The Official Journal of the Italian Society for the Study of Hemostasis and Thrombosis
SISET
Società Italiana per lo Studio dell'Emostasi e della Trombosi
Corso Nazionale SISET
Bergamo | 23-25 ottobre 2025

29TH NATIONAL CONFERENCE OF THE ITALIAN SOCIETY FOR THE STUDY OF HEMOSTASIS AND THROMBOSIS, 2025

ABSTRACT N. CO44

ALTERAZIONI DELLE PIASTRINE E CONDIZIONI GENETICHE

FIRST INTERLABORATORY VALIDATION WORKSHOP OF IMMUNOFLUORESCENCE MICROSCOPY ON THE PERIPHERAL BLOOD SMEAR FOR RECOGNIZING PATIENTS WITH INHERITED PLATELET DISORDERS.

C. Zaninetti[1], L. Bury[2], V. Bozzi[3], A. Zamora-Cánovas[4], K. Althaus[5], G. Uzun[5], S. Brown[6], A. Norman[6], D. Faille[7], A. Stepanian[8], C. Freyer[1], L. Kager [9], T. Bakchoul[5], A. Pecci[3], P. Gresele[2], T. Thiele[1], J. Rivera[4], A. Greinacher[1].

1Institut für Immunologie und Transfusionsmedizin, Universitätsmedizin Greifswald; [2]Department of Medicine, Section of Internal and Cardiovascular Medicine, University of Perugia, Perugia; [3]Department of Internal Medicine, University of Pavia, Pavia; General Medicine 1, IRCCS Policlinico San Matteo Foundation, Pavia; [4]Servicio de Hematología, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonacio´n, Universidad de Murcia, Spain; [5]Institute for Clinical and Experimental Transfusion Medicine, University Hospital of Tuebingen, Tuebingen, Germany; Center for Clinical Transfusion Medicine, Tuebingen, Germany; [6]Centre for Children's Health and Research CCHR, South Brisbane, Australia; [7]Laboratoire d'Hématologie, Assistance Publique Hôpitaux de Paris, Hôpital Bichat-Claude-Bernard, Paris, France; [8]AP-HP, Service d'Hématologie Biologique, Hôpital Lariboisière, Paris Cité University, Paris, France; [9]St. Anna Kinderspital, Universitätsklinik für Kinder- und Jugendheilkunde der Medizinischen Universität Wien, Vienna, Austria;.

Background and aims:
Recognizing patients with inherited platelet disorders (IPD) still represents a challenge, and a multidisciplinary approach is routinely required. We have established a method for phenotyping platelets on the peripheral blood smear using immunofluorescence- and light microscopy, which we have proposed as a screening-tool for IPD. The method has been validated by blinded comparison with molecular testing for 9 different disorders, for which typical changes of platelet structure can be identified. However, an interlaboratory validation of this approach has not been performed so far. To test the reproducibility of the technique and to implement its use in different Countries, we promoted a first, interlaboratory validation workshop of immunofluorescence microscopy on the blood smear for recognizing IPD.
Methods:
Unstained and unfixed, native air-dried blood smears were prepared and shipped by regular mail to each participating Center, where they were fixed and stored at -20°C upon delivery. Subsequently, the slides were stained using a standard panel of 13 mouse or rabbit anti-human primary antibodies against different platelet structures (alpha- and dense granule, surface glycoproteins (GP) Ib/IX and IIb/IIIa, cytoskeleton, and the stem-cell antigen CD34) and 2 fluorescence-labelled goat anti-mouse or -rabbit secondary antibodies. Each participating Laboratory performed the investigation independently, reported results according to a defined evaluation-grid, and eventually formulated a possible diagnosis documenting the suspicion with pictures.
Results:
Six European and one Australian Institution performing diagnosis of bleeding disorders participated in the project. By investigating blood films obtained from healthy controls, the reproducibility of preanalytical conditions, as well as of the staining- and observation method was initially ensured. Subsequently, blood smears from healthy controls or patients with IPD confirmed at molecular level were blindly shipped to each Center, which independently performed the investigation. Seven out of seven Laboratories correctly predicted the diagnosis of MYH9-related thrombocytopenia, Glanzmann thrombasthenia, and GFI1B-related thrombocytopenia; 6/7 of Bernard-Soulier syndrome, delta-storage pool disorder, and TUBB1-related thrombocytopenia; 5/7 of GATA1-related thrombocytopenia. Moreover, most of the participating groups correctly recognized the healthy controls, which only in five cases were wrongly reported as possible pathological samples.
Conclusions:
The validation workshop confirmed the technical reproducibility of immunofluorescence microscopy on the blood smear - even when performed by some Laboratories for the first time. Even in this multicenter setting, the technique provided reproducible results. Additional training is expected to further increase the sensitivity and specificity of this approach.

Email: *carlo.zaninetti@uni-greifswald.de*