that microfilaments within the inclusion bodies were the subcellular structures recognized by the antibody. *Conclusions*. The expression of an abnormal NM-MHCIIA due to MYH9 mutation causes an alteration of its distribution in the cytoplasm of granulocytes and platelets. The formation of *Döhle-like* inclusions is related to the presence of zones of NM-MHCIIA accumulation in the cytoplasm. We previously pointed out that all identified MYH9 mutations are expected to have a role in the correct assembly or stability of the quaternary myosin complex: this could explain the altered NM-MHCIIA localization in MHA and FTNS patients and its possible aggregation into abnormal paracrystalline arrays. Until now the diagnosis of MHA and FTNS has been based on the morphological recognition of Dohle-like inclusions, which often proves difficult. Our results show that immunocytochemical analysis is a sensitive, specific and time-saving tool for the diagnosis of MYH9-related disorders.

CO086
FLOW CYTOMETRY INVESTIGATION OF RETICULATED PLATELET PERCENTAGE AND PLATELET ASSOCIATED IMMUNOGLOBULINS IN THROMBOCYTOPENIC PATIENTS: DIAGNOSTIC AND CLINICAL CORRELATIONS

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In a prospective study we have compared the usefulness of flow cytometry investigation of platelet associated immunoglobulins (PAIg) and reticulated platelet percentage (RP%) in the routine work-up of 145 thrombocytopenic patients. Reticulated platelets, which represent newly synthesized platelets, were assessed by flow cytometry using fluorochrome thiazole-orange. Seventy patients were classified as having an immunologic thrombocytopenia (IMT) while 31 and 44 patients had a diagnosis of infective (INT) and malignant (MAT) thrombocytopenia respectively. IMT was characterized by a lower PLT count (p=0.0386) and a higher RP% (p=0.0007). Platelet count significantly correlated with RP% among patients with less than 50 $\times 10^9$/L platelets (PLT<50; p=0.005) and in the group of patients with PLT<50 and diagnosis of IMT (p=0.001). PAIg determination, in our hands, was a specific (88%) but not sensitive test (sensitivity 25.7%) with an overall test efficiency of 57.9%. The efficiency of PAIg determination in the PLT<50 group was 51.9%. RP% (cut off value of 4%) was a more efficient test (efficiency 69.6 %, p<0.0001) with a sensitivity of 51.4% and a specificity of 86.6%. At bone marrow examination (n=81), megakaryocytic hyperplasia and dysplastic features were associated with IMT (p=0.00027) and MAT respectively (p=0.00097). Response (complete and transient) to corticosteroid first line treatment in the PLT < 50 group (n=35) was associated with higher RP% (p=0.003) and megakaryocytic hyperplasia (0.002) but not with PAIg positivity (p=0.083). Overall our analysis showed that PAIg investigation is an unnecessary and perhaps inappropriate test to be performed in the diagnostic investigation of thrombocytopenic patients. By contrast, RP%, which is a useful even though not perfect indicator of marrow megakaryocytopoiesis, is a more efficient test which could be used not only for the diagnostic evaluation of thrombocytopenic disorders but also to predict the response (complete or transient) to first line treatment.

CO087
MOLECULAR GENOTYPING IN A COHORT OF SEVERE HEMOPHILIA PATIENTS WITH INHIBITORS

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The development of inhibitors against transfused factor VIII still remains the main complication of replacement therapy in patients with hemophilia A. This problem has been correlated in the past with the type of replacement therapy, the amount of therapy as well as to MHC status. To date none of these factors seems to fully explain the inhibitor formation. More recently patients carrying diverse FVIII gene defects have shown different proportions of inhibitor development. Large gene deletions, factor FVIII gene inversion and nonsense mutations display an incidence of antibodies formation of approximately 35%, compared with only 5-7% of patients with small gene deletions or missense mutations. The objective of this study was the identification of the molecular defects in a cohort of 21 severe A hemophiliacs with a history of inhibitor. FVIII gene inversion detection by multiplex long range PCR according to Liu (1998) revealed the presence of this common mutation in 4 (19%) patients. The other patients were analyzed by conformation sensitive gel electrophoresis (CSGE), a heteroduplex based method for nucleotide mismatch detection requiring amplification of the gene coding and regulative sequences (26 exons and 5' and 3' flanking regions) as separate fragments. In 2 patients we were unable to obtain any PCR product for a portion of the FVIIII gene (exons 2 to 25 and exons 5 to 10), suggesting a large deletion for both. By long range PCR, a specific product was obtained, using primers for 5' and 3' sequence flanking the breakpoint intronic regions. In the remaining patients 9 single nucleotide substitutions, 2 small deletions (4-pb and 7-pb), 2 insertions (1-pb) and 1 single nucleotide substitution in a splice junction were identified. The FVIII gene inversion represents a well known risk factor for inhibitor development but in this cohort it is not present as expected because of the bias in the selection criteria. Nevertheless other genetic defects probably interfering with the synthesis of a normal FVIII protein are well represented in this group especially deletions, insertions and nonsense mutations (57%). These results support the hypothesis that gene defects producing a severe phenotype can be frequently found in association with higher risk of inhibitor development.

CO088
MULTIPLE METHODS FOR THE CHARACTERIZATION OF HEMOPHILIA A AND B GENE MUTATIONS

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Hemophilia A (HA) and B (HB) are X-linked bleeding disorders that result from reduced or absent functional proteins in the plasma. Both display heterogeneity of mutations throughout the