



Dr **Annette Bowyer**

Accurate assessment of the concentration of FVIII:C is essential both for the diagnosis and treatment of hemophilia A and von Willebrand's disease (VWD). FVIII:C is usually measured by one of three methods: one-stage clotting assay, two-stage clotting assay, and the two-stage chromogenic method. Most laboratories use the one-stage assay due to its simplicity and ease of automation. Discrepancies between the one and two-stage assays have been described for many years. The two-stage and chromogenic assays are less likely to be affected by activation.

We have evidence that the diagnosis of hemophilia will be missed if only the one-stage assay is employed. Thrombin generation assays are increasingly employed and may be useful in the assessment of the hemostatic defect. We wish to compare the different currently used assays with each other and with the thrombin generation assay in patients with defined genetic defects and following

The utility of different assays in the assessment of the concentration of factor VIII:C

treatment with concentrates.

Areas to be investigated will be:

- a) The proportion of patients (diagnosed with the two-stage assay) that would have been missed if the one-stage or chromogenic assay was employed
 - b) The comparison of the different assays with each other, their correlation to the molecular defect and the thrombin generation test results
 - c) The role of von Willebrand factor in the performance of the different assays
 - d) The re-examination of the different variables with the different assays, e.g. incubation time and the correlation of these to the molecular defects
- Assays to be employed:
- a) Two-stage clotting assay
 - b) One-stage clotting assay
 - c) Two-stage chromogenic assay
 - d) Thrombin generation test

In Sheffield we are in an almost unique position to investigate this problem, since:

- a) The Centre is a World Health Organisation collaborating center for the diagnosis and comprehensive care of patients with bleeding disorders as well as an international hemophilia training center
- b) We have an excellent genetic set-up with almost all of our hemophilia A, VWD type 2 and 3 already characterised at the DNA level
- c) All of our patients were diagnosed with the two-stage clotting assay since this is the assay routinely used in our laboratory
- d) Many of our patients have donated plasma that was distributed by the

quality control schemes (UK NEQAS and WHO EQA) that are run from Sheffield and we have data on their test results from hundreds of laboratories worldwide who use different assays.

Funding is requested to allow a Biomedical Scientist to spend 50% of their working time to investigate this problem.

Expected results: The one-stage clotting assay, currently used by >90% of clinical laboratories worldwide, underestimates the severity of up to a third of mild hemophiliacs and can fail to diagnose the disorder in some patients with mild disease. It is impossible to predict which assays better correlate with the thrombin generation results.

“

We have evidence that the diagnosis of hemophilia will be missed if only the one-stage assay is employed

”

Royal Hallamshire Hospital
UK