Abstract Submission

3. Platelets

ECTH-144

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Please indicate your presentation preference: Oral Presentation

Please indicate your type of research: Basic Laboratory Research

Please indicate if you are under 35 years of age at the time of ECTH 2019: Yes

Background: Immune thrombocytopenia (ITP) is an autoimmune condition characterized by an isolated low platelet count in the absence of other underlying causes. One of the ITP causes is phagocyte-mediated accelerated clearance of platelets coated by antibodies to platelet receptors in the reticuloendothelial system. Being a relatively rare disorder, ITP is poorly diagnosed. Furthermore, distinguishing between different ITP causes is also challenging and thus appropriate therapy selection is complicated. Developing a comprehensive diagnosis approach would help to facilitate clinical decision and promote ITP nature understanding.

Aims: To determine signaling and functional defects of the platelets from patients with immune thrombocytopenia alongside inherited platelet disorders.

Methods: 12 patients with ITP, 1 patient with Glanzmann thrombasthenia(NGS confirmed), 1 patient with GPVIdeficiency (NGS confirmed) and 1 patient on clopidogrel therapy were included in the research. Blood was collected in hirudin-tubes (Sarstedt monovette©) in accordance with the Declaration of Helsinki. Whole patient blood was loaded by 2 mM of calcium sensitive dye Fura-Red in the presence of apyrase. PRP was collected and diluted in a Tyrode's buffer (pH7.3, $1x10^6$platelets/ml). Labelled fibrinogen and Annexin-V were added prior or during the experiment. Flow cytometry was performed using BD FACS Canto II flow cytometer (BD Biosciences). Alternatively, functional platelet analysis (Ignatova et all., Platelets 2018) was performed. Statistical analysis of the data was performed using Python 3.6.

Results: We evaluated PAR1, P2Y1, P2Y12, GPVI, CLEC-2 receptors and their synergy. Platelet functional response was assessed using cytosolic calcium mobilization, integrin activation state (fibrinogen binding) and platelet procoagulant activity (Annexin-V binding). Continuous flow cytometry analysis of the patients with the known diagnosis was in a moderate agreement with platelet functional analysis and aggregometry. Analysis of the ITP patients allowed to identify 4 groups: weak GPVI induced activation (GPVI group); normal calcium response to ADP stimulation, while defected integrin activation (P2Y12 signaling defects group); defected calcium response to TRAP6 stimulation (PAR1 signaling malfunctioning group); weak platelet integrin activation (GPIIb/IIIa activation group). Comparison of patients from these groups to patients with NGS-confirmed inherited disorders revealed similar behavior of the GPVI deficiency patient with patients from GPVI group and Glanzmann thrombasthenia patients with GPIIb/IIIa group. These findings allowed us to predict that these patients might be suffering from the antibodies to GPVI or GPIIb/IIIa, respectively. Results of the analysis of the patients from P2Y12 group were corresponding to patients on clopidogrel therapy. However, no direct analogy was found for patients from PAR1 group and additional study is required for these cases.

Summary/Conclusion: Here we have developed a comprehensive flow cytometry approach, suitable for the characterization of platelets from patients with inherited and acquired platelet function disorders. This method corresponded with conventional diagnosis methods as platelet functional analysis and aggregometry. This approach was capable to distinguish patients with known diseases and, specifically, differentiate ITP-patients into different groups. Thus, our method could be used for biomedical research needs.