Abstract:

QUANTIFYING LOW TITRE FVIII INHIBITORS: INFLUENCE ON RESIDUAL FVIII, HEAT INACTIVATION AND FVIII:C ASSAY TYPE.

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Objectives. Inhibitor development against coagulation factor VIII (FVIII) is a major complication in many patients with haemophilia A treated with substitution products. A correct laboratory investigation with detection and determination of the inhibitor titre is of great importance for correct clinical decisions. The recommended assay to determine the inhibitor titre is the Bethesda-Nijmegen assay. Although the assay principle is well described, there are still open issues, especially in the case of low titre inhibitors, how to handle the presence of endogenous FVIII in the patient sample and the influence of the method to determine the residual FVIII activity, i.e. the one-stage clotting assays or chromogenic ones.

Methods. Twenty samples with low titre inhibitors against FVIII (range 0.5 – 2.0 BU/mL), and varying amounts of FVIII (range 0 – 20 IU/dL) were prepared in vitro and used to evaluate the effect of pre-analytical heat inactivation of the FVIII activity prior to the Bethesda-Nijmegen assay. Furthermore, the residual FVIII activity in all samples was determined with both a one-stage and a chromogenic assay in order to evaluate the effect on the calculated inhibitor titre.

Results. Addition of FVIII had a dose dependent effect in lowering the calculated antibody titre and the pre-analytical heat-inactivation procedure had no effect on the results. For non-heat inactivation samples, results for the one-stage and chromogenic assays varied between 0.4 - 1.8 and 0.4 -1.9 BU/mL, respectively. The corresponding results for one-stage and chromogenic assays for the heat-inactivated samples ranged between 0.4 – 2.0 and 0.5 – 1.9 BU/mL, respectively.

Conclusions. Addition of FVIII had a dose-dependent negative effect on the calculated Bethesda titre irrespective of heat-inactivation procedure was applied or not prior to analysis. Furthermore, there was no significant difference between the one-stage and chromogenic assays results.