Oral Abstracts: Disorders of Hemostasis and Thrombosis: Updates and Innovations in Laboratory Assessment

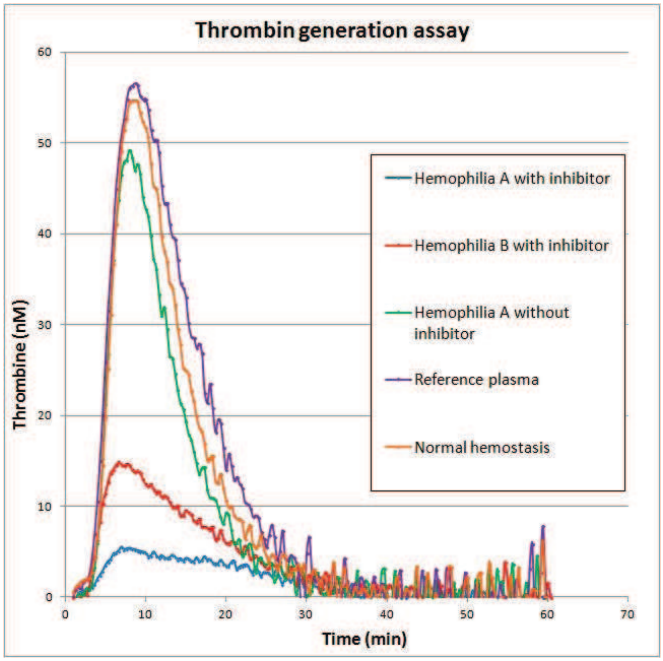
O30

DETECTION OF FVIII AND FIX INHIBITORS USING THROMBIN GENERATION ASSAY: A NEW DIAGNOSTIC APPROACH

Ellen Van Even, Dominique Latinne, Catherine Lambert, Cédric Hermans, Stéphane Eeckhoudt

Laboratory of Hematology, University Hospital Saint-Luc Brussels, Belgium

Introduction: Factors VIII/IX inhibitors are IgG auto- or allo-antibodies that bind functional epitopes and can inhibit the coagulant activity of factor VIII or IX. The Bethesda-Nijmegen assay is commonly used for detection of inhibitors, but is labour intensive and has a poor sensitivity for low titer inhibitors. Thrombin Generation Assay (TGA, Thrombinoscope, Synapse BV, Maastricht, The Netherlands) is being revived as an overall functional assay of plasmatic hemostasis and allows measuring the influence of inhibitors on global thrombin generation. In our study, the use of TGA for detection of factor VIII and IX inhibitor antibodies was evaluated. Methods: The study was performed using plasma samples from 42 patients with normal hemostasis and from 45 hemophilia (41 type A and 4 type B) patients. Of these hemophilia samples, eight were diagnosed FVIII inhibitor positive and one FIX inhibitor positive using the Bethesda-Nijmegen method. One patient with acquired hemophilia A showed a residual FVIII activity of 79% on Bethesda-Nijmegen assay with suspected presence of an inhibitor. Plasma samples were heated at 56°C to inactivate all clotting factors, whereas IgG antibodies are heat resistant leaving the potential inhibitor levels unchanged. Heat inactivated plasma samples were mixed in equal parts with commercial pooled plasma (CryoCheck™). TGA was performed on those samples and results were expressed in endogenous thrombin potential (ETP) ratio. This ratio was obtained by dividing ETP results of clinical sample mixtures with ETP results of reference plasma. This reference sample was composed by mixing FVIII deficient control plasma and normal pooled plasma in a 1:1 dilution. Results: ETP ratio values were between 0,70 and 1,23 (mean±SD: 0,89±0.14) for patients with normal hemostasis (n=42) and between 0,63 and 1,06 (mean±SD: 0,78±0.09) for hemophilia patients without inhibitors (n=36). ETP ratio values were between 0,19 and 0,54 (mean±SD: 0.32±0.14) for the hemophilia patients with presence of an inhibitor detected using the Bethesda-Nijmegen assay (n=9). The patient with acquired hemophilia A exhibited an ETP ratio of 0,42. Kinetics of thrombin generation are shown in figure 1. Conclusions: Our preliminary results demonstrate the use of TGA for detection of factor VIII and IX inhibitors in mixing experiments. TGA is also able to detect weak inhibitors or an inhibitory effect of antibodies with complex kinetics. Complementary studies should be performed to evaluate the influence of different variables such as pH.



O31

UPDATE ON PROFICIENCY TEST RESULTS FOR HEPARIN-INDUCED THROMBOCYTOPENIA: A REPORT FROM THE NORTH AMERICAN SPECIALIZED COAGULATION LABORATORY ASSOCIATION (NASCOLA)

Kristi Smock[1], Marlies Ledford-Kraemer[2], Piet Meijer[3], Peihong Hsu[4], Elizabeth Van Cott[5]

[1]University of Utah Department of Pathology, ARUP Laboratories Salt Lake City, UT, USA, [2]CLOT-ED, Inc. Islamorada, FL, USA, [3]ECAT Foundation Leiden, Netherlands, [4]Department of Laboratory Medicine, Hofstra North Shore-Long Island Jewish School of Medicine Lake Success, NY, USA, [5]Department of Pathology, Massachusetts General Hospital Boston, MA, USA

Introduction: In 2011 NASCOLA distributed two proficiency testing challenges to evaluate laboratory testing for heparin-induced thrombocytopenia (HIT). We analyzed results from both surveys and also compared those 2011 results to the performance of NASCOLA laboratories in 2010. Methods: Results were submitted for 4 samples (2 positive; 2 negative). Qualitative classification was compiled for all methods. For ELISA assays, statistical analysis was performed on the optical density (OD) value and OD/cutoff ratio. Results: 140 immunologic results were submitted by 31 laboratories in 2011, including 134 ELISA results and 6 qualitative gel agglutination. 96% were from commercial kits. In 2011, slightly over half of ELISAs utilized polyvalent antibody detection and the remainder were IgG-specific. In 2010, approximately two-thirds were from polyvalent kits. Similar to the 2010 data, the vast majority (99%, n= 71) of results on the 2 positive samples were classified by the laboratories as positive. For the first positive sample, the mean ELISA OD/cutoff ratio was 3.26 (SD = 0.90, range 1.68 - 5.00). For the second, the mean ELISA OD/cutoff ratio was 4.99 (SD = 1.04, range 2.88 - 6.73). Polyvalent and IgG-specific ELISAs had similar mean OD/cutoff ratios in the positive samples (3.18