



THE ROLE OF TISSUE FACTOR IN ACTIVATION COAGULATION SYSTEM AT PREGNANCY COMPLICATIONS

Martin Prochazka^a, Jana Procházková^b, Marek Lubušský^a, Eliška Hostinská^a, Petr Polák^c, Luděk Slavík^b, Jana Úlehlová^b, Martin Novák^b, and Radovan Pilka^a

^a Department of Obstetrics and Gynecology, Palacký University Faculty of Medicine and Dentistry and University Hospital Olomouc, Czech Republic

^b Department of Hemato-Oncology, Palacký University Faculty of Medicine and Dentistry and University Hospital Olomouc, Czech Republic

^c Department of Obstetrics and Gynecology, Palacký University Faculty of Medicine and Dentistry and University Hospital Olomouc and USGPOL s.r.o. - Antenatal clinic, Czech Republic

Aims

As part of our project to determine the causes of preeclampsia and other obstetric complications, we focused on the role of tissue factor in the activation of these pathophysiological processes. We attempted, on based recent findings, to design appropriate markers for the determination of contribution of individual processes on clinical expression of these conditions.

TF is an integral membrane protein constitutively expressed in many types of cells outside the blood vessels, but it is not normally expressed on cells which are in contact with flowing blood. It is a specific and high-affinity receptor for factor VII / VIIa and acts as a cofactor for Factor VIIa. Exposure of activated TF in coagulation system triggers the physiological blood coagulation and thrombosis in a number of thrombotic diseases. The blood cells especially monocytes don't have the ability to constitutively express functional TF, but are capable of synthesis and expression of TF in the stimulation of either lipopolysaccharide (LPS) or inflammatory cytokins. TF expression on monocytes is involved in the mechanism of thrombosis in a number of conditions including sepsis, atherosclerosis, an oral contraceptives, cancer and hyperhomocysteinemia.

Recent findings attribute a significant proportion of the activation coagulation system to autoantibodies. When the mechanism is activated, the antibodies induced expression of tissue factor (TF CD142) on monocytes and vascular endothelial cells. There is also increasing evidence that certain types of antiphospholipid antibodies, especially those directed against beta-2-glycoprotein I (beta-2GPI), stimulate tissue factor expression on monocytes.

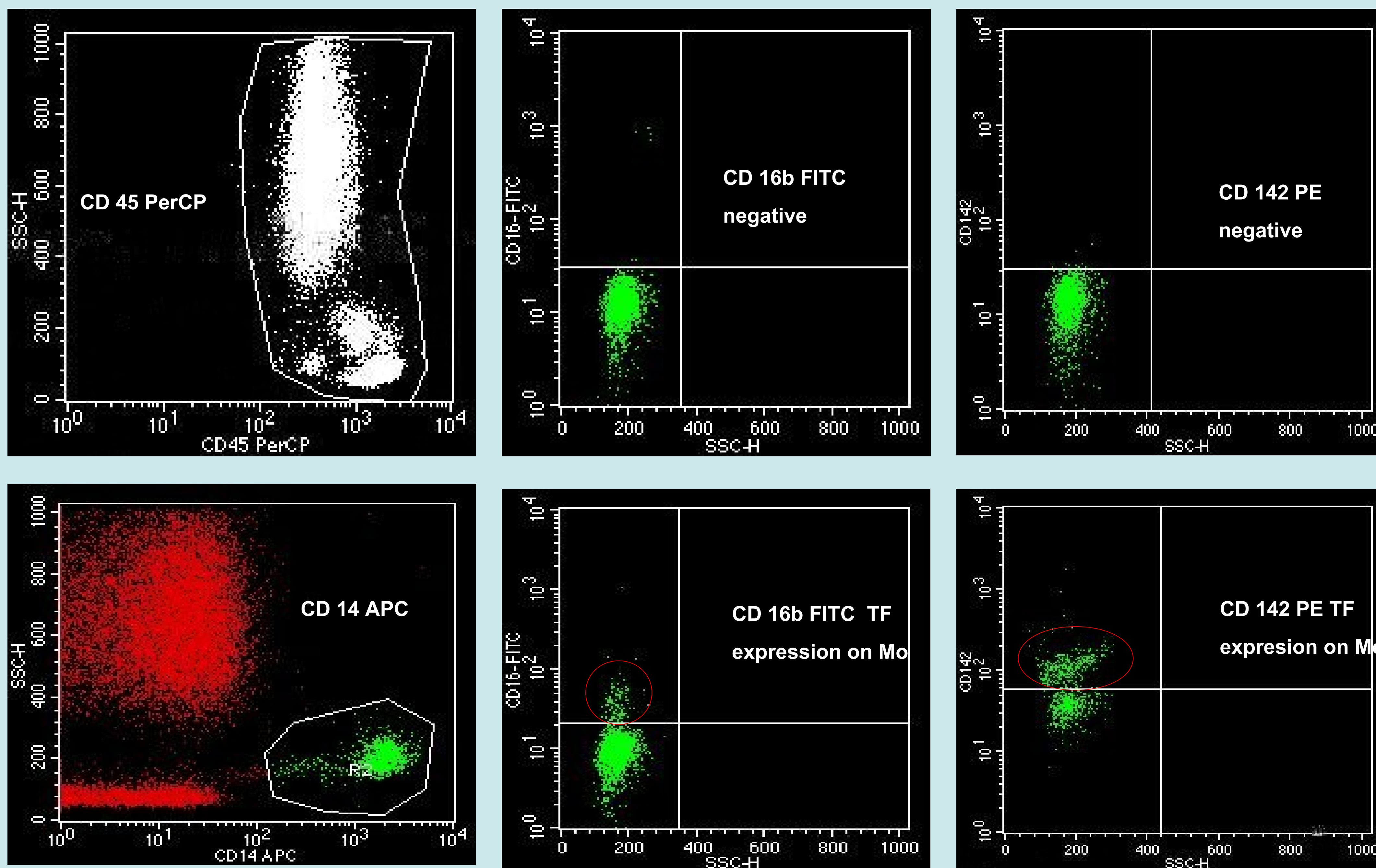
Methods

For this reason, we have proposed a model monitor activation of the coagulation system in preeclampsia and other pregnancy complications with TF expression on monocytes by flow cytometry, and simultaneously fixing the TF-induced thrombin generation in plasma. To determine expression of tissue factor (CD142) on monocytes, we used the method of multicolor flow cytometry using anti CD45 PerCP, clone MEM-28 (EXBIO Praha), anti CD14 APC clone MEM-15 (EXBIO Prague), CD16b FITC clone MEM-154 (Exbio Prague), anti CD142 PE (BD Pharmingen) and the appropriate isotope control.

Analyses were performed on samples of peripheral blood stabilized with K3EDTA. The material that can't be processed within two hours of sampling was also fixed by Transfix (Cytomark, Caltag Medsystems Ltd.).

The model has been verified in patients with severe antiphospholipid syndrome, in which the high expression of antibodies, especially anti-beta-2GPI. We have demonstrated enhanced expression of TF expression on monocytes and a significant increasing of thrombin generation in plasma.

Results



TF expression on Mo	Pregnant women	Preeclampsia	%	TGT	TGT preeclampsia
0 - 15 %	27	2	7,41	2460	1733
15 - 35 %	7	1	14,29	2277	N/A
35 - 55 %	11	3	27,27	2452	2081
55 - 75 %	8	3	6,67	2151	1759
75 - 100 %	8	3	6,67	2628	1759

We present preliminary data from 86 pregnancies. Pregnant women were prospectively followed and blood test were done in each trimester / 12th, 24th, and 36 th weeks of gestation/. There were no internal, neurological and other serious diseases at the time of the first sampling (at the end of the first trimester) in the medical history of all patients.

Conclusion

- ✓ The model has been validated on patients with antiphospholipid syndrom, which is a high expression of antibodies, and particularly against beta-2GPI.
- ✓ We demonstrated enhanced expression of TF expression on monocytes and a significant increasing of thrombin generation in plasma.
- ✓ In light of these findings, the detection of CD142 expression by flow cytometry to be an effective method of monitoring the activation of the coagulation system and methodology developed appears to be very promising for further uncovering the mechanisms of preeclampsia and conditions associated with it.