

The aim of our recent studies was to identify innate immune mechanisms associated with acute TTP, therefore, complement-, neutrophil-, and endothelial cell activation were investigated in the setting of acute TTP.

Multiple EDTA-plasma and serum samples of 38 TTP patients were investigated together with samples of 20 healthy controls. ADAMTS13 activity and anti-ADAMTS13 inhibitory antibodies were measured by the VWF-FRET73 assay. Complement parameters (C3, Factors H, I, B and total alternative pathway activity) together with complement activation fragments (C3a) or complexes (C1rs-INH, C3bBbP, sC5b9), PMN-Elastase-proteinase-inhibitor complex were measured by ELISA or RID. CT-pro-Endothelin-1 was measured with immunoassay.

Increased levels of C3a, SC5b9 and endothelin-1 were observed in TTP during acute episodes, as compared to healthy controls. Decreased complement C3 levels indicative for complement consumption occurred in 15% of acute TTP patients. The sustained presence of anti-ADAMTS13 inhibitory antibodies in complete remission was associated with increased complement activation. Furthermore, acute TTP was also associated with increased PMNE levels, increased PMNE levels and deficient ADAMTS13 activity together characterized hematologically active disease. PMNE concentration inversely correlated to disease activity markers platelet count ($r = -0.349$, $p = 0.032$) and hemoglobin levels ($r = -0.382$, $p = 0.018$). There was positive correlation between PMNE or endothelin-1 levels and complement activation markers.

Activation of two important arms of innate immunity, the complement and neutrophils, was shown in acute TTP, and there was positive correlation with endothelial activation. These results support the 'multiple hit' model of the pathogenesis of TTP, where activation of innate immunity and endothelial cells may contribute to the acute precipitation of TTP episodes in ADAMTS13 deficient patients.

Funding: OTKA K100687

SE5.3

Vascular calcification and chronic kidney disease

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Vascular calcification, especially in patients diagnosed with chronic kidney disease, is the major pathophysiologic process in cardiovascular diseases. Calcification develops in two main distinct sites within arteries. While in atherosclerosis the intima is affected focally at predisposed areas of vessels, in media sclerosis the media layers of the large- and medium-sized arterial wall are mineralized in a diffuse fashion. In calciphylaxis the small cutaneous arterioles are affected and the mineralization of vessels almost exclusively occurs in patients with advanced chronic kidney disease. Abnormalities in mineral metabolism are important regulators of vascular calcification. It is an actively regulated multistep process in which the trans-differentiation of smooth muscle cells (SMC) into osteoblast-like cells is induced by change in inorganic phosphate (Pi) level. After osteoblastic differentiation these cells lack characteristics of SMC, and develop osteoblast features. At physiological environment, cells express smooth muscle lineage markers, SM 22 α and SM α -actin. After exposure to elevated phosphate, high glucose level, activated vitamin D, reactive oxygen, a dramatic loss of markers for SMC lineage occurs and simultaneously a gain of osteochondrogenic markers such as alkaline phosphatase, osteocalcin and core-binding factor-1 (cbfa-1) develops. Phosphate uptake through a sodium-dependent phosphate co-transporter, Pit-1, is implicated in vascular SMC calcification and phenotypic modulation. While types I and II transporters are restricted to the kidney and intestine, type III transporters are ubiquitously present in tissues including kidney, heart, lung, and bone. Pit-1 and Pit-2 represent the type III transporter. Of these known transporters, Pit-1 was found to be expressed in human SMC as well as in human aorta that facilitates entry of Pi into vascular cells. Vascular calcification being a delicately regulated cellular process where SMC gain an osteoblastic phenotype is also indicated by the fact that increased expression of cbfa-1 is observed in cells exposed to high phosphate or platelet-derived growth factor. Cbfa-1 is an essential regulator of osteoblast differentiation and fulfills a dominant function for other gene products. Osteocalcin is the major non-collagenous protein in bone matrix. The calcium binding properties of osteocalcin and its pattern of expression in bone suggests an important function in bone mineralization. Osteocalcin, one of the gamma-carboxy-glutamic acid containing proteins is present in calcified atherosclerotic lesions and mineralized heart valves at high concentration. Other important factors acting directly (fetuin, BMP, MGP), or indirectly (FGF23, klotho, leptin, cytokines, LDLox) on mineralization will be reviewed as well.

SE5.4

Diagnostic considerations based on the experience of genetic analysis in Protein C deficiency and molecular characterization of different mutations

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Protein C (PC) serves as a major anticoagulant and numerous distinct mutations in its coding gene result in type I (quantitative) or type II (qualitative) PC deficiency with high thrombosis risk. Screening for PC deficiency is executed by clotting or chromogenic functional tests. Both methods have several disadvantages. FV Leiden mutation (FVL) interferes with the clotting assay leading to low PC activity and FVL positive patients seem to have type II PC deficiency. Chromogenic tests do not suffer from this problem, however they may not detect some type II cases caused by certain mutations.

We evaluated the functional clotting and chromogenic PC assays from the point of view of FVL interfering effect and determined the mutation spectrum of PC deficiency in the Hungarian population. Moreover, the molecular consequences of certain novel or uncharacterized mutations were also investigated.

Non-related individuals having 70% or lower PC activity measured by the clotting test were recruited (n=109). PC activity by the chromogenic method and PC antigen were also determined. The gene encoding PC (PROC) was analyzed by direct DNA sequencing and by MLPA method in those cases if larger genetic abnormality was assumed. The fate of mutant proteins (p.Asp77Gly, p.Ala163Glu and p.Ala163Val) expressed in HEK cells was monitored by ELISA and Western blotting. Their intracellular localization was examined by immunostaining and confocal laser scanning microscopy. Structural consequences of the mutations were investigated by molecular modeling and dynamics simulations.

Most of the patients with low PC clotting activity were carriers of the FVL (n=72) and only 12.5% of them were positive for PROC mutations. As opposite, 78.4% of FVL negative patients had causative mutations in the PROC gene. Altogether 28 different mutations were detected including 15 novel ones (two of them were identified by MLPA). The 163Val and 163Glu mutant PC had undetectable levels in the culture media of HEK cells and showed intracellular co-localization with the 26S proteasome. The secretion of Gly77 mutant into the media was slightly decreased. The 163Val and 163Glu mutations caused significant changes in the relative positions of the EGF2 domains suggesting misfolding, while no major structural alteration was observed in case of the Gly77 mutant.

Funding: OTKA PD101120

SE5.5

Progressive chromogenic anti-factor Xa assay and its use in the classification of antithrombin deficiencies

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Antithrombin (AT) is a slow-acting progressive inhibitor of activated clotting factors. It is particularly effective inhibitor of thrombin and activated factor X (FXa). The presence of heparin or heparan sulfate accelerates its effect by several magnitudes. AT deficiency is a severe thrombophilia, which is classified as type I (quantitative) and type II (qualitative) deficiency. Mutations causing type II deficiencies may influence the reactive site, the heparin binding-site (HBS) and exert pleiotropic effect. Heterozygous type II-HBS deficiency is a less severe thrombophilia than other heterozygous subtypes. However, as opposed to other subtypes, it also exists in homozygous form representing a very high risk of venous thromboembolism. There are no commercially available tests for the differential diagnosis of type II-HBS deficiency.

We developed a modified anti-FXa chromogenic AT assay for this purpose. The assay determines both the progressive (p) and the heparin cofactor (hc) AT activities. The assays showed excellent reproducibility and were not influenced by high concentrations of triglyceride, bilirubin and hemoglobin. Reference intervals for p-anti-FXa and hc-anti-FXa AT activities were 84-117% and 81-117%, respectively. The usefulness of the assay in detecting type II-HBS AT deficiency was tested on 78 AT deficient patients including 51 type II-HBS heterozygotes and 18 type II-HBS homozygotes. Heterozygous type II-HBS AT deficient patients demonstrated low hc-anti-FXa activity with normal p-anti-FXa activity. Homozygotes had very low hc-anti-FXa activity and only slightly decreased p-anti-FXa activity. The hc/p ratio clearly distinguished wild type controls, type II-HBS heterozygotes and homozygotes.

Parallel determination of p-anti-FXa and hc-anti-FXa activities provides a reliable tool for the clinically important diagnosis of type II-HBS AT deficiency and distinguishes between homozygotes and heterozygotes.

SE5.6

Changes of coagulation parameters in patients with end-stage renal disease

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There is a significant risk of both thrombotic and hemorrhagic disorders in patients with end-stage renal disease (ESRD). Changes in the hemostatic system might contribute to the pathogenesis of these disorders. Our aim was to test the level of hemostatic factors, which might associate with the risk of thrombosis in patients with ESRD.

In the study 30 patients being on hemodiafiltration (HDF) treatment for at least 3 months were included. Treatment of the same patients was switched to conventional hemodialysis (HD) for 2 weeks and hemostasis parameters were also investigated after this period. Blood samples were taken just before and 1 and 4 hours after the initiation of HDF or HD treatment. Factor VIII (FVIII) activity, antithrombin (AT) activity, factor XIII (FXIII) activity and FXIII antigen levels were determined. The levels of the coagulation parameters were adjusted for serum albumin concentration.