Performances of the Hydragel 5 von Willebrand multimers- a new within-day von Willebrand factor (VWF) multimer screening method


Sheffield Haemophilia and Thrombosis Centre, UK
Sebia R&D Department, Lisses, France
‘Angelo Bianchi Bonomi’ Hemophilia and Thrombosis Center, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico and Luigi Villa Foundation, Milan, Italy

OBJECTIVES

Analysis of VWF multimers is essential for the diagnosis and classification of Von Willebrand disease (VWD). Multimers analysis is currently non-standardised and laborious. The local in-house method takes 4 days to produce interpretable results. A novel semi-automated within-day VWF multimer test has recently become available. The aim of this study was to assess the performance of the semi-automated Hydragel 5 VW multimers system (Sebia) as compared to in-house multimers in VWD diagnosis and classification.

METHODS

Multimers analysis of 161 well characterised VWD patients, 30 patients without VWD (non-VWD) and 25 normal donors were performed in parallel. VWD patients comprised types 1 n=56, 2A n=30, 2B n=16, 2M n=26, 2N n=11, 3 n=3, Acquired VW n=19. The in-house method routinely used 1.6% SDS agarose gel electrophoresis, followed by visualisation with alkaline phosphatase-conjugated antibody. A 1.0% agarose was used where differences were observed. Hydragel 5 VWF multimers were performed on the Hydrasys 2 semi-automated system. Patients were grouped according to the VWF Activity (Innovance, Siemens) and ratio of VWF Activity/antigen with a ratio of ≤0.6 equating to type 2 VWD.

RESULTS

Concordance of 98.4% was demonstrated between methods. A normal multimer pattern was observed using Hydragel in all normal donors, 28/30 non-VWD patients, 43/46 type 1 VWD, 20/26 2M and all type 2N (see patient 1). Five 2M samples had normal multimer distribution using the in-house method and a slightly flattened high molecular weight multimer (HMWM) peak with the Hydragel method (see patients 3/4). Loss of multimers was observed in all types 2B, 2A and 3 VWD, (see patient 2). Three individuals with normal VWF Activity had normal in-house multimers but loss of HMWM using Hydragel. One was subsequently diagnosed with type 2B VWD the other two are unknown. Three subjects categorised as type 1 VWD due to ratio of Activity/antigen of >0.6 had a loss of HMWM with both methods; one had a combined 2A and 2B VWD, two had type 2B (see patient 5).

CONCLUSIONS

The Hydragel 5 von Willebrand multimers demonstrated excellent agreement with the in-house method for VWD classification and subtyping. The clinical significance of a unique pattern for some type 2M individuals using the Hydragel 5 von Willebrand multimers is not known but may be related to the genetic mutation of the patient. Hydragel 5 von Willebrand multimers is suitable for inclusion in a routine screening program for VWD. The method saved on staff time, produced highly reproducible results and allowed for easy interpretation due to in-built densitometry.