

Microangiopatie trombotiche

EVALUATION OF PLATELET ACTIVATION MARKERS IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA

M. Di Fuorti[1], M.R. Cozzi[2], A. Steffan[3], I. Quaglia[1], C. Sacco[1], C. Lodigiani[1], M. Bacci[1]

[1]Center for Thrombosis and Hemorrhagic Diseases, IRCCS Humanitas Research Hospital, Rozzano, Italy; [2]Immunopathology and Cancer Biomarkers Unit, Centro di Riferimento Oncologico di Aviano IRCCS, Aviano, Italy; [3]Clinical Pathology Unit, ASFO General Hospital, Pordenone, Italy

Background and Aims. Thrombotic Thrombocytopenic Purpura (TTP) is a rare disease characterized by deficiency of ADAMTS13, a metalloprotease involved in the cleavage of high-molecular-weight (HMW) multimers of von Willebrand factor (VWF). This process regulates the interaction between VWF and platelet (PLT) membrane receptors, thereby supporting PLT adhesion to the sub endothelium and aggregation. In the onset and progression of TTP, the interaction between VWF and PLT plays a pivotal role. It is known that PLT glycoproteins (Gp), including GpIbα, GpIIb/IIIa, and GpVI, as well as the contents of alpha and dense granules and the ability of PLT to degranulate, contribute to PLT plug formation. In some conditions, these mechanisms may be overexpressed, leading to the development of thrombotic events. In this study, we aimed to investigate the contribution of PLT to the onset of TTP.

Methods. Six patients with acquired TTP associated with the presence of anti-ADAMTS13 antibodies (Abs) were enrolled at the IRCCS CRO of Aviano. Two patients were evaluated during the disease acute phase, while the others during routine follow-up. The diagnostic work-up included assessment of ADAMTS13 activity, anti-ADAMTS13 Abs, VWF levels, and VWF multimer analysis. PLT activation markers were studied using an indirect method at the IRCCS Humanitas Research Hospital. Briefly, two whole-blood samples from healthy donors (HDs) were treated with 1 µM prostaglandin E1 and centrifuged twice at low speed to obtain a PLT pellet. One PLT sample was resuspended in plasma derived from TTP patients (PLT-TTP), while the other in plasma from HDs (PLT-HD), used as an autologous control. We investigated the expression of PLT GpIbα, GpIIb/IIIa, GpVI, P-selectin, PAC-1, mepacrine, and CD63 at steady state and after activation with the agonists ADP 10µM, collagen 5µg/mL, and TRAP 50µM. We also assessed VWF binding to PLT (VWF-PLT) after stimulation with ristocetin, by flow cytometry. Results were analyzed using descriptive statistics, reporting mean values, SEM, and p-values ($p<0.05$).

Results. Patients with TTP showed low levels of ADAMTS13 activity during the acute phase, with an increase during follow-up. Anti-ADAMTS13 Abs were variable, ranging from 2 to 30 IU. Two patients showed increased HMW multimers, which were associated with ADAMTS13 activity below 50%. PLT-TTP appeared more activated than PLT-HD, showing decreased levels of GpIbα, GpIIb/IIIa, GpVI, and mepacrine (42%vs72%, $p=0.02$) at steady state. In contrast, P-selectin expression was increased in PLT-TTP compared with PLT-HD (30% vs 16%, $p=0.03$). Upon stimulation with agonists, these markers were not further upregulated. VWF-PLT binding was not constitutive but was induced by agonist stimulation. It was higher in PLT-TTP than in PLT-HD, reaching 32.6% vs 15.5% upon stimulation with ristocetin.

Conclusions. Our results suggest that PLT from HDs, when exposed to plasma from TTP patients, undergo downregulation of surface Gps, possibly reflecting the shedding of these receptors, together with increased expression of P-selectin, PAC-1, and CD63. Moreover, the reduced mepacrine signal at steady state suggests that PLT are activated and externalize dense granule contents. Overall, these findings indicate that plasma from TTP patients may enhance the activation state of resting platelets. Further studies are needed to confirm these preliminary results during follow-up and in additional patients with TTP who will be enrolled.