

ISTH 2007 Abstracts
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P-S-282 – A COMPARISON OF TWO COMMERCIAL ADAMTS-13 ACTIVITY ASSAYS WITH A REFERENCE LABORATORY METHOD

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Introduction: ADAMTS-13 is the enzyme responsible for cleaving the ultra large von Willebrand factor multimers. Deficiency of ADAMTS-13 is associated with thrombotic thrombocytopenic purpura (TTP), a serious disorder usually diagnosed with clinical criteria. Previous test methods used to measure ADAMTS-13 activity were time consuming and technically challenging. Recently, commercial kits have been developed which use fluorescence resonance energy transfer (FRET) methodology to measure ADAMTS-13 activity. In this study, we compared results from two recently released commercial kits with results obtained from a reference laboratory method using FRET technology to assay samples from patients referred for a possible diagnosis of TTP.

Methods: Method comparison testing was performed using two commercial assay kits: ADAMTS-13 Activity Assay (GTI, Waukesha, Wisconsin, USA) and the Technozym ADAMTS-13 ELISA kit (Technoclone, Vienna, Austria). Tests were read on a SpectraMax M5 microplate reader (Molecular Devices Corp., Sunnyvale, California, USA). Sodium citrate plasma specimens were tested on both commercial methods according to the assay protocol, and data was analyzed using Microsoft Excel spreadsheets obtained from each kit manufacturer. All three assays report results as a percentage of normal.

Results: A total of 29 samples were assayed and compared. Six samples had less than 5% of normal activity identified by the reference laboratory. The GTI kit identified four of these six samples, while the Technoclone kit identified all six. The correlation coefficient comparing the reference laboratory vs. GTI was 0.82. The correlation coefficient comparing the reference laboratory vs. Technoclone was 0.91.

Conclusions: In this comparison study, the Technoclone ADAMTS-13 activity kit identified 100% of the severe deficiency samples, while the GTI kit identified 67%. The availability of reliable ADAMTS-13 assays will improve the rapid and accurate diagnosis of TTP.

P-M-299 – SERIAL MONITORING OF ADAMTS13- AND VWF-RELATED PARAMETERS IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA: PREDICTION OF RESPONSE TO THERAPY, RISK OF RELAPSE, AND LONG-TERM COURSE

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Introduction: Severely reduced activity of ADAMTS13 due to inhibitory autoantibodies is a key feature of acquired thrombotic thrombocytopenic purpura(TTP), leading to the persistence of ultralarge(UL)VWF multimers, platelet aggregation and disturbance of microcirculation.

Methods: We followed 27 patients(3 male,24 female,mean age 40 years) with clinical signs of TTP for 1-16 years and observed a total of 39 episodes of TTP. ADAMTS13 activity was measured with a collagen-binding assay(Gerritsen) and the Technozym ADAMTS-13 assay, also used for determination of ADAMTS13:Ag. Anti-ADAMTS13 antibodies were measured with a modified Bethesda method with both the above mentioned assays and with the Technozym ADAMTS-13 INH ELISA. VWF:Ag, VWF:RCo, and multimeric analysis were performed by standard methods.

Results: 23 patients had autoimmune TTP, from these 24 episodes were available for analysis. During acute episodes of TTP, ADAMTS13 activity was severely reduced (<0.05 U/ml), as well as ADAMTS13:Ag. Anti-ADAMTS13 antibodies could be detected in all episodes.

Response to therapy (plasma exchange, steroids, ASS) could not reliably be predicted by ADAMTS13- or VWF-related parameters. For this purpose, platelet counts, LDH levels, and reticulocyte counts were better predictors. Anti-ADAMTS13 antibodies and inhibited ADAMTS13 activity persisted during remission (normal blood cell counts and normal LDH) for up to 2 years. During this time UL-VWF multimers were present. All relapses occurred during this period. After elimination of the antibodies and restoration of ADAMTS13 activity no further relapses were observed. 13 patients are in remission with normal ADAMST13 values, 4 are in remission but have low ADAMST13 levels, and 2 still have smoldering TTP.

Conclusions: Determination of ADAMTS13-related parameters is necessary to distinguish between autoimmune, hereditary, and secondary TTP and to choose an appropriate therapy. It may also be valuable for predicting the risk of relapse in patients with TTP in remission.

P-T-196 – ADAMTS13 LEVELS ARE ASSOCIATED WITH THE RISK OF ARTERIAL THROMBOSIS IN YOUNG SUBJECTS

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Introduction: A deficiency of the von Willebrand factor (VWF)- cleaving protease ADAMTS13 may play a role in arterial thrombosis because reduced ADAMTS13 activity will result in less degradation of VWF and thereby increased VWF activity. Our aim is to study the relationship of levels of ADAMTS13 and VWF with the occurrence of arterial thrombosis.

Methods: We performed a case control study in which the cases had a first-ever arterial thrombosis resulting in coronary heart disease (CHD), cerebrovascular disease (CVD) or peripheral arterial disease (PAD). The upper age limit was 45 years for males and 55 years for women. Blood was drawn one to three months after the ischemic event for the measurement of ADAMTS13 activity and antigen (Technozym ADAMTS13 ELISA) and VWF collagen binding (CB) activity and antigen. Controls did not have a cardiovascular history and were age and sex matched.

Results: A total of 374 patients of which 218 patients with CHD, 108 patients with CVD and 47 with PAD and 332 controls were included. ADAMTS13 activity and antigen were significantly lower in cases than in controls. Higher levels of VWF were seen in cases than in controls. These effects were strongest in the CHD and PAD groups. An inverse correlation was seen for VWF act and ADAMTS13 act (Rs-0.20). The relative risk of arterial thrombosis was highest in individuals in the upper quartiles of VWF:Ag (OR 2.0, 95%CI 1.3-3.2), VWF:CB (OR 2.0, 95%CI 1.3-3.1) and in the lowest quartiles of ADAMTS13 antigen (OR 3.3, 95%CI 1.8-6.1) and ADAMTS13 activity (OR 5.1, 95%CI 2.5-10.4), with adjustment for age and gender. The relative risk for the ratio VWF act/ADAMTS13 act was significantly higher in the highest quartiles compared with the lowest quartile (OR 3.4, 95%CI 1.8-6.5).

Conclusions: In conclusion, both low levels of ADAMTS13 (antigen and activity) and high levels of VWF (antigen and activity) are associated with risk of arterial thrombosis. This indicates a pathogenic role of ADAMTS13 in arterial thrombosis.

307 – ADAMTS-13 ACTIVITY, ANTIGEN AND INHIBITOR ANTIBODIES IN TTP PATIENTS – A SINGLE CENTER EXPERIENCE

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Introduction: Thrombotic thrombocytopenia purpura (TTP) is associated with inherited or acquired reduction in ADAMTS-13 activity. Evaluation of ADAMTS-13 activity and the potential causes for its reduction may be relevant for the diagnosis and treatment of TTP. The aim of this study was to examine ADAMTS-13 activity, antigen and corresponding inhibitor antibodies levels in TTP patients during acute episodes and following treatment with fresh frozen plasma or plasma pheresis (PP).

Methods: Levels of ADAMTS-13 activity, antigen and antibodies were evaluated in 18 TTP patients by the TECHNOZYM ADAMTS-13 ELISA kits.

Results: In 9 patients with stem cell transplantation and/or chemotherapy related TTP normal-border line levels of ADAMTS-13 activity, antigen and antibodies ($54\pm 8\%$, $60\pm 9\%$, 10 ± 4 U/ml, respectively) were found. In contrast, in 7 idiopathic TTP patients high levels of ADAMTS-13 antibodies (>70 U/ml) were detected. Levels of antibodies against ADAMTS-13 were in correlation with LDH levels and inverse correlation with ADAMTS-13 activity and antigen levels ($<30\%$ of the normal) and platelets count. Levels of ADAMTS-13 antibodies were reduced after 6-7 PP sessions to normal levels (<15 U/ml) leading to recovery of ADAMTS-13 activity ($>60\%$) and increase in platelets count.

Antibodies against ADAMTS-13 (>100 U/ml) were also associated with TTP in two unrelated females with familial TTP (ADAMTS-13 activity level $<5\%$) that were reduced to normal levels after PP sessions.

Conclusions: High levels of antibodies against ADAMTS-13 and low activity and antigen levels correlated with severity of episodes in idiopathic and familial TTP but not in cancer related TTP. Levels of ADAMTS-13 activity and antibodies might predict clinical outcome and might assist tailoring treatment, however, this should be further studied.

P-W-362 – ALTERED ULTRALARGE VON WILLEBRAND FACTOR LEVELS AND ADAMTS-13 ACTIVITY AFFECT RESIDUAL PLATELET REACTIVITY IN HIGH RISK VASCULAR PATIENTS ON DUAL ANTIPLATELET THERAPY

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Introduction: We have hypothesized the presence of ultralarge vWF eventually accompanied by a reduced activity of ADAMTS-13 as one of the mechanisms of residual platelet reactivity (RPR).

Methods: We investigated 159 patients with acute coronary syndromes on dual antiplatelet therapy. Platelet function was assessed by optical aggregometry on platelet-rich-plasma induced by arachidonic acid (AA-PA) and 10 micromol/L ADP (ADP-PA). RPR was defined in the presence of AA-PA >20% and/or ADP-PA >70%. vWF levels and collagen binding assay (CBA) were measured by a conventional ELISA system. ADAMTS-13 antigen levels and activity were measured by a fluorogenic assay (Technozym ADAMTS-13, Technoclone).

Results: 64/159 patients had RPR by AA-PA (40.2%), 36/159 had RPR by ADP-PA (22.6%) and 25/159 (15.7%) by both AA-PA and ADP-PA (dual RPR). Patients with RPR by AA-PA and by ADP-PA had significantly higher levels of both vWF antigen levels and CBA [vWF: 327%(108-780) vs 200 (74-854), p<0.0001; CBA: 271%(7.5-934) vs 195%(21-978), p<0.0001/vWF: 330%(145-645) vs 227 (74-854), p=0.01; CBA: 235%(12.5-828) vs 211%(7.5-978), p<0.05]. Also patients with dual RPR were found to have significantly higher levels of vWF antigen and CBA: vWF= 339%(159-645) vs 226 (74-854), p<0.0001; CBA= 295%(12.5-828) vs 212.5%(7.5-978), p<0.05. A significantly decreased ADAMTS-13 activity was demonstrated in patients with RPR induced by AA-PA (82.1% (24.7-129.8) vs 93.1%(10.3-172.7), p<0.005), by ADP-PA (78.4%(52.8-137.7) vs 90.7% (10.3-172.7), p<0.05) and in patients with dual RPR (76.3%(52.8-117.3) vs 90.6%(10.3-172.7), p<0.05). No significant differences were found in ADAMTS-13 antigen levels.

Conclusions: These results demonstrate that platelet function in response to antiplatelet therapy might be modulated by the presence of significantly higher levels of CBA. One of the possible mechanisms explaining the presence of these forms of vWF, in addition to the endothelial activation, is the presence of reduced levels of ADAMTS-13.