ASSESSMENT OF THE VON WILLEBRAND FACTOR MULTIMERS PROFILE IN PATIENTS REFERRED FOR BLEEDING TENDENCY EVALUATION IN ESTONIA: A PRELIMINARY REPORT OF THE VON WILLEBRAND DISEASE DIAGNOSTICS PROJECT

Marika Pika, Kadri Saks, Mirja Varik, Maria Hytti, Kreete Ilves, Mariken Ross

1Department of Laboratory Medicine, North Estonia Medical Centre, Tallinn, 13419 Estonia
2Department of Health Technologies, Tallinn University of Technology, Tallinn, 12616 Estonia
3Hematology Department, Tallinn Children’s Hospital, Tallinn, 13419 Estonia
4Hematology Department, North Estonia Medical Centre, Tallinn, 13419 Estonia

Received: 11 March 2021/Revision: 18 March 2021/Accepted: 05 April 2021

ABSTRACT: Introduction: VonWillebrand disease (VWD) is considered the most common autosomal inherited bleeding disorder. Laboratory testing for diagnosis or exclusion of VWD is based on a complex of different diagnostic assays. In the diagnostic workup of patients with suspected VWD, the von Willebrand factor (VWF) multimer assay is one of the most important indicators for VWF quality. This study aims to assess the VWF multimers profile in patients with bleeding tendency and increase knowledge and awareness of VWD laboratory diagnosis in Estonia. Methods: This retrospective study investigated the laboratory results of 131 individuals who were selected from the laboratory information system based on the request of VWF tests profile and 31 healthy volunteers for comparison. Results: Control group, non-VWD patients and patients suspected with VWD type 2N or mild haemophilia A demonstrated normal VWF multimer (VWF:MM) pattern. Patients with low VWF and suspected with VWD type 1 also showed normal VWF:MM distribution with reduced intensity. All cases suspected with VWD type 2A or 2M had a decrease of high molecular weight multimers (HMWM); one of them showed a loss of intermediate molecular weight multimers and HMWM and low VWF activity to antigen ratio (<0.7). Furthermore, multimers were undetectable in patients suspected with VWD type 3 or severe type 1. Conclusions: This is the first report of VWD laboratory evaluation in Estonia to provide insight into the potential clinical significance of using VWF: MM. The interpretation of VWF multimers should be necessarily complemented by the quantification of fractions of multimers by densitometry additional to visual gel’s examination.

KEYWORD: von Willebrand disease, von Willebrand factor, von Willebrand factor multimers
INTRODUCTION:

Von Willebrand disease (VWD) is considered to be the most common autosomal inherited bleeding disorder caused by a deficiency or functional abnormality of von Willebrand factor (VWF) [1]. VWD is classified into partial and total quantitative deficiencies of VWF (VWD types 1 and 3) and qualitative variants (VWD types 2A, 2B, 2M and 2N) [1].

The National Heart, Lung and Blood Institute Expert Panel report published in 2008 [2] suggested that VWD type 1 can be diagnosed when VWF antigen (VWF:Ag) or VWF activity is <30%, and levels of VWF:Ag between 30% and 50% should be classified as low VWF.

An evaluation of the patient personal and family bleeding history is recommended using a Bleeding Assessment Tool (BAT) before laboratory tests request [3]. Laboratory testing for the diagnosis or exclusion of VWD is based on a complex of different diagnostic assays: [4,5] platelet count, patient skin bleeding time or the platelet function analyser closing time, prothrombin time, activated partial thromboplastin time (APTT), VWF:Ag, VWF activity, coagulation factor VIII (FVIII:C), VWF multimer analysis (VWF:MM), VWF collagen binding assay (VWF:CB), VWF-FVIII binding assay (VWF:FVIIIIB), propeptide of VWF (VWFpp), ristocetin-induced platelet agglutination assay (RIPA) and molecular analysis of VWF gene. The treatment of VWD bleeding involves the use of tranexamic acid (TA), desmopressin (DDAVP) and plasma derived and recombinant VWF concentrates [6].

The estimated prevalence of VWD appears to be between 0.01% and 1% [1]. Most of the patients are asymptomatic or with mild type 1 VWD and may be difficult to distinguish from healthy individuals [1]. According to the present knowledge, the prevalence of VWD in Estonia is unknown. Estonia is situated in north-eastern Europe with around 1.3 million inhabitants.

Furthermore, making a definite diagnosis of VWD subtypes or severe forms of haemophilia A in Estonia until 2016 was not possible because of the limited availability of laboratory-specific tests. The VWD hypothesis was based on routine coagulation screening tests, and the measurement of VWF antigen level applied reference ranges. In 2016, a new fully automated assay protocol for VWF activity measurement (INNOVANCE® VWF Ac, Siemens, Marburg, Germany) was adapted on STA-R Evolution analyser (Diagnostica Stago, Asnieres, France), and a new VWF multimer electrophoresis assay (Sebia, Lisses, France) was evaluated preclinically, and their analytical performance was evaluated [7-9].

This retrospective study aimed to assess the VWF multimers profile in patients with bleeding tendency and to increase the knowledge and awareness of VWD laboratory diagnosis in Estonia.

MATERIALS AND METHODS:

Participants/Samples

The laboratory results of 131 individuals who were selected from the laboratory information system (LIS) based on the request of VWF tests profile, were investigated between May 2016 and December 2020. The samples were from patients visiting the outpatient clinic and from hospitalised patients. Moreover, the background clinical information of the patients, provided by clinicians, was available in LIS. The basic data of patients were anonymously collected.

The median age of the patients was 17 (range, 1–77 years). The control group included 31 healthy volunteers (seven men and 24 women) without known bleeding disorders. Samples were collected into 3.2% sodium citrate tubes (BD Vacutainer; BD Diagnostics, Plymouth, UK) for coagulation assays and hirudin blood tubes (Roche Diagnostics, Basel, Switzerland).
or hirudin tubes (Sarstedt, Nümbrecht, Germany) for platelet aggregation evaluation.

The study was performed according to the Declaration of Helsinki and was approved by the Tallinn Ethical Committee on Medical Research (approval number 680).

**Laboratory Investigations**

The investigation performed included VWF antigen (VWF:Ag; Liatest-VWF:Ag; Diagnostica Stago, Asnieres, France), and the VWF activity was measured as VWF binding to the glycoprotein Ib receptor on the platelet surface (VWF:GPIbM; Innovance® VWF Ac kit; Siemens Healthcare Diagnostics, Marburg, Germany) and FVIII:C determined by a one-stage, clot-based assay (Diagnostica Stago), which were measured using an automated coagulometer STA-R Evolution (Diagnostica Stago).

Whole blood aggregation (WBA) was performed using the impedance Multiplate® platelet aggregometry analyser (Roche). Ristocetin-induced platelet aggregation in whole blood (WB-RIPA) was performed with two final ristocetin concentrations (high, 0.77 mg/mL; low, 0.2 mg/mL) following the standard Multiplate® RISTOtest protocol.

The measurements VWF:Ag, VWF:GPIbM, FVIII:C and whole blood ristocetin-induced platelet agglutination (WB-RIPA) were repeated (minimum twice) on a separate new sample to confirm or refute initial investigation results.

VWF:MM was measured by gel electrophoresis (Sebia) and separates VWF according to molecular size (low molecular weight multimers (LMWM), intermediate molecular weight multimers (IMWM) and high molecular weight multimers (HMWM) as previously described) [8,9]. All parameters were analysed using a standard methodology in an accredited laboratory.

**Algorithm of VWD Subtype Classification**

The diagnostic criteria for VWD were based on the current revised classification by the International Society on Thrombosis and Haemostasis (ISTH) [10,11]. A diagnostic algorithm for VWD was created [12] and used in this study based on available laboratory assays in Estonia.

**Statistical Analysis**

The baseline patients’ characteristics were presented as median and interquartile range (IQR) or number of cases (in percentage, counting data). Spearman’s correlation coefficient was calculated to test the association between HMWM vs. VWF:GPIbM/VWF:Ag and RistoHigh vs. VWF:GPIbM. The difference between variables was tested using the Mann–Whitney test. Statistical significance was considered if p<0.05. Statistical analysis was conducted with the Statistical Package for the Social Sciences, version 23 (IBM, Armonk, NY, USA).

**RESULTS:**

This study analysed the results of 131 patients. Table 1 presents the main characteristics of the study subjects.
Table 1. Baseline characteristics of study subjects (refer to Table 2)

<table>
<thead>
<tr>
<th>Laboratory findings, units, reference ranges, p value (in comparison with group 1)</th>
<th>Group-1 (n = 31)</th>
<th>Group-2 (n = 50)</th>
<th>Group-3 (n = 46)</th>
<th>Group-4 (n = 17)</th>
<th>Group-5 (n = 10)</th>
<th>Group-6 (n = 6)</th>
<th>Group-7 (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>male/female</strong></td>
<td>7/24</td>
<td>15/35</td>
<td>19/27</td>
<td>5/12</td>
<td>4/6</td>
<td>5/1</td>
<td>-/2</td>
</tr>
<tr>
<td><strong>Age range, years</strong></td>
<td>18–69</td>
<td>4–66</td>
<td>1–54</td>
<td>4–52</td>
<td>1–77</td>
<td>4–43</td>
<td>7–13</td>
</tr>
<tr>
<td><strong>VWF:Ag, % 50%–160%</strong></td>
<td>86 (65–102)</td>
<td>69 (59–99)</td>
<td>43 (39–47)</td>
<td>24 (20–28)</td>
<td>25 (17–33)</td>
<td>82 (68–116)</td>
<td>2–8</td>
</tr>
<tr>
<td>p = 0.68</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.621</td>
<td>p &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>VWF:GPIbM, % 46%–146% (0 group) 61%–179% (non-0)</strong></td>
<td>85 (71–105)</td>
<td>77 (65–120)</td>
<td>51 (46–57)</td>
<td>26 (20–34)</td>
<td>11.5 (9–13.3)</td>
<td>89 (65–138)</td>
<td>3–12</td>
</tr>
<tr>
<td>p = 0.285</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.918</td>
<td>p &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>VWF:GPIbM /VWF:Ag &gt;0.7</strong></td>
<td>1.04(0.97–1.15)</td>
<td>1.09(1.01–1.25)</td>
<td>1.17(1.09–1.32)</td>
<td>1.05(0.92–1.22)</td>
<td>0.51(0.39–0.59)</td>
<td>1.04(0.95–1.16)</td>
<td>1.50</td>
</tr>
<tr>
<td>p = 0.078</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.931</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>FVIII:C, % 60%–150%</strong></td>
<td>101 (82–124)</td>
<td>103(88–126)</td>
<td>72 (69–83)</td>
<td>65 (45–86)</td>
<td>37 (26–45)</td>
<td>29 (13–35)</td>
<td>5–31</td>
</tr>
<tr>
<td>p = 0.787</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>FVIII:C/VWF:Ag &gt;0.7</strong></td>
<td>1.17(1.06–1.35)</td>
<td>1.42(1.22–1.64)</td>
<td>1.64(1.47–2.04)</td>
<td>2.81(2.07–3.59)</td>
<td>1.45(0.93–1.89)</td>
<td>0.33(0.12–0.52)</td>
<td>2.50–3.88</td>
</tr>
<tr>
<td>p = 0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.430</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>RistoHigh, U 98–180 U</strong></td>
<td>not determined</td>
<td>118 (97–139)</td>
<td>97 (87–116)</td>
<td>66 (25–111)</td>
<td>20 (9–51)</td>
<td>109 (74–142)</td>
<td>5–10</td>
</tr>
<tr>
<td><strong>RistLow, U 0–20 U</strong></td>
<td>not determined</td>
<td>8 (6–10)</td>
<td>7 (4–11)</td>
<td>5 (3–9)</td>
<td>6 (3–11)</td>
<td>6 (6–8)</td>
<td>4–5</td>
</tr>
<tr>
<td><strong>VWF:MM fractions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LMWM, % 10.4%–22.5%</strong></td>
<td>15.0 (12.7–17.2)</td>
<td>17.9 (14.7–19.9)</td>
<td>16.9 (14.4–20.3)</td>
<td>22.2 (18.5–30.0)</td>
<td>44.1 (32.7–53.9)</td>
<td>16.7 (14.2–21.6)</td>
<td>undetectable</td>
</tr>
<tr>
<td>p = 0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.209</td>
<td></td>
</tr>
<tr>
<td><strong>IMWM, % 22.6%–37.6%</strong></td>
<td>29.2 (26.7–31.2)</td>
<td>29.9(26.3–33.3)</td>
<td>26.4 (22.8–30.6)</td>
<td>25.6 (32.1–29.7)</td>
<td>25.5 (21.0–31.1)</td>
<td>30.6 (24.3–33.9)</td>
<td>undetectable</td>
</tr>
<tr>
<td>p = 0.537</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.137</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.837</td>
<td></td>
</tr>
<tr>
<td><strong>HMWM, % 45.6%–66.6%</strong></td>
<td>55.4 (51.1–60.2)</td>
<td>53.7 (47.5–56.9)</td>
<td>55.6 (50.1–60.3)</td>
<td>50.8 (45.5–56.1)</td>
<td>32.0 (20.6–36.9)</td>
<td>53.3 (44.8–58.7)</td>
<td>undetectable</td>
</tr>
<tr>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.724</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p &lt;0.05</td>
<td>p = 0.333</td>
<td></td>
</tr>
</tbody>
</table>
The median age within the cohort was 17 years (range, 1–77 years) with 63.4% female patients. The analysis of the data of indications for VWD testing found the following reported reasons: nose bleeding (23%); menorrhagia with or without anaemia (24%); easy bruising (16%); bleeding after an invasive procedure, dental extractions, or surgery (8%); positive family history without bleeding symptoms (7%); prolonged APTT (5%) and request for investigations (5%) from general practitioners with the comment ‘for bleeding disorders evaluation’.

As shown in Table 2, all participants were divided into different groups based on the laboratory investigation. Patients from groups 4 to 7 were designated as suspected because all results in the relationship with genetic testing will be analysed in the future.

Table 2. Definition of study groups and laboratory phenotype of participants.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Laboratory phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td>Control group</td>
</tr>
<tr>
<td>Non-VWD</td>
<td>Patients who do not currently fulfil the diagnostic criteria for VWD</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td>Low VWF</td>
</tr>
<tr>
<td>Patients with VWF:Ag and/or VWF:GPIbM values 50%–30% and normal VWF:GPIbM/VWF:Ag (&gt;0.7) ratio</td>
<td></td>
</tr>
<tr>
<td><strong>Group 4</strong></td>
<td>Suspected VWD type 1</td>
</tr>
<tr>
<td>Patients with VWF:Ag and/or VWF:GPIbM values &lt;30% and normal VWF:GPIbM/VWF:Ag (&gt;0.7) ratio</td>
<td></td>
</tr>
<tr>
<td><strong>Group 5</strong></td>
<td>Suspected VWD type 2A or 2M</td>
</tr>
<tr>
<td>Patients with ratio of VWF:GPIbM/VWF:Ag&lt;0.7 and WB-RIPA results without enhanced response with low-dose ristocetin</td>
<td></td>
</tr>
<tr>
<td><strong>Group 6</strong></td>
<td>Suspected VWD type 2N or mild haemophilia A</td>
</tr>
<tr>
<td>Patients with VWF:Ag and VWF:GPIbM values within reference intervals and decreased FVIII:C results</td>
<td></td>
</tr>
<tr>
<td><strong>Group 7</strong></td>
<td>Suspected VWD type 3 or severe type 1</td>
</tr>
<tr>
<td>Patients with VWF:Ag values &lt;10%</td>
<td></td>
</tr>
</tbody>
</table>

The factor VIII level in healthy individuals was comparable with that in group 2 (p = 0.787) and was reduced in other groups (p < 0.05). In group 4, the ratio of FVIII:C/VWF:Ag has shown higher values (2.81; 2.07–3.59) compared with those in the other groups. The ratio of FVIII:C/VWF:Ag has the lowest results (0.33; 0.12–0.52) in group 6 and was statistically different compared with the other groups (p < 0.05).

Figure 2 illustrates the RistoHigh testing results in the different study groups. However, WB-RIPA was not performed in the control group. Patients in groups 2,3 and 6 had partly overlapping results with reference intervals provided by the manufacturer. Platelet aggregation was reduced at the 0.77 mg/mL ristocetin concentration in group 4 and progressively reduced in group 5. Moreover, these patients had lower VWF:GPIbM results. No response to ristocetin was demonstrated in group 7. RistoHigh positively correlated with VWF:Ag (r = 0.518, p < 0.01) and VWF:GPIbM (r = 0.484, p < 0.01) in all study populations.
Figure 2. WB-RIPA by Multiplate at ristocetin concentration of 0.77 mg/mL in different study groups (refer to Table 2). The dashed lines indicate reference intervals. WB-RIPA: whole blood ristocetin-induced platelet agglutination; RistoHigh: ristocetin-induced platelet aggregation in whole blood with final ristocetin concentration 0.77 mg/mL.

VWF multimeric analysis was conducted in all patients. The normal ranges for HMWM were 45.6%–66.6% as previously reported [13]. However, the HMWM decrease was defined as < 40% using values 40%–45% as the grey zone. Furthermore, Fig. 3 shows the distribution of HMWM multimers in the study population.

Figure 3. HMWM in the different study groups (refer to Table 2). The dashed line indicated the cut-off of 40%. HMWM: high molecular weight multimers.

The control group demonstrated a normal VWF:MM pattern, and the multimers fractions were within reference intervals. Group 2 (50 of 131 samples; 38%) showed a normal multimeric pattern with a normal VWD phenotypic profile, do not currently fulfil the diagnostic criteria for VWD and were therefore defined as non-VWD, but HMWM was 36% (lower than the cut-off of 40%) in one patient.

In group 3, 46 of 131 (35%) suspicious patients were identified to have low VWF. In this group, 43 samples had normal multimeric distribution. However, VWF:MM interpretation was difficult/impossible in three cases (single-family members) because a smeary appearance was visible with a gel, HMWM ranged from 25 to 37 by densitometry, and these family members had a normal ratio of VWF activity to antigen and normal platelet aggregation results.

Furthermore, group 4 has 17 of 131 (13%) patients categorised as suspected VWD type 1. All samples showed a normal multimeric pattern, but HMWM was 36% (lower than the cut-off of 40%) in one patient.

In group 5, 10 of 131 (8%) patients were grouped as suspected VWD type 2A or 2M. A visible HMWM decrease (range, 39–1.3 by densitometry) was found in all cases. However, one of them showed a visual loss of IMWM and HMWM on the gel as well as quantitatively (IMWM, 7.1%; HMWM, 1.3%). In this group, patients had low VWF activity to antigen ratio (<0.7).

In group 6, 5% of all patients were classified as suspected VWD type 2N or mild haemophilia A. All samples showed a normal multimeric pattern, but HMWM was 38% (lower than the cut-off of 40%) in one patient.

Furthermore, in group 7, two patients were categorised as suspected VWD type 3 or severe type 1.
Consequently, VWF:MM was undetectable in both cases.

This study found that the decreased levels of the VWF ratio activity to antigen were related to the reduction of HMWM. Moreover, VWF:GPIbM/VWF:Ag positively correlated with HMWM (r = 0.35, p < 0.01) in all study populations.

**DISCUSSION:**

All measurements of the VWF profile were repeated (minimum twice) on a separate new sample to confirm or refute the initial investigation results [14]. The VWF:Ag level was <50% and <30% in 52% and 18% of the patients, respectively. Moreover, the values of VWF:GPIbM were <50% and <30% in 36% and 17% of the cases, respectively.

The calculated ratio between VWF activity and antigen can aid in identifying the qualitative VWF abnormalities and help differentiate type 1 from type 2-like. In the present study, the cut-off used was <0.7 [15].

The results showed that the ratio of FVIII:C/VWF:Ag was increased to > 2 in patients suspected to have VWD type 1, demonstrating a defect in VWF secretion as the main cause of quantitative deficiency [16]. At the same time, the ratio of FVIII:C/VWF:Ag was <0.7 in patients suspected with VWD type 2N or mild haemophilia A, supporting the hypothesis of defective FVIII:C-VWF binding or FVIII:C deficiency [17].

Multiplate® platelet aggregometry analyser (Roche) is widely used for screening of platelet function disorders (PFDs) [18]. Published data about the usefulness of WB-RIPA in VWD diagnosis are controversial. Moreover, it has potential diagnostic value for VWD by performing ristocetin-induced platelet aggregation in whole blood [19]. Diagnostic accuracy has been proven for patients with previously diagnosed VWD and an agreement exists with Born aggregometry results [20]. A study with 30 previously characterised VWD patient population showed that WBA was as sensitive as Light Transmission Aggregometry (LTA) in detecting VWD with a 76% correlation between the two methods [20]. Furthermore, the clinical usefulness of Multiplate as a screening assay for PFDs is limited, and this method may represent an alternative to LTA only for Glanzmann’s thrombasthenia or other severe PFDs, whereas WBA is poorly sensitive in detecting mild PFDs [21]. Moreover, researchers from Sweden [19] evaluated the diagnostic accuracy of WB-RIPA, performed at a high ristocetin concentration, in a study with 100 VWD patients and reported that reduced WB-RIPA correlated with low-VWF activity and is a sensitive screening test to exclude VWD. Nummi et al. [22] proposed the use of Multiplate-based WB-RIPA to rule out VWD. This study also found that ristocetin-induced platelet aggregation was decreased in patients suspected to have VWD type 1, 2A or 2M.

RIPA testing has been reported [19] to demonstrate no response to ristocetin in VWD type 3. Similar results were found in this study in patients assumed to have VWD type 3 or severe type 1.

In the diagnostic workup of patients suspected VWD, the VWF multimer assay is one of the most important indicators for VWF quality [23]. The HMWM interpretation using the new VWF:MM assay (Sebia) in clinical practice is based on individual decisions, and no consensus currently exists for that. The cut-offs of 40% and 38% were used for patients’ samples and lyophilised samples, respectively based on the results from the External Quality Assessment [8,9]. However, these suggestions need to be clarified further.

Healthy individuals, non-VWD, low VWF and patients suspected to have VWD types 1 and 2N showed the normal distribution of VWF multimer fractions. The HMWM decrease is associated with impaired VWF function [24]. Moreover, several authors [25] have demonstrated a loss of HMWM in patients with VWD types 2A and 2B (in most cases) and also type 2A is sometimes associated with IMWM loss. Similar results were found in this study in group 6 patients. However, recognition of 2A and 2M subtypes based on multimer pattern is sometimes ambiguous because detecting
2M phenotype with non-proteolysed multimers is not possible using low-resolution gels \(^{[26]}\). Thus, the VWD type 2M is misdiagnosed and under-recognised, depending on the laboratory test panel used \(^{[25]}\). VWF multimers were undetectable in patients suspected to have VWD type 3 or severe type 1, which corresponds to the sensitivity of the method \(^{[9]}\). According to the results of this study, the VWF:GPIbM/VWF:Ag ratio positively correlated with HMWM. Moreover, a recent study conducted by Favaloro et al. \(^{[27]}\) showed that VWF activity to antigen ratios was positively related to HMWM. Their findings suggest that the highest correlation was found with the chemiluminescence method.

DDAVP, TA, or replacement VWF therapy are used for managing patients with VWD \(^{[28]}\). A DDAVP test–dose infusion at the time of diagnosis is recommended to evaluate the individual response, which depends on various factors (e.g., phenotype and genotype) \(^{[29]}\). Usually, patients with VWD type 1 demonstrate a good response to DDAVP \(^{[30]}\). Moreover, the replacement therapy is the treatment of choice for non-responders to DDAVP or type 2B patients for whom the DDAVP is contraindicated \(^{[29]}\). Previously, the response to DDAVP was assessed \(^{[31]}\) in seven patients: six were defined as good responders, and one patient demonstrated a partial response to DDAVP.

The genetic evaluation was not yet routinely used for VWD type 1. However, it is often performed for VWD types 2 and 3 \(^{[5]}\). Genetic testing for VWD type 2N vs. haemophilia A was done in four patients wherein two of them were previously diagnosed with HA. Differential diagnosis between VWD and HA is important because the HA therapy is monospecific (e.g. recombinant FVIII) and management of VWD may be less effective if DDAVP or VWF replacement therapy is not provided \(^{[32]}\).

Medical information initially provided by clinicians is required for correct laboratory evaluation for patients with bleeding disorders \(^{[32]}\). Moreover, non-specific results are very difficult to interpret. Additionally, patient-related preanalytical issues should be taken into consideration \(^{[33]}\). For selective approach in laboratory request, the adult and paediatric ISTH-BAT \(^{[34]}\) was translated into Estonian and incorporated into routine practice to identify individuals with clinically relevant bleeding tendency/symptoms. Furthermore, regular meetings and discussions focusing on clinical cases were established between clinicians and the laboratory.

**CONCLUSIONS AND FUTURE PERSPECTIVES:**

This is the first report of VWD laboratory evaluation in Estonia to provide insight into the potential clinical significance of using VWF: MM. The interpretation of VWF multimers has to be complemented by the quantification of fractions of multimers by densitometry additional to visual gel’s examination. It is hoped that this work supports the improvement in VWD diagnosis in Estonia, and it is suggested that the real VWD prevalence should be evaluated in the future.

**Acknowledgments**

The authors would like to express gratitude to the laboratory staff of North Estonia Medical Centre for their excellent technical assistance related to sample testing.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Funding Sources**

Sebia (Lisses, France) donated von Willebrand multimers kits.

**Author contributions**

MP: study concept and design, data acquisition, statistical analysis, analysis and interpretation of data, manuscript drafting, study supervision and critical revision of the manuscript for important intellectual content. KS, MV, MH, KI and MR: study concept and design, analysis and interpretation of data and critical
revision of the manuscript for important intellectual content. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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