphisms)-array to a series of patients with hereditary coagulation defects. The study subjects were 5 patients with hereditary thrombophilia (HT), 10 male patients with hemophilia, and 1 woman from a family of hemophilia. Five patients with HT were 3 with protein S deficiency and 2 with antithrombin deficiency. Ten patients with hemophilia were 6 with hemophilia A and 4 with hemophilia B. One female was a mother of a patient with hemophilia A. The diagnosis of specific coagulation defect in each patient was made based on coagulation tests and was confirmed by exon dosage mutation analyses involving multiple ligation-dependent probe amplification (MLPA). Array experiments were performed using Affymetrix Genome-Wide Human SNP arrays 6.0, and the CN status of the target gene and adjacent region was visually inspected by Genotype Console 4.0.

Results: SNP array revealed CN mutations in 10/11 (90.9%) patients. The genomic segment involved in HT mutations identified by SNP array ranged from a single exon to unexpectedly multiple genes (contiguous gene deletion). Ten patients with hemophilia were 6 with hemophilia A and 4 with hemophilia B. One female was a mother of a patient with hemophilia A. The diagnosis of specific coagulation defect in each patient was made based on coagulation tests and was confirmed by exon dosage mutation analyses involving multiple ligation-dependent probe amplification (MLPA). Array experiments were performed using Affymetrix Genome-Wide Human SNP arrays 6.0, and the CN status of the target gene and adjacent region was visually inspected by Genotype Console 4.0.

Conclusions: To the best of our knowledge, this is the first report on the application of SNP array in hereditary coagulopathy in human. SNP array could detect CN mutations in most cases of hereditary coagulopathy, with fine delineation of aberrant genomic segment involving from a single exon to multiple genes. Further investigation on the genomic regions involved in CN mutations could delineate rearrangement hotspots or genetic susceptibility.

Corresponding Author: Hee-Jin Kim, Dept. of Laboratory Medicine & Genetics, Samsung Medical Center, 50 Ilwon-dong, Gangnam-gu, Seoul, South Korea, heejinkim@skku.edu; hee-jin.kim@samsung.com
Cerebrovascular and peripheral atherothrombosis

Wednesday 7th July 2010

P523

PLATELET ACTIVATION PROFILE IN PATIENTS WHO UNDERWENT CAROTID VERSUS CORONARY STENT REVASCULARIZATION

M. Camera 1,2, P. Montorsi 1,3, E. Tizlioni 1, D. Trabattoni 1, M. Brambilla 1, S. Ghulam Ali 1, P. Cazano 4, A. Bartorelli 1, E. Tremoli 1,2
1 Centro Cardiologico Monzino IRCCS, Milan, Italy; 2 Dept. of Pharmacological Sciences, University of Milan, Milan, Italy; 3 Institute of Cardiology, University of Milan, Milan, Italy

Keywords: platelet activation, tissue factor, stent revascularization

Background: Platelet activation occurs in both coronary and carotid artery stenting as a result of vessel wall damage. The dual antiplatelet regimen (aspirin–thienopyridine) has a significant impact on reducing stent thrombosis and adverse outcomes. Whether differences exist in the degree of platelet activation among stent-treated coronary and carotid vessels is not known.

Aims: To compare platelet activation in patients who underwent carotid versus coronary revascularization.

Patients and methods: 20 patients with carotid stenosis and 20 stable angina patients who underwent BMS implantation were studied. To assess platelet function, blood was withdrawn after a washout period (T1) after stenting procedure and 2 months after thienopyridine discontinuation (T2). Platelet activation markers (PAC1, CD62 and tissue factor [TF]) and the % of monocyte-platelet aggregates [MPA]) were assessed by whole blood flow cytometry in resting conditions and upon in vitro ADP stimulation.

Results: Results of platelet activation in carotid revascularization, T2, positive (+) platelets were comparable in carotid and coronary-treated patients. By contrast, TF+ platelets as well as TF+ MPA were 3 fold higher in carotid vs coronary-treated patients, both under resting conditions and upon ADP stimulation (p<0.001). At T2, when thienopyridine was discontinued, the platelet activation profile was comparable to that observed at T1, with TF+ platelets and TF+ MPA being still significantly higher (2-3 fold) in coronary vs carotid-treated patients, both under resting conditions and upon ADP stimulation (p<0.01). No significant differences in the platelet activation markers expression were observed between T1 and T2 both in carotid- as well as in coronary-treated patients.

Conclusions: Significant higher levels of TF+ platelets and TF+ MPA were observed in coronary patients who underwent revascularization with stent implantation compared to patients with carotid artery stenting, both 1 month after stenting and 2 months after thienopyridine discontinuation. This prothrombotic platelet phenotype may have implications for thrombotic complications in coronary patients.

Corresponding Author: Marina Camera, Centro Cardiologico Monzino IRCCS, Milan and Dept. of Pharmacological Sciences, University of Milan, Via Farae, 4, Milan, Italy; marina.camera@unimi.it

P538

THE RISK FACTORS OF STROKE AMONG PERIPARTUM IN TAIWAN

P.H. Chu 1, C.H. Tang 2,3
1 The Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University, College of Medicine, Taipei, Taiwan, Taipei, Taiwan; 2 School of Health Care Administration, Taipei Medical University, Taipei, Taiwan; 3 Gynecology Research Center, Taichung Medical University Hospital, Taichung, Taiwan

Keywords: stroke, peripartum

Background/Aims: Stroke is a recognized complication of pregnancy, contributing to more than 12% of all maternal deaths. Estimated incidence rates vary considerably from 4.3 to 210 strokes per 100,000 deliveries. However, few studies have evaluated stroke risk in Asian populations and followed women beyond the early postpartum period. Thus, the present study determined the risk of stroke in women in Taiwan during pregnancy and the first postpartum year.

A population-based cohort study was performed on 1,132,019 parturients during 1999-2003 using a dataset linking birth certificates and National Health Insurance hospital discharge data. Stroke-free survival rates were estimated using the Kaplan-Meier method, and the log-rank test was used to examine the effect of risk factors on the prevalence of stroke. Socio-demographic factors and obstetric complications were used in multivariate logistic regression models to determine the adjusted odds ratios of the risk of hemorrhagic and ischemic stroke during pregnancy and within the first postpartum year.

Results: There were 139 cases of hemorrhagic stroke and 107 cases of ischemic stroke. The three most important risk factors were cesarean delivery, systemic lupus erythematosus and preeclampsia-eclampsia. The individual respective relative risk of cesarean delivery was 1.56 (95% CI, 1.25-1.96), systemic lupus erythematosus 7.22 (95% CI, 2.68-19.47), and preeclampsia-eclampsia 7.69 (95% CI, 5.06-11.67).

Conclusions: Women with cesarean delivery, systemic lupus erythematosus and preeclampsia-eclampsia have a significantly higher risk of stroke during pregnancy.

Corresponding Author: Pao-Hsian Chiu, 199 Tun-Aw-North Road, Taipei, Taiwan.chiu@gmail.com

P461

PREDICTIVE VALUE OF CARDIOVASCULAR RISK FACTORS, PATIENT FOR AMEN OVALE, AND PROTHROMBOTIC GENOTYPES ON MYOCARDIAL INFARCTION RESULTS IN YOUNG ADULTS WITH ISCHEMIC STROKE

A. Pizzini 1, C. Lodigiani 2, R. Patella 1, G. Gando 1, F. Casoni 3, R. Musolino 1, R. S. Calabrò 4, P. Bovi 7, A. Adami 8, A. Giossi 3, A. Zini 10, P. Cerrato 11, M. Magoni 1, L. Iacovelli 11, M. Grassi 12, A. Padovani 1
1 Stroke Unit, Vascular Neurology, Dept Medical and Surgical Sciences, Spedali Civili and University of Brescia, Italy; 2 Dept Internal Medicine, IRCCS Ist Clinico Humanitas, University of Milan, Rozzano, Italy; 3 Stroke Unit, Azienda Ospedaliera Sant’Andrea, Rome, Italy; 4 Neurosciences Dept, University of Genova, Italy; 5 Neurological and Psychiatric Sciences Dept and 4 IRCCS, Centro Neurolesi Bonito-Pulejo,University of Messina, Italy; 6 Neurology Unit, University Hospital of Verona, Verona, Italy; 7 Stroke Center, Dept Neurology, Sacro Cuore Negrar Hospital, Verona, Italy; 8 Neurology Unit, Ospedale di Circolo, University of Insubria, Varese, Italy; 9 Stroke Unit, Neurological Clinic, Nuovo Ospedale Civile “S. Agostino Estense”, AUSL Modena, Italy; 10 Stroke Unit, Dept Of Neurosciences, University of Turino, Italy; 11 Research Laboratory, Center of Research and Formation of High Technology in Biomedical Sciences, University Cattolica del Sacro Cuore, Campobasso, Italy; 12 Dept Sanitary Applied Sciences, Section of Biostatistic and angi Epidemiology, University of Milan, Italy

Keywords: ischemic stroke, PFO, prothrombotic genotype

Background/Aims: The mechanisms underlying the relation between migraine and ischemic stroke remain uncertain. The aim of the present study was to investigate the predictive value of cardiovascular risk factors, migraine, cardiac interatrial abnormalities, and additional biologic markers on migraine subtypes in a large series of young adults with ischemic stroke.

Patients and methods: Ischemic stroke patients aged ≤ 45 years were consecutively enrolled as part of the Italian Project on Stroke in Young Adults (IPSYS). A comprehensive evaluation of cardiovascular risk factors, migraine, procoagulant state, and genotyping to detect factor V Leiden and the G20210A mutation in the prothrombin gene.

Results: 981 patients (mean age, 36.0 ± 7.6 years; 50.7% women) were included. The risk of migraine with aura (MA)-related infarcts increased with decreasing number of cardiovascular risk factors (OR, 0.50; 95% CI, 0.24 to 0.99 for two factors or more), increasing number of thrombophilic variants (OR, 2.21; 95% CI, 1.05 to 4.68 for carriers of at least one of the two), and the presence of PFO (OR, 2.41; 95% CI, 1.37 to 3.45), as compared to stroke patients without migraine. None of these factors had influence on the risk of migraine without aura (MO)-related infarcts.

Conclusions: In young adults with ischemic stroke, low cardiovascular risk profile, PFO and an underlying procoagulant state are strong predictors of MA. The biologic effects of these factors should be considered in future studies aimed at investigating the mechanisms linking migraine to brain ischemia.

Corresponding Author: Alessandro Pizzini, Brescia, Italy, ale_pizzini@hotmail.com

P527

SERUM OSTEOPROTERTUGIN PREDICTS FUTURE MYOCARDIAL INFARCTION, ISCHEMIC STROKE, AND TOTAL MORTALITY IN A GENERAL POPULATION

A. Vik 1, E. Mathiesen 2, J. Brox 3, T. Wilsgaard 2, I. Njostad 3, L. Jørgensen 3, J.B. Hansen 1
1 Hematological Research Group, Department of Clinical Medicine; 2 Cerebrovascular Research Group Department of Clinical Medicine; 3 Department of community medicine; University of Tromsø, Tromsø, Norway

Background: The osteoprotegerin (OPG) concentration in serum is associated with the presence and severity of atherosclerosis, and predicts cardiovascular disease and mortality in high-risk populations. In contrast, no association between OPG and ischemic stroke was reported in a case control study in the general population. The present study was undertaken to investigate the association between serum OPG levels and risk of future myocardial infarction (MI), ischemic stroke (IS) and total mortality.

Methods: Serum OPG was measured in serum samples from 6267 persons without cardiovascular diseases aged 25 to 84 years, who participated in a population health survey. The Tromso Study, in Troms county, in Northern Norway. Total mortality were registered from the date of inclusion until 31th of December 2005. Cox regression models were used to estimate crude and adjusted hazard ratios (HR) for clinical events. Results: There were 575 MI, 284 incident IS, and 824 deaths during a median of 10.6 years of follow-up. Serum OPG concentrations (per SD (1.13 mg/ml) increase in OPG) were associated with increased risk of MI (HR 1.20; 95% CI 1.11-1.31), IS (HR 1.32; 95% CI 1.18-1.47), and total mortality (HR 1.41; 95% CI 1.29-1.54) after adjustment for traditional cardiovascular risk factors such as age, sex, current smoking, systolic blood pressure, BMI, HDL cholesterol, total cholesterol, creatinine, hs-CRP and diabetes mellitus.

Conclusion: In our large population-based cohort study serum OPG concentration at baseline was associated with future MI, IS and total mortality independent of traditional cardiovascular risk factors. It may be suggested that OPG has a prothrombotic impact on the cardiovascular system as long as it is known that serum OPG does not promote plaque growth or formation.

Corresponding Author: Anders Vik, Tromsø, Norway, Anders.Vik@unn.no
P120  A MUTATION IN THE INNER MITOCHONDRIAL MEMBRANE PEPTIDASE 2-LIKE GENE (IMMP2L) INCREASES INFARCT VOLUME AFTER A TRANSIENT CEREBRAL FOCAL ISCHEMIA

A. Li 1, Y. Ma 2, B. Lu 3
1 Department of Pharmaceutical Sciences, BRITE, North Carolina Central University, Durham, NC, USA; 2 Department of Pathology, College of Basic Sciences, Ningxia Medical University, Yinchuan, PR China; 3 Institute of Reproductive Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA;

Keywords: mitochondrial, cerebral ischemia, oxidative stress, gene mutation, brain damage

Mutation of Immp2l gene affected the signal peptide sequence processing of mitochondrial proteins, cytochrome c1, and glycerol phosphate dehydrogenase 2. Mutant Immp2l impairs fertility by enhancing oxidative stress [1]. Although mutation of Immp2l gene is associated with Tourette syndrome [2] its influence in the CNS is unknown. The objectives of this study are to explore the effects of mutant Immp2l on ischemic outcome and to determine the effects of hyperglycemia on brain damage in both WT and mutant mice. Male Immp2l mutant and WT mice were subjected to 1 hour MCAO under normo- and hyperglycemic conditions. Their brains were harvested after 5- and 24-hrs of reperfusion. Cerebral infarct volumes, edema, and production of superoxide were measured. The results showed that average infarct volume increased from 12% of hemisphere in the WT to 30.9% in the mutant mice (p=0.004). Hyperglycemia enlarged infarct volume in the WT but did not increase the damage in the mutant mice. There was no significant difference observed in cerebral edema among the experimental groups. In situ detection of superoxide revealed a significant elevation of superoxide production in the mutant mice compared to the WT animals. Our results suggest that mutation of Immp2l gene increases ischemic brain damage by enhancing superoxide production and that hyperglycemia enhances ischemic brain damage in WT animals but did not further enhance the damage in mutant mice because maximum damage was already reached in the mutant animals.

References

Corresponding Author: Andy Li, Department of Pharmaceutical Sciences, BRITE, North Carolina Central University, 302 E. Lawrence Street, Durham, NC, USA, bl@ncsu.edu

P175  EFFECT OF INTRAHOSPITAL TREATMENT WITH ANTIPLATELETS AND OTHER CARDIOVASCULAR DRUGS ON THE SEVERITY OF ACUTE ISCHEMIC CEREBROVASCULAR EVENTS. THE GIFA STUDY

A. Tuttolomondo 1, 2, D. Di Raimondo 2, R. Di Sciacca 2, 3, C. Pedone 2, A. Pinto 2, G. Licata 1
1 Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo, Palermo, Italy; 2 Cattedra di Gerontologia, Campus Biomedico Roma, Rome, Italy

Background/Aims: The pathophysiology of ischemic stroke dictates that treatments be administered shortly after symptom onset to be beneficial. No information exists, to our knowledge, about the possible role of cardiovascular drugs administration in the acute phase of ischemic stroke and possible effects on stroke outcome. On this basis the aim of our study was to evaluate the relationship between intra-hospital treatment with cardiovascular drugs in patients with acute ischemic stroke on some outcome indicators.

Methods: 1096 subjects enrolled in the GIFA study, who had a main discharge diagnosis of ischemic stroke represent the final sample. Drugs considered for the analysis were the following: ACE-inhibitors (ACE-I), angiotensin II receptor blockers (ARBs), statins, calcium-channel-blockers (CCBs), antiplatelet (AFL) drugs, antivitamin-k (VKAs), and heparins. As outcome indicators we choose in-hospital mortality, functional indicators such as cognitive and functional performance at discharge.

Results: Subjects with no-intrahospital mortality, HAMT > 6 and 0 ADL impaired were more likely to have: a lower age, lower blood glucose level at admission, higher SBP at admission, higher plasma levels of total cholesterol, lower white blood cell count, lower Charlson index. Moreover, patients with a good outcome showed a higher rate of intra-hospital treatment with Ace-inhibitors, calcium-channel blockers and antiplatelet drugs and a lower rate of pre-treatment with heparin.

Conclusions: Our study suggests that if a patient with acute ischemic stroke has higher SBP at admission, higher total plasma cholesterol levels, a lower Charlson index and if it is treated with ace-inhibitors, calcium channel blockers and antiplatelet drugs the short term outcome is better in terms of intrahospital mortality and of functional indicators such as cognitive and functional performance at discharge.

Corresponding Author: Antonio Tuttolomondo, Campus Biomedico Roma (Italy), Piazza delle Cliniche, n.2, Palermo, Italy, brunotto@unipa.it

P154  NO ASSOCIATION BETWEEN CHROMOSOME 12P13 SINGLE NUCLEOTIDE POLYMORPHISMS AND EARLY-ONSET ISCHEMIC STROKE.

L. A. Lotta 1, B. Giusti 2, C. Saracini 3, A. Vestrini 3, S. Massamonti 3, M. Rausa 1, M. Volpe 2, S. Rubattu 4, R. Abbate 5, P.M. Mannucci 6, P. Feyvand 1
1 A. Bianchi Bonomi Hemophilia and Thrombosis Centre, L. Villa Foundation, Dept Medical and Medical Specialities, Fondazione IRCCS Ca’ Granda – Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; 2 Dept Medical and Surgical Critical Care, University of Florence – Atherothrombotic Disease Unit, AOU Careggi, Florence, Italy; 3 Dept Neurology-Stroke Unit, II School of Medicine, Sapienza University, Ospedale S. Andrea, Rome, Italy; 4 Dept Cardiology, II School of Medicine, Sapienza University, Sant’Andrea Hospital, Rome, Italy; 5 IRCCS Neuromed, Pozzilli, Isernia, Italy.

Keywords: ischemic stroke, genetic, chromosome 12p13

Background/Aims: There is little information on whether or not early-onset ischemic stroke shares similar disease pathways and genetic predisposition with late-onset disease. A recent genome-wide association study, conducted in the frame of prospective population-based cohorts, found a strong association between two common single nucleotide polymorphisms at chromosome 12p13 and the incidence of ischemic stroke. This finding is as yet not replicated. We assessed the association of chromosome 12p13 single nucleotide polymorphisms with ischemic stroke in a case-control study of early-onset ischemic stroke.

Methods: rs11833579 and rs12425791 were genotyped in 419 Italian patients with early-onset ischemic stroke (median age at first stroke: 43 years, range: 1-65 years) and 1077 healthy controls, comparable for age and gender with the cases.

Results: Before and after stratification for covariates no significant association between the variants and disease was found.

Conclusions: The lack of association between chromosome 12p13 single nucleotide polymorphisms and ischemic stroke in this study indicates that rs11833579 and rs12425791 probably affect (or tag variants that affect) the risk of ischemic stroke with disease mechanisms that are not shared by early-onset disease.

Corresponding Author: Luca A Lotta, University of Milan, Via Pace 9, Milan, Italy, lotta@unimi.it

P176  EFFECT OF PRE-HOSPITAL TREATMENT WITH ANTIPLATELETS CARDIOVASCULAR DRUGS ON THE SEVERITY OF ACUTE ISCHEMIC CEREBROVASCULAR EVENTS. THE GIFA STUDY

A. Tuttolomondo 1, 2, D. Di Raimondo 2, R. Di Sciacca 2, 3, C. Pedone 2, A. Pinto 2, G. Licata 1
1 Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo, Palermo, Italy; 2 Cattedra di Gerontologia, Campus Biomedico Roma, Rome, Italy

Background/Aims: Previous data have underlined the possible prognostic role of demographic and clinical variables at admission in stroke patients, but few studies have examined the role of drugs with a known cerebrovascular preventive effect on acute ischemic stroke prognosis. The aim of this study is to evaluate the relationship between some clinical and laboratory variables and pre-treatment with cardiovascular drugs and a favourable outcome in subjects with acute ischemic stroke.

Methods: 1096 subjects enrolled in the GIFA study, who had a main discharge diagnosis of ischemic stroke represent the final sample. All drugs prescribed during pre-hospital time were taken from hospital charts and codified according to the anatomical therapeutic chemical classification. Drugs considered for the analysis were the following: ACE-inhibitors (ACE-I), angiotensin II receptor blockers (ARBs), statins, calcium-channel-blockers (CCBs), antiplatelet (AFL) drugs, antivitamin-k (VKAs), and heparins. As outcome indicators we choose in-hospital mortality, functional indicators such as cognitive and functional performance at discharge.

Results: Subjects with no-intrahospital mortality, HAMT > 6 and 0 ADL impaired were more likely to have: a lower age, lower blood glucose level at admission, higher SBP at admission, higher plasma levels of total cholesterol, lower white blood cell count, lower Charlson index. Moreover, patients with a good outcome showed a higher rate of intra-hospital treatment with Ace-inhibitors, calcium-channel blockers and antiplatelet drugs and a lower rate of pre-treatment with heparin.

Conclusions: Our study suggests that if a patient with acute ischemic stroke has higher SBP at admission, higher total plasma cholesterol levels, a lower Charlson index and if it is treated with ace-inhibitors, calcium channel blockers and antiplatelet drugs the short term outcome is better in terms of intrahospital mortality and of functional indicators such as cognitive and functional performance at discharge.

Corresponding Author: Antonio Tuttolomondo, Campus Biomedico Roma (Italy), Piazza delle Cliniche, n.2, Palermo, Italy, brunotto@unipa.it
THROMBOPHILIC RISK FACTORS AND OUTCOME IN PATIENTS UNDERGOING ENDOVASCULAR INTERVENTION FOR PERIPHERAL ARTERIAL DISEASE

M. Sartori, E. Conti, E. Favaretto, C. Legnani, C. Pili, G. Palareti

Angiology and Haemostasis Unit, University Hospital, Bologna, Italy

Keywords: peripheral arterial disease, arterial thrombosis, thrombophilic risk factors

Aims: Few data are available on thrombophilic risk factors and clinical outcome in patients undergoing percutaneous transluminal angioplasty (PTA) for peripheral arterial disease (PAD). We investigated the role of homocysteine, fibrinogen, Factor VIII (FVIII), von Willebrand factor (vWF), FII G20210A, and FV R506Q (FV Leiden) mutations, known to be associated with thrombosis risk, as prognostic factors in 199 patients who underwent PTA for PAD (Fontaine’s stages: II through IV; aged 69 ±1 years, male/female 119/78).

Design and methods: A longitudinal study. End-points of the study were total mortality, cardiovascular events and restenosis after PTA. Patients were followed up for an average time of 32±2 months.

Results: During the follow-up, total mortality was 16%, 45.5% of patients had a cardiovascular event. According to Cox regression analysis, age and the presence of critical limb ischaemia were predictors of mortality and cardiovascular events, whereas diabetes, hyperlipidaemia, homocysteine, and PAC were predictor of cardiovascular events. Considering as dichotomous the following variables: fibrinogen, homocysteine, FVIII, presence of PAC, FII G20210A, and FV Leiden mutations, the frequency of patients with at least two thrombophilic alterations was 31%. During the follow-up, cardiovascular events were more frequent in the patients with at least two thrombophilic alterations versus those with one or without thrombophilic alterations (37 vs. 17% log-rank p=0.001). Rates of restenosis during the follow-up were not different in the two groups (26 vs. 20%, p=ns).

Conclusions: The presence of two or more thrombophilic risk factors in patients who underwent PTA for PAD is associated with increased risk of arterial thrombotic events. Intervention trials are required to show the benefit of different therapeutic approaches in such patients at high risk of clinical deterioration.

Corresponding Author: Michelangelo Sartori, Angiology and Haemostasis Unit, University Hospital Policlinico Sant’Orsola Malpighi, Pad. 2, Via Albertoni, 15, Bologna, Italy, michelangelo.sartori@aosp.bo.it

CHANGES OF HAEMOSTATIC PARAMETERS IN PATIENTS WITH CAROTID STENOSIS

I.M. Canic, L. Koncar, N. Antonijevic, B. Beletic, M. Golubovic, L. Davidovic, N. Majkic-Singh

1 Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia; 2 Clinicals for Vascular Surgery, Clinical Center of Serbia, Belgrade, Serbia; 3 Clinicals for Cardiology, Clinical Center of Serbia, Belgrade, Serbia.

Keywords: haemostasis, carotid stenosis

Background/Aims: Disturbances in various components of the haemostatic system may account for clinical manifestations of atherothrombosis and are considered as valuable prognostic factors. The aim of our study was to evaluate changes in fibrinogen concentrations and activities of antithrombin III, plasminogen, PAI 1 and von Willebrand factor in patients with carotid stenosis.

Materials and methods: The study compared levels of the above mentioned parameters between a group of 80 patients with carotid stenosis (43 men and 37 women) and a control group of 55 age-matched controls (29 men and 26 women). Plasma samples were analyzed using standard methods and results were compared using Student’s t-test.

Results: Mean fibrinogen levels in patients (5.43 g/L) and in controls (2.86 g/L) were significantly different (p<0.001). Comparison of antithrombin III activity between patients (98.5 %) and controls (106.6%) revealed significant difference (p=0.001). Plasminogen activity in patients (133.8%) was significantly higher (p<0.001) compared to that measured in controls (103.9%), while the difference in PAI 1 activities (3.99 U/mL in controls vs. 4.14 U/mL in patients) did not reach the level of significance. Activity of von Willebrand factor was significantly higher (p<0.001) in patients (164.8%) than in controls (90.1%). Gender related differences in levels of all measured parameters had no significance.

Conclusions: We can conclude that patients with carotid stenosis have increased fibrinogen levels and decreased antithrombin III activity. Plasminogen activity is also increased in these patients, while the PAI 1 activity is not significantly affected. Additionally, we can state that patients with carotid stenosis have increased activity of von Willebrand factor.

Corresponding Author: Ivana M. Canic, Center for Medical Biochemistry, Clinical Center of Serbia, Vrthigradzka 26, Beograd, Serbia, ivana.klinika@gmail.com

PROSPECTIVE ANALYSIS OF THE USE OF THROMBOELASTOGRAPHY IN PREDICTION OF HEMORRHAGE IN STROKE PATIENTS


University of Texas-Houston Medical School, Houston, Texas, USA

Keywords: thromboelastography, stroke, hemorrhage

Background: Thromboelastography (TEG) provides dynamic information about the balance of thrombosis and lysis, and therefore may be useful to predict bleeding and guide lytic or hemostatic therapy. Up to 10% of ischemic strokes (IS) bleed after TPA therapy, and more than 33% of hemorrhagic strokes (HS) have spontaneous hematoma enlargement (HE). Such bleeding results in poor outcome, and if correctly predicted, might be avoided by withholding or reducing the dose of TPA (IS) or administering hemostatic drugs (HS). No test has been shown to predict such bleeding after IS or HS. This study was designed to measure TEG at the onset of IS and HS, and to see if it might predict bleeding.

Methods: IS and HS patients arriving at our emergency department within 3 hours of symptom onset have blood drawn for TEG measurement. If patients meet inclusion criteria (IS-meet established criteria for, and receive, IV TPA within 3 hrs; HS-non-traumatic hematoma volume 5-65ml and not requiring surgery), patients or families are consented and TEG analysis carried out including R time (fibrin formation), K time (clot strengthening), a angle (speed of clot formation), and MA (maximum clot strength). TEG values are compared to age-matched non-stroke patients (C). TPA treated patients have repeat TEG 10 min after bolus. Follow up brain imaging is obtained and assessed for bleeding (IS) or hematoma enlargement (HS) at 36 ± 12 hours. Sample size (24 per group) is sufficient to detect a 40% difference in the proportion of patients with abnormal TEG values between IS, HS, or C patients, and in the proportion of pts with abnormal TEG with or without HE.

Results: To date, 15 patients have been tested and final results on all patients and controls will be reported at the time of this presentation.

Conclusion: TEG values may be abnormal in some IS and HS patients and may predict bleeding complications.

Corresponding Author: James C. Grotta, University of Texas-Houston Medical School, 6431 Fannin St, 77030 Houston, TX, USA, james.c.grotta@auth.tmc.edu

ACTIVATED FACTOR XI AND TISSUE FACTOR IN CIRCULATING BLOOD ARE ASSOCIATED WITH WORSE CLINICAL OUTCOMES IN PATIENTS WITH A HISTORY OF ISCHEMIC CEREBROVASCULAR EVENTS

A. Undas, A. Slovik, R. Topör-Madry, M. Gisell, K.G. Mann, S. Butenas

1 Institute of Cardiology, 2 Department of Neurology, 3 Institute of Public Health, Jagiellonian University School of Medicine, Krakow, Poland; 4 Department of Biochemistry, University of Vermont, Burlington, VT, USA

Keywords: tissue factor, factor XI, stroke

Background/Aims: Elevated factor (FXI) and tissue factor (TF) have been reported to occur in patients with acute ischemic stroke. Data on these parameters following stroke are sparse. The aim of the current study was to determine levels of active FXI and TF and correlate these with clinical outcomes in patients with ischemic cerebrovascular events.

Patients and methods: We studied 241 patients, aged 65 years or less, recruited between 6 months and 4 years following such events (162 strokes and 79 transient ischemic attacks [TIAs]). Plasma FXIa, FIXa and TF were determined in clotting assays by measuring the response to inhibitory monoclonal antibodies.

Results: Detectable active TF (>0.5 pM), FXIa and FIXa were observed in 10.4%, 11.4% and 37% of patients, respectively. Patients with detectable TF or FIXa or FXIa had higher modified Rankin scale score, and a lower prevalence of FXIa in patients with small vessel disease compared with the remainder (16.4% vs 29.6%, p=0.01). Patients with detectable TF or FXIa, but not FXIa, had a higher NIHSS score at discharge at the time of the index event and at enrollment, a higher modified Rankin scale score, and a lower Barthel Index compared to those without circulating active TF or FXIa (all p<0.01).

Conclusions: Detection of active TF (>0.5 pM), FXIa and FIXa may provide useful information for planning antithrombotic or hemostatic therapy. Up to 10% of ischemic strokes (IS) bleed after TPA therapy, and more than 33% of hemorrhagic strokes (HS) have spontaneous hematoma enlargement (HE). Such bleeding results in poor outcome, and if correctly predicted, might be avoided by withholding or reducing the dose of TPA (IS) or administering hemostatic drugs (HS). No test has been shown to predict such bleeding after IS or HS. This study was designed to measure TEG at the onset of IS and HS, and to see if it might predict bleeding.

Corresponding Author: Anetta Undas, Institute of Cardiology, Jagiellonian University School of Medicine, 80 Pradnik St, Krakow, Poland, anettundyass@yahoo.com
WHAT IS MORE EFFECTIVE AND SAFE: LOW MOLECULAR WEIGHT HEPARIN OR UNFRACTIONATED HEPARIN DURING ARTERIAL RECONSTRUCTIVE SURGERY?

A. Vladimirovich Pokrovsky 1, V. Nikolaievich Gontarenko 1, M. Ivanovna Titova 2, V. Vladimirovna Egorova 1, V. Semenovna Demidova 2

1 Department of Vascular Surgery, A.V. Vishnevsky Institute of Surgery, Moscow, Russian Federation; 2 Laboratory of Clinical Biochemistry, A.V. Vishnevsky Institute of Surgery, Moscow, Russian Federation.

Aims:

To compare iliac and femoro-popliteal percutaneous transluminal angioplasty (PTA) procedures in patients with peripheral arterial disease (PAD) with regards to rates of periprocedural and antithrombotic treatment-related complications, long-term (3 years) restenosis and rethrombosis, need of further revascularization procedures and leg amputations.

Methods: Consecutive PAD patients undergoing PTA were prospectively followed-up at 1-6-12 months, then yearly. Visits included echo color doppler with Ankle-Brachial-Index (ABI) measurement. After PTA double antiplatelet therapy was administered for 30 days, then aspirin alone, for infrainguinal procedures LMWH (half therapeutic dosage) was added for a week. All patients received statins for at least six months.

Results: 201 patients were enrolled. Mean age was 68.5 y and 60.2% were male. Mean follow-up was 36 months. Indications for PTA were claudication (58.2%), rest pain (10.9%), tissue loss (18.4%), acute thrombosis (12.4%). In 396 lesions in 272 limbs, 220 procedures were performed. Iliac procedures were 141 (51.8%), infrainguinal ones (36.4%), and both sites 32 (11.8%). The last two groups were analyzed together. Stent was used in 79% of 200 iliac lesions and in 6% of 196 infrainguinal ones. Thirty-day mortality was 0.5%. Periprocedural and 30-day antithrombotic treatment-related haemorragic complications rates were 9.1% and 2.7% respectively (Table). One-year and 3-year ≥75% restenosis or rethrombosis cumulative rates were 1.3% and 7.7% respectively in iliac-treated patients (Figure).

Conclusions: Iliac PTA has a long-term sustained better prognosis than infrainguinal procedures, the second strongest predictor of recurrent disease being baseline ABI. Periprocedural and 30-day antithrombotic treatment-related haemorragic complications are relevant.

<table>
<thead>
<tr>
<th>Procedure complications (232 procedures, 12 for restenosis treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Pseudoneurysm</td>
</tr>
<tr>
<td>Large inguinal haemotoma</td>
</tr>
<tr>
<td>Thrombus dislocation</td>
</tr>
<tr>
<td>Dissection</td>
</tr>
<tr>
<td>Cholesterol embolization</td>
</tr>
<tr>
<td>Balloon rupture</td>
</tr>
<tr>
<td>Closure device-related thrombosis</td>
</tr>
<tr>
<td>Catheter access A-V fistula</td>
</tr>
<tr>
<td>Brachial a. thrombosisys</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Figure: Kaplan-Meier restenosis risk curves in relation to treated lesion localization.
Background/Aims: Thrombin-activatable fibrinolysis inhibitor (TAFI) gene polymorphism is a possible risk factor in Egyptian patients suffering from ischemic cerebral stroke or not.

Patients and methods: Eighty-four adult males and females aged from (25-60 years) were classified into 2 groups: Group 1: 42 patients with ischemic cerebral stroke; Group 2: normal control persons. All samples were prepared for laboratory investigations (fasting blood glucose, serum cholesterol, serum triglycerides, HDL-cholesterol, LDL-cholesterol) and genomic DNA study.

Results: Thr325lle polymorphism of the TAFI gene, hyperlipidemia, hypertension, old age and family history of stroke showed an association as risk factors with ischemic stroke (P<0.05). For the genotype of Thr325lle TAFI gene polymorphism in patients, there were (71.4%) heterozygous patients (CT) and (28.6%) were homozygous for the low risk allele (CC). TT allele was not found in a homozygous form in both groups. The present study revealed that the frequency of the high risk allele (T) in ischemic stroke patients was 35.7% compared with 19.05% in control group. Odds ratio (OR) for the high risk allele (T) = 2.4 with 95% confidence interval (CI) from 1.17 to 4.77.

Conclusions: Our data suggest that the 325Thr/lle genotype of the TAFI gene might be a possible risk factor for cerebral ischemic stroke in Egyptian patients. However, more studies on the relationship between the other genetic polymorphisms of the TAFI gene and stroke are warranted.

Corresponding Author: Ola Farouk Lehet, Clinical Pathology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt, olaleheta@yahoo.com

Keywords: percutaneous transluminal angioplasty, peripheral artery disease

Our understanding of the mechanisms of restenosis/reoclusion of the superficial femoral artery after percutaneous transluminal angioplasty (PTA) is incomplete. No associations between blood constituents and success of PTA have been documented yet, and were therefore, tested in this study.

142 consecutive patients treated by femoropopliteal PTA because of disabling claudication or critical limb ischemia were followed-up by vascular ultrasound imaging at 1, 6 and 12 months after the procedure. The technical success of PTA was assessed by peri-procedural angiography. Adverse outcome of PTA was defined by restenosis ≥50% that was confirmed by at least doubling of the maximal systolic velocity in comparison to a proximal, non-occluded arterial segment, and reocclusion was determined by the absence of Doppler flow signal. At six months blood was drawn for routine laboratory analysis. In addition, closure time, fibrinogen, von Willebrand factor, D-dimer, homocysteine, P-selectin, VCAM-1, tissue plasminogen activator were determined by the absence of Doppler flow signal. At six months blood was drawn for routine laboratory analysis. In addition, closure time, fibrinogen, von Willebrand factor, D-dimer, homocysteine, P-selectin, VCAM-1, tissue plasminogen activator were determined by the absence of Doppler flow signal.

Keywords: percutaneous transluminal angioplasty, peripheral artery disease

Background/Aims: Infectious agents, especially the intracellular Chlamydia pneumoniae, have been supposed to be involved in the atherosclerotic process. We performed a cross-sectional, multicenter, outpatient protocol to study Chlamydia pneumoniae DNA in leukocytes measured by a real-time PCR in patients with type 2 diabetes with different degrees of atherosclerosis evaluated by carotid ultrasound.

Methods: One hundred thirty-five consecutive type 2 diabetic patients were studied. Clinical, metabolic (HbA1c, lipids) and inflammatory (high-ultrasensitive C-reactive protein, tumor necrosis factor-alpha, interleukin-6) variables were measured. Previous clinical macrovascular disease was registered and B-mode ultrasound was performed. Real-time PCR protocol for Chlamydia pneumoniae (Tib Molbiol, Berlin, Germany) in a LightCycler thermocycler (Roche, Basel, Switzerland) was performed in all patients, using adequate positive and negative internal controls.

Results: Patients mean age was 62 ± 7 years. Mean diabetes duration was 16 ± 9 years. Mean HbA1c was 7.1 ±1.1%. In relation to carotid ultrasound results, 40.7% patients presented carotid plaque, 32.5% subclinical atherosclerosis and 26.6% no evidence of atherosclerosis. All groups were homogeneous in anthropometric data. Biochemical determinations were similar in all groups except for cholesterol and non-HDL-cholesterol levels. Patients with clinical atherosclerosis had greater carotid intima-media thickness compared to the other two groups. No Chlamydia pneumoniae DNA was detected in any of the type 2 diabetes patients regardless of the presence of clinical or subclinical atherosclerosis.

Conclusions: The lack of detection of Chlamydia pneumoniae DNA in leukocytes suggests that this bacterium does not have an active systemic role in the pathogenesis of atherosclerosis in middle-aged type 2 diabetic patients, and it is not a reliable marker for atherosclerosis in high risk patients.

Corresponding Author: Juan-Carlos Reverter, Department of Hemotherapy and Hemostasis. Hospital Clinic, Barcelona, Spain., Villarroel 170, Barcelona, Spain, reverter@clinic.ub.es

Keywords: micro particles, atherothrombosis, systemic lupus erythematosus

Background/Aims: Microparticles (MPs) are a key component in the haemostatic response. We analyzed the procoagulant activity of MPs and its correlation with arterial thrombosis and preclinical arteriosclerosis in patients with systemic lupus erythematosus (SLE).

Methods: We included 100 patients with SLE (27 with associated APS, 36 with antiphospholipid antibodies -aPL- but without APS and 37 with aPL). Early arteriosclerosis was evaluated by ultrasonographic study of carotids measuring intima-media wall thickness and presence of arteriosclerotic plaque. Procoagulant MPs were assessed by a functional assay in which MPs were captured through annexin V and then thrombin was formed by the addition of activated factor X, activated factor V and prothrombin (Hyphen BioMed, Neuville, France).

Results: A total of 16 episodes of arterial thrombosis in 8 patients with APS, 7 without aPL and 1 patient with aPL have been registered. SLE patients with associated APS had greater prevalence of plaque than patients without aPL or with aPL but without APS (51.9% vs 24.3 % vs 22.2 %). No differences were seen in procoagulant MPs according the gender or the age of the patients. Overall, there was a significant relationship between procoagulant MPs and the presence of carotid plaque (16.7 ±8.6 nM vs 12.7 ±7.3 nM; p<0.02) and the number of carotid plaques (14.5 ±4.0 nM in patients with one plaque and 17.3 ±10.3 nM in patients with 2 or more plaques; p=0.02). In addition, a relationship between arterial thrombotic events and the procoagulant activity (18.7 ±9.5 nM with events vs 13.0 ±7.2 nM without events, p=0.007).

Conclusions: Procoagulant MPs may be implicated in arterial thrombosis and arteriosclerosis in SLE patients.

Corresponding Author: Dolores Tássies, Department of Hemotherapy and Hemostasis, Hospital Clinic, Barcelona, Spain, Villarroel 170, Barcelona, Spain, dtassies@clinic.ub.es

Keywords: Procoagulant Microparticles and Arterial Atherothrombotic Disease in Patients with Systemic Lupus Erythematosus

Background/Aims: Chlamydia pneumoniae DNA was detected in any of the type 2 diabetes patients regardless of the presence of clinical or subclinical atherosclerosis.

Conclusions: The lack of detection of Chlamydia pneumoniae DNA in leukocytes suggests that this bacterium does not have an active systemic role in the pathogenesis of atherosclerosis in middle-aged type 2 diabetic patients, and it is not a reliable marker for atherosclerosis in high risk patients.
EFFECT OF PROTHROMBIN 19911 A>G POLYMORPHISM ON THE RISK OF CEREBRAL SINUS-VENOUS THROMBOSIS

S.M. Passamonti 1, P. Bucciarelli 1, V. De Stefano 2, M. Menegatti 1, D. Tormene 1, A. Tosetto 1, I. Martinelli 1

1. A. Bianchi Bonomi Haemophilia and Thrombosis Center, Department of Internal Medicine, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, University of Milan, Italy; 2. Institute of Hematology, Catholic University of Rome, Rome, Italy; 1. Department of Medical and Surgical Sciences, University of Padua, Padua, Italy; 2. Hematology Department, S. Bortolo Hospital, Vicenza, Italy

Keywords: cerebral sinus-venous thrombosis, thrombophilia, factor V Leiden, prothrombin 19911 A>G

Background/Aims: The A>G polymorphism at position 19911 of the prothrombin gene is associated with a mildly increased risk of venous thromboembolism, alone or in association with such common thrombophilia mutations as factor V Leiden and prothrombin 20210 GA. Its role in cerebral sinus-venous thrombosis is not known.

Materials and methods: The presence of prothrombin 19911 A>G was investigated in a case-control study of 108 patients with cerebral thrombosis and factor V Leiden (n=25), prothrombin 20210 GA (n=48), without thrombophilia (n=35) and 842 healthy individuals with the corresponding coagulation profile.

Results: Prothrombin 19911 A>G did not increase the risk of cerebral sinus-venous thrombosis in carriers of factor V Leiden (adjodds ratio 1.6, 95%CI 0.6-4.7), prothrombin 20210 GA (odds ratio 1.2, 95%CI 0.6-2.4), nor in patients without thrombophilia (odds ratio 1.3, 95%CI 0.5-3.1).

Conclusions: Prothrombin 19911 A>G polymorphism does not appear to be a risk factor for cerebral sinus-venous thrombosis, alone or in association with factor V Leiden or prothrombin 20210GA.

Corresponding Author: Serena M. Passamonti, A. Bianchi Bonomi Haemophilia and Thrombosis Centre, Department of Internal Medicine, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, University of Milan, Via Pace 9, Milan, Italy, serena.passamonti@unimi.it

P174

HOMOCYSTEINE LEVELS AND INCIDENCE OF HYPERHOMOCYSTEINEMIA IN PATIENTS WITH CAROTID STENOSIS

A. Beletic 1, I. Koncar 1, I. Canic 1, N. Antonijevic 1, D. Mirkovic 1, M. Golubovic 1, L. Davidovic 2, N. Majkic-Singh 1

1. Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia; 2. Clinics for Vascular Surgery, Clinical Center of Serbia, Belgrade, Serbia

Keywords: hyperhomocysteinemia, carotid stenosis

Background/Aims: Hyperhomocysteinemia is considered one of the factors related to atherothrombosis. The aim of the study was to evaluate changes in homocysteine concentrations (Hcy) and incidence of hyperhomocysteinemia (HHcy), defined as Hcy concentration above 12 μmol/L, in patients with carotid stenosis.

Materials and methods: The study compared Hcy level and HHcy incidence between the group of 80 patients with carotid stenosis (43 men and 37 women) and control group of 55 age-matched controls (29 men and 26 women). Hcy was measured in serum, using HPLC method with fluorescence detection. Results were compared by Student’s t and chi-square tests.

Results: Mean Hcy levels in patients (14.3 μmol/L) and in controls (10.8 μmol/L) were significantly different (p=0.001). HHcy was present with significantly higher incidence (p=0.037) in patients (65.2%) than in controls (23.2%). The difference in Hcy concentrations between male (14.9 μmol/L) and female (13.1 μmol/L) patients was not significant, while the opposite was observed in controls (males 12.2 μmol/L vs. 9.3 μmol/L, p=0.013). Men with carotid stenosis had significantly higher Hcy levels compared with healthy men (14.9 vs. 12.2 μmol/L, p=0.012). Similar results were observed in female population of our study: Hcy levels were significantly (p=0.001) higher in patients (13.1 μmol/L) than in controls (9.3 μmol/L).

Conclusions: We conclude that patients with carotid stenosis have increased Hcy levels and higher HHcy incidence. Generally, gender related differences in homocysteinemia increase in these patients are not significant, but there is a need to highlight the fact that patients of both sexes have higher Hcy levels than their matches in control group.

Corresponding Author: Andjelo Beletic, Center for Medical Biochemistry, Clinical Center of Serbia, Visegradska 26, Belgrade, Serbia, abeletic@eunet.rs

P171

RECURRENCES OF STROKE IN PATIENTS WITH ATRIAL SEPTALANEURYSM AND PATENT FORAMEN OVALE: LONG-TERM FOLLOW-UP

A. V. Mattioli 1, S. Pennella 1, R. Lonardi 1, A. Farinetti 2

1. Department of Biomedical Science, University of Modena and Reggio Emilia, Modena, Italy; 2. Department of General Surgery and Surgical Specialty, University of Modena and Reggio Emilia, Modena Italy

Keywords: atrial septal aneurysm, patent foramen ovale, stroke

Background/Aims: It is uncertain whether anticoagulants and antiplatelet agents are effective therapy in preventing stroke among patients with atrial septal aneurysm (ASA) and/or patent foramen ovale (PFO). The aim of the present study was to evaluate in a long term follow-up recurrences of stroke in patients with ASA and PFO.

Patients and methods: We prospectively evaluated 490 patients: 245 patients who had a previous stroke (Group A) and a control group (B) of 245 patients. Transesophageal echocardiography showed ASA in 104 patients; 68 patients (27.7%) in group A and 36 (14.7%) in Group B (p<0.001). The prevalence of PFO was 22.8% in group A and 12.6% in group B (p=0.001). PFO+ASA was found in 72 patients (69%). The frequency of ischemic events was evaluated over 7 years. Treatment assignments were at the discretion of the consulting cardiologist.

Results: In group A 140 patients received aspirin (14 had ASA, 2 had PFO and 16 had ASA+PFO) and 98 received warfarin (2 patients with ASA, 2 with PFO and 36 patients with ASA+PFO). In group B 200 patients received aspirin. In group A 21 patients had percutaneous closure of PFO. In a 7-year follow-up we do not observed a significant reduction of recurrent stroke and death in patients with ASA treated with aspirin compared with those treated with warfarin (17% vs 11%; OR 0.46 95%CI 0.3-2.71). Similarly we did not find differences in patients with PFO treated with aspirin compared with warfarin (19% vs 12%). No embolic events or deaths in patients who underwent percutaneous closure of PFO were reported.

Conclusions: No reduction of recurrent stroke and death was observed in patients receiving aspirin compared with those receiving anticoagulants, suggesting that both are reasonable therapy. The limitations of the study were: therapeutic regimen was not randomized, nevertheless the follow-up was done for a long period.

Corresponding Author: Anna Vittoria Mattioli, Department of Biomedical Science University of Modena and Reggio Emilia, Via del Pozzo, 71, Modena, Italy, annavit@unimore.it

P194

POLYMPH OF PRO-INFLAMMATORY/ANTI-INFLAMMATORY AND THROMBOTIC/FIBRINOLYTIC GENES IN PATIENTS WITH ACUTE ISCHEMIC STROKE AND RELATIONSHIP WITH TOAST SUBTYPE

A. Tutulomudo 1, 2, 3, D. Di Raimondo 1, A. Casuccio 1, L. Vaccarino 1, L. Scola 1, S. G. I. Forte 2, S. Sanacore 1, D. Lio, A. Pinto 1, G. Licitra 1

1. Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo, Palermo, Italy; 2. Dipartimento di Biopatologia e Medicologie Biomediche, Università degli Studi di Palermo, Palermo, Italy; 3. Dipartimento di Neuroscienze Cliniche, Università degli Studi di Palermo, Palermo, Italy

Keywords: SNPs, stroke

Background/Aims: The genetic basis of complex diseases like ischemic stroke probably consists of several predisposing risk factors that can interact with environmental factors to produce the disease phenotype. Inflammation and thrombotic pathways have been involved in stroke pathogenesis. In this study our aim was to evaluate the role of SNPs (single nucleotide polymorphisms) of some pro-inflammatory/anti-inflammatory and thrombotic/fibrinolytic genes in patients with acute ischemic stroke.

Methods: We enrolled consecutive patients with a diagnosis of acute ischemic stroke admitted to the Internal Medicine Department at the University of Palermo between November 2006 and January 2009, and control patients without a diagnosis of acute ischemic stroke.

Results: We observed a significant higher frequency of IL-10 1082 AA genotype in stroke patients vs (p=0.033), with a significant trend at regression analysis (HR=3.521; p=0.005). We also reported a higher frequency with a significant hazard ratio at regression analysis, in stroke subjects in comparison to controls, of the TPA 7351-CT genotype (p=0.019) (HR=3.706; p=0.001) and of IL-1 VNTR 86bp 2/2 genotype (p=0.017) (HR=7.50; p=0.011). We reported a significant relationship with TOAST subtype with a significant risk trend only between CC-TPA (HR=8.00, p=0.001) and 1/1 IL-1 VNTR 86bp (HR=3.929, p=0.021) genotypes and lacunar TOAST subtype.

Conclusions: Ischemic stroke is a common multifactorial disease, which is affected by a number of genetic mutations and environmental factors. Our findings showing a relationship between pro-inflammatory/anti-inflammatory and thrombotic/fibrinolytic genes SNPs and ischemic stroke mat contribute to delineate a possible stroke risk profile in subjects with cerebrovascular risk factors.

Corresponding Author: Antonio Tutulomudo, Università degli Studi di Palermo, Piazza delle Cliniche, 2, Palermo, Italy, brunottuto@unipa.it
Background: The etiology of stroke in children is still undetermined in up to one third of cases. There is increasing evidence that inherited or acquired prothrombotic disorders may be implicated in the etiology of stroke in childhood.

Aims: The aim of this study was to determine the frequency of common inherited and acquired prothrombotic risk factors in children and adolescents with arterial ischemic stroke (AIS) and transient ischemic attacks (TIA) in Croatia, and to identify the possible association of two or more risk factors with the disease.

Methods: We investigated 17 prothrombotic risk factors in blood samples from 124 children with an established diagnosis of AIS (N=47) and TIA (N=77) and in 42 children who represented the control group. Prothrombotic risk factors were classified into five groups: natural coagulation inhibitors (antithrombin, protein C, and free protein S antigen), blood coagulation factors (factor V Leiden and factor II (G20210A), homocysteine or methylenetetrahydrofolate reductase C677T, vitamin B12, serum folate and methylenetetrahydrofolate reductase C677T), lipid and lipoprotein profile (lipoprotein a, triglycerides, total, high- and low-density lipoprotein cholesterol) and antiphospholipid antibodies (lupus anticoagulant, antiphospholipid and antiphosphatidylserine antibodies).

Results: At least one prothrombotic risk factor was identified in 87.2% children with AIS, in 88.3% with TIA and in 88.1% controls. A high number of various individual and combined prothrombotic risk factors, distributed among all risk factor groups, was found either in children with AIS or TIA. Three most common prothrombotic risk factors: low serum folate, MTHFR C677T and elevated Lp(a), were identified in approximately 30% of children in both patient groups. More than a two-fold higher frequency of positive IgG antiphospholipid antibody titer, was identified in children with AIS (17.2%), compared to controls (7.1%). The overall rate of three or more prothrombotic risk factors was significantly higher in children with AIS compared to controls (p=0.016).

Conclusions: High frequency of multiple prothrombotic risk factors found in our study corroborates previous reports that a combination of risk factors rather than individual risk factors could contribute to AIS in children.

Corresponding Author: Jasna Lenicek Krleza, Children’s Hospital Zagreb, Kliačeva 16, Zagreb, Croatia, jasna.lenicek.krleza@zg.hnet.hr

Keywords: Atherosclerotic disease with local delivery of paclitaxel after balloon angioplasty

Background: Percutaneous tranluminal angioplasty (PTA) and percutaneous tranluminal coronary angioplasty (PTCA) are established, proven methods for re-opening stenotic or occluded arteries in a minimally invasive way. The balloon is placed in the stenotic segment of the artery and then expanded until the lumen reaches its original diameter. To this end, very high pressure is applied, which unavoidably causes vessel wall injury. Hyperplasia resulting in lumen narrowing is the natural reaction to this injury. A single short contact of tissue with a small dose of paclitaxel has been shown to efficaciously inhibit local cell proliferation antiproliferative taxanes such as paclitaxel seem to be suitable due to their high lipophilicity and tight binding to various cell constituents, resulting in effective local retention at the site of delivery. Paclitaxel as a hydrophobic compound possesses preferential tissue binding.

Methods: 23 patients, all subjects between 65 and 86 years of age with symptomatic claudication (Rutherford category 1-6) with TASC II type A, B, or C lesions in lower limbs were invited to participate in this study under signed informed consent. The vascular pheripherical stenoses were localized in FSA, 16 lesions, popliteal arteries 9 lesions, anterior tibial artery, 6 lesions, posterior tibial artery, 12 lesions, peroneal artery 2 lesions. All stenoses were treated with classic balloon angioplasty (PTA) with adequate diameter balloon for each arterial diameter, after successful procedure stent was placed in the stenotic segment of the artery and then expanded until the lumen reaches its original diameter. The procedure was completed without complications and no collateral effects of the procedure, and it would be the new way to treat lower limbs arterial disease.

Corresponding Author: Roberto Jorgy Fernandez Vina, Fernandez Vina Foundation & Maimonides University, San Nicolas clinic, Mirte 345, San Nicolas, Argentina, robertofernandezvina@hotmail.com

Keywords: peripheral vascular atherosclerotic disease
AUDIT ON APPROPRIATE ANTI-PLATELET THERAPY IN THE PREVENTION OF OCCLUSIVE VASCULAR EVENTS

T. Ong, S. Bikmalla, F. Siddiqui, C. Maity, M. Salchin
Department of Medicine for the Elderly, Leighton Hospital, Mid Cheshire Hospitals Foundation Trust MCHFT, Crewe, UK

Aims: Anti-platelets play an important role in the prevention of occlusive vascular events (OVEs). OVEs includes myocardial infarction (MI), transient ischaemic attack (TIA), ischaemic stroke and symptomatic peripheral arterial disease (PAD). We reviewed our own clinical practice based on current existing guidelines and hope to make appropriate recommendations to improve practice.

Methods: A prospective audit was conducted for 5 weeks, 2 March 2009 in patients who presented to the inpatient or outpatient service of the hospital with an OVE. Data was sourced from the medical records. Standard deviation was used to gauge our current practice based on NICE TA090 - clopidogrel and modified release diprydamole in the prevention of occlusive vascular events, JBS 2, National Clinical Guidelines for Stroke-2008 and the ESC guideline.

Results: A total of 168 patients (103 male and 65 female) were audited. A majority presented with only a single vascular bed affected (41% presented with either a TIA or an ischaemic stroke; 30.4% with a MI; and 24.4% with symptomatic PAD). The remainder presented with an OVE with a pre-existing involvement of another vascular bed (2.4% presented with a TIA or ischaemic stroke and pre-existing heart disease due to MI; and 1.8% with a MI and a pre-existing PAD). No patients in our audit had 3 vascular beds affected. All patients presented with either a MI or symptomatic PAD were prescribed antplatelet according to current guidelines. Only 86% of patients with TIA or ischaemic stroke were prescribed antplatelets based on current guidelines. Deviation from guidelines in TIA and ischaemic stroke was due to the use of clopidogrel instead of aspirin alone in patients unable to take diprydamole MR and is supported by ProFESS trial. Guidelines on antiplatelet therapy in TIA or ischaemic stroke need updating in the light of current evidence.

Table: Audit standards for antplatelet prescribing

<table>
<thead>
<tr>
<th>TIA / Ischaemic stroke</th>
<th>Aspirin 300mg for 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>Aspirin 75mg and Diprydamole MR after 2 weeks</td>
</tr>
<tr>
<td>Symptomatic PAD</td>
<td>Clopidogrel / Aspirin only if intolerant to Aspirin</td>
</tr>
</tbody>
</table>

Corresponding Author: Terence Ong. Department of Medicine for the Elderly, Leighton Hospital, Mid Cheshire Hospitals Foundation Trust (MCHFT), Middowech Road, Crewe, UK, terenceo@doctors.org.uk

KEYWORDS: morphine withdrawal, syndrome, cerebral ischemia, opiates, addiction

ASSOCIATION BETWEEN PULSE PRESSURE AND BLOOD RHEOLOGY IN HEALTHY AND HYPERTENSIVE SUBJECTS

A. Rekhviashvili 1, G. Labakhua 2, M. Nadareishvili 3
1 I. Javakhishvili Tbilisi State University, Tbilisi, Georgia; 2 National Center of Surgery, Tbilisi, Georgia; 3 Chavchavadze Tbilisi State University, Tbilisi, Georgia

Keywords: pulse pressure, blood rheology, hypertension

Aims: Study purpose was to determine whether blood rheological factors are associated with pulse pressure (PP) level among hypertensive and healthy middle-aged men and women.

Methods: A population-based sample of 57 hypertensive and 17 normotensive (35-60 years of age) subjects was studied. Ambulatory monitoring of blood pressure and haemorrhological investigation were performed in each case. Hypercholesterolemic subjects, diabetics, smokers, patients with manifested heart disease, Raynaud’s phenomenon, history of clinical evidence of cardio-, cerebro- and peripheral vascular diseases, coagulopathy, renal and liver diseases were excluded from the study.

Results: Compared with normotensive control subjects, hypertensive patients had significantly higher level of platelet aggregative and adhesive activity (97.68±2.42 vs. 87.18±4.67; P=0.000 and 40.8±3.31 vs. 28.35±3.89; P=0.000); fibrinogen concentration (3.71±0.15 vs. 2.97±0.29; P=0.000), hematocrit (40.2±6.06 vs. 38.24±0.94; P=0.001), erythrocyte aggregability (27.2±3.71 vs. 14.59±3.78; P=0.000) and plasma viscosity (1.75±0.07 vs. 1.54±0.04; P=0.002). Total pulse pressure, as well as average daytime pulse pressure did not show any correlations with haemorrhological characteristics in the hypertensive group. Among hypertensive patients, nighttime average pulse pressure correlated with fibrinogen concentration (r=0.275; P=0.039), plasma and whole blood viscosity (r=0.0274; P=0.038; respectively). Average daytime pulse pressure in healthy subjects correlated with platelet aggregative and adhesive activity (r=0.057; P=0.020 and r=0.542; P=0.025; respectively). Night-time average pulse pressure in control subjects showed significantly high correlations with most haemorrhological indices, namely platelet aggregation (r=0.583; P=0.014), platelet adhesion (r=0.616; P=0.008), erythrocyte aggregation (r=0.847; P=0.000), erythrocyte deformation (r=-0.532; P=0.028), plasma and whole blood viscosities (r=0.621; P=0.008 and r=0.654; P=0.004; respectively).

Conclusions: Obtained data indicate the high importance of nighttime pulse pressure in health and hypertensive subjects. We can conclude that subjects with normal blood pressure level, but high nighttime pulse pressure have higher tendency of thrombotic complications. Therefore, this population needs more attention to avoid future vascular complications.

Corresponding Author: Anna Rekhviashvili. Iv. Javakhishvili Tbilisi State University, 28, Al. Kazbegi Ave., 1 block, H. Houra, Apart. 9, Tbilisi, Georgia, anna_rekhviashvili@hotmail.com
THE ROLE OF APC RESISTANCE AND PATENT FORAMEN OVALE IN PATIENTS WITH ISCHEMIC STROKE

M.E. Schleef 1, R. Feurer 2, S. Sadikovic 2, A. Bockelbrink 1, H. Poppert 1

1 Institut für Klinische Chemie und Pathobiologie, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany;
2 Neurologische Klinik und Poliklinik, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany;
3 Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie, Charité, Berlin, Germany

Keywords: stroke, APC, PFO

Background: As patent foramen ovale (PFO) is a frequent finding in cryptogenic stroke, paradoxical embolism has been discussed as pathogenic mechanism. APC resistance as a well known risk factor for venous thromboembolism may cause paradoxical emboli and contribute to the risk of ischemic stroke in patients with PFO.

Materials and methods: The records of 973 patients with definite diagnosis of cerebral ischemia at discharge were analyzed retrospectively. All patients had undergone PFO detection based on transcranial Doppler ultrasonography and were screened for APC resistance, factor V Leiden mutation was demonstrated by polymerase chain reaction. Stroke origin has been subtyped using the TOAST classification criteria. All patients are being contacted for follow up by e-mail or telephone.

Results: The prevalence of APC resistance was not higher in the group of patients with PFO than among those without (6.8% vs. 8.5%, p=0.4). APC resistance was not more frequent among patients with cryptogenic stroke than among those with non-cryptogenic stroke (7.2% vs. 8.3%, p=0.6). Among patients with cryptogenic stroke, APC resistance was not more prevalent in patients with PFO than in patients without (7.1% vs. 7.3%, p=0.9).

Follow-up is still ongoing; to date complete follow up was obtained in 540 patients (55%). Among the responding patients with PFO (n=179), recurrence of cerebral ischemia was observed in 1 patient (11.1%) positive for factor V Leiden and in 7 patients (4.5%) with no evidence of APC resistance (p=0.4).

Discussion: Our results question the theory of paradoxical embolism because the prevalence of APC resistance did not differ significantly between patients with cryptogenic stroke and those with non-cryptogenic stroke. Moreover no association between APC resistance and PFO could be proven among patients with cryptogenic stroke.

Conclusions: Our data suggest that APC resistance is not a strong risk factor for ischemic stroke among patients with PFO.

Figure: Prevalence of factor V Leiden mutation and patent foramen ovale among 973 patients with ischemic stroke.

Corresponding Author: Michael E. Schleef, Institut für Klinische Chemie und Pathobiologie, Klinikum rechts der Isar der Technischen Universität München, Ismaninger Str. 22, Munich, Germany, schleef@gmx.net

THE ROLE OF FVII PROMOTER POLYMORPHISMS IN TURKISH ATHEROSCLEROSIS PATIENTS

O. Cumaoğulları 1, A. Ozturk 1, R. Akar 2, N. Akar 1

1 Department of Pediatric Genetics, School of Medicine, Ankara University, Ankara, Turkey; 2 Department of Cardiovascular Surgery, Heart Center, Ankara University, Ankara, Turkey

Background/Aims: Several polymorphisms in the promoter region of the F7 gene associated with low or high plasma levels of FVII have been previously identified. Some studies have reported altered plasma FVII levels in groups with manifest or risk of coronary arterial disease (CAD), whereas others did not. Although -323ins10bp polymorphism is known to be associated with low FVII levels and has been suggested that a protective role against CAD and allele association with -401G/T polymorphism in Caucasians, -402 G/A polymorphism has been associated opposite effect on FVII plasma level. The studies showed that the effect of the three FVII polymorphisms are ethnicity-dependent. In this study we aimed to evaluate the effect of the three F7 gene polymorphisms on CAD in Turkish population and find out the related haplotypes.

Methods: Three polymorphisms of the F7 gene (-323ins10bp, -401G/T, -402