Clinical evaluation of the Sebia Hydragel von Willebrand factor assay in comparison to Electrophoresis and blotting based Multimer analysis

H. Seidel1, P. Westhoffen1, H. Bautista2, G. Beullier2, G. Nouadje2, J.P. Kruppchenbacher2
1 Centrum für Blutgerinnungsstörungen und Transfusionsmedizin, Bonn,
2 Sebia, Research and Development department, Lisses, France

BACKGROUND and AIMS

Laboratory diagnosis of von Willebrand disease (VWD) requires measuring von Willebrand Factor (VWF) by immunological (VWF-Ag), VWF-Collagen-binding (VWF-CB) and functional tests (VWF activity (VWF:Act), ristocetin cofactor activity VWF:RCO)). A reduced VWF ratio of functional and immunological assays often indicates a qualitative VWF defect. Knowledge of VWD subtype determines or modifies therapeutic options. The gold standard to identify qualitative VWD is the analysis of the patients’ distribution.

However, as conventional multimer analysis is a time and personal consuming process (2 – 3 days) and limited to specialized centers, urgent therapeutic decision making often has to be performed without its results.

Recently, a new rapid test (< 6 hours) Hydragel 5, von Willebrand Multimers (Sebia, Lisses, France) has been developed and is now commercially available. We investigated the impact of the Hydragel semi-automated system (in comparison to the conventional method).

PATIENTS and METHODS

Citrated plasma samples were loaded in a simple 2% agarose gel system (no stacking and running gel) and electrophoresed on the Hydrasys 2 Scan within 110 minutes. Multimers were probed in gel by immunofixation using horse-radish peroxidase (HRP) conjugated rabbit anti-VWF (90 minutes). Visualization of multimers was achieved by colorimetry using commercially available TTF1/TTF2 Sebia reagents. Curves were produced using GelScan and Sebia Phoresis software.

Conventional multimer analysis involves preparation low- and intermediate-resolution gels combined with an optimized visualization system.

We analyzed 101 patients with suspected or confirmed VWD. Clinical and laboratory phenotype were determined by standardized questionnaire and VWF parameter, respectively.

Ethical approval. The study was approved by the Ethical Committee of the Aöko, Dusseldorf, Germany.

RESULTS

Kits of Hydragel 5 von Willebrand Multiplier (HSVWM) were ready to use and reagents were provided. Results were received within one working day (< 6 hours). Based on laboratory findings we found 22 patients with VWD type 1 and 12 patients with VWD type 2. The discrepant findings were shown in table 1: HSVWM failed diagnosing in a patient with cardial acquired VWD. As HSVWM can not visualize triplet structure, VWD type 2M can not be excluded by HSVWM (normal distribution of large multimers). False-positive results using the conventional assay were suspected likely due to transportation artefact in four cases. In one case clinically and laboratory based diagnosing of VWD was not sufficient with conflicting results of the multimer analysis. Probably different timepoints of blood samples explained discrepant findings for multimer analysis in a patient with essential thrombocythaemia (ET).

| Table 1: Patients with discrepant findings between conventional multimer analysis and HSVWM, and all patients with confirmed diagnosis of VWD type 2: |
| o False diagnosis of VWD type 2 probably due to transportation artefact in 4 patients (cases 1, 2, 3 and 6) |
| o Concordant results for both assays in 9 patients with VWD type 2 |
| o HSVWM failed diagnosing in a patient with acquired (aortic valve stenosis) VWD type 2 (case 19) |
| o Multimers of triplet structure that may allow subclassification of VWD type 2M cannot be seen with the HSVWM system (case 10). Type 2M is characterized for normal multimer distribution. |
| o Discrepant findings in case 12 were probably explained by different timepoints of multimer analysis (e.g. before and after treatment of ET) |
| o Diagnosis and classification of VWD failed due to conflicting clinical and laboratory results in case 19 |


CONCLUSIONS

This study confirms the reliability of HSVWM in lab’s routine. It detects diminution or loss of multimers in the majority of samples. For more complex samples it helps the lab in orientating the decision towards more specialized laboratory tests (i.e. visualization of triplets, genotyping).

The risk of false-positive results, e.g. due to transport to the extern specialized lab, could be excluded.

VWD type 2M can not be detected by HSVWM, as this subtype is characterized by alterations of triplet structure and not by any decrease of high-molecular-weight multimers (HMMWM).

The new assay was easy and rapid to perform (< 6 hours) and could be performed on a commercially available instrument (Hydrasys 2 and GelScan or Hydrasys 2 Scan).

Due to the contemporary results it could be possibly useful particularly for patients with urgent surgery or even in emergency situations when VWD type 2 is suspected (e.g. by decreased VWF ratio) and before patients VWD subtype is determined by conventional method.