Methods: Circulating MPs were isolated from plasma of thrombotic thrombocytopenic purpura or cardiovascular disease patients and from healthy subjects. MPs were also obtained from purified human blood cell subpopulations. Identification of plasminogen activators on MPs was performed by zymography and their capacity to generate plasmin by chromogenic assay.

Results: Circulating MPs isolated from patients generated a range of plasmin activity at their surface. This property was related to a variable content in uPA and/or tPA. Using distinct MP subpopulations derived from endothelial cells, platelets, leukocytes and erythrocytes, we demonstrated that plasmin is generated on endothelial- and leukocyte-derived MPs, but is absent on MPs from platelet or erythrocyte origins. Leukocyte-derived MPs bear uPA and its receptor uPAR whereas endothelial-derived MPs carry tPA and tPA/ inhibitor complexes.

Comment: Endothelial- and leukocyte-derived MPs, bearing respectively uPA or tPA, support at least a part of fibrinolytic activity in the circulation that is modulated in pathological settings. This blood-borne fibrinolytic activity conveyed by MPs provides a more comprehensive view on the role of MPs in the haemostatic equilibrium and put forward the basis for a potential new biomarker.

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CO128 Decrease of microparticles and hemostasis improvement after the treatment in hemophilia A. coincidence or causation?

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Background: Microparticles (MPs) are small membrane vesicles (0.1 – 1 μm) released from various cell types (platelets, endothelial cells, leucocytes, erythrocytes) after activation and/or apoptosis. Different types of MPs are increased in atherosclerotic diseases but there are limited data about their role in hemophilia A.

Methods: Samples were taken before and 30 minutes after FVIII injection in 18 patients with severe hemophilia A treated on-demand with 1000–2000 IU highly purified plasma-derived FVIII concentrate. MPs were assayed using a Beckman Coulter Gallios flow cytometer. The MP population was gated and visualised on a dot plot using labeled antibodies directed to phalloidin as a quality control, lactadherin for determination of total MP (TMP), CD42a (platelet MP – PMP), CD 144 (endothelial MP – EMP) and CD 45 for leucocyte MP – LMP. The results were compared with endogenous thrombin potential (ETP), overall hemostatic potential (OHP), fibrin gel permeability (presented as KS) and thrombin activable fibrinolysis inhibitor (TAFI) (those results were part of a larger study).

Results: Total amount of MPs was relatively low and majority of MPs belonged to the subgroup of PMP. TMP and PMP decreased after the treatment 1015 ± 221 and 602 ± 134 in comparison to the values before the treatment 2373 ± 618 and 1316 ± 331 (p<0.01). EMP also decreased after the treatment (78 ± 12 vs. 107 ± 13, p<0.05). Very low level of LMP (21 ± 1) was not influenced by the treatment (20 ± 1). Both TMP and PMP inversely correlated moderately but statistically significant with OHP, ETP and TAFI/TAFIIa (r = −0.30, −0.32, −0.32 for TMP and r = −0.28, −0.32, −0.30 for PMP; p<0.05 for all). TMP and PMP correlated also with KS (0.34 and 0.32, p<0.05). EMP correlated only with ETP (r = −0.29, p<0.05), while LMP did not express any correlation. TMP and PMP also correlated with the FVIII level (r = −0.29, p<0.05).

Comment: TMP, PMP and EMP decreased after on-demand treatment with FVIII concentrate in hemophilia A patients. Decrease of circulated MP which inversely correlated with hemostatic activation and fibrin gel tightness (and also TAFI activation which is well known to be placed within the thrombus) may potentially implicate that those MP may be incorporated in the hemostatic plug formed after FVIII substitution on the site of injury. Nevertheless to confirm that this is not just pure coincidental finding, but there is causation behind it, additional mechanistical experiments have to be done.

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CO431 Microparticle TF/TFPI Hemostatic Balance in Physiological and Pathological States

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Background: Microparticles (MPs) are cell surface membrane vesicles, which are mainly formed by cell apoptosis or cell activation and found in blood under normal physiological conditions; their levels increase in a variety of diseases. MPs bear coagulation proteins and promote thrombus formation. Normal coagulation is based on hemostasis between pro- and anti-coagulant mechanisms. A major portion of circulating tissue factor (TF) is found on MPs. Tissue factor pathway inhibitor (TFPI) controls TF activity. We assume that MP TF/TFPI ratio can predict hypercoagulability states and therefore, need to be studied in high risk settings.

Methods: Blood samples were collected from healthy subjects, pregnant women and pregnant women with gestational vascular complications (GVC). Additionally, samples were drawn from patients with cardiovascular complications (CAD), diabetic CAD (DCAD), diabetic foot, diabetic retinopathy and from cancer patients at stage III or IV of the disease, including cancer of the lung, liver, bone, brain and pelvis. MP-TF and MP-TFPI levels in poor platelet plasma were measured by flow cytometry.

Results: We found that the MP hemostatic ratio was < 1 in non-pregnant women (n = 65), or healthy controls above age 45 years, increased in healthy pregnant (~1.86; n = 89, p<0.0001) and was highest in the GVC group (2.51; n = 59, p = 0.02).

The MP TF/TFPI ratio was < 1 in the healthy group (n = 43), similar among the diabetic retinopathy group (n = 25), but significantly increased in CAD (~2; n = 37), DCAD (~1.5; n = 39) and diabetic foot (n = 19, p<0.0002) cohorts.

MP TF/TFPI ratio was significantly elevated in cancer patients (n = 30, p = 0.0001) compared to healthy controls.

Comment: The MP hemostatic ratio can predict hypercoagulable state and may be used for monitoring anticoagulant therapy.

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Thrombophilia

CO0075 Type II antithrombin deficiency caused by a large in-frame insertion. Structural, functional and pathological relevance

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