**Fifth Åland Island conference on von Willebrand disease**

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**Summary**

The fifth Åland Island meeting on von Willebrand disease (VWD) was held on the Åland Islands, Finland, from 22 to 24 September 2016 – 90 years after the first case of VWD was diagnosed in a patient from the Åland Islands in 1926. This meeting brought together experts in the field of VWD to share knowledge and expertise on current trends and challenges in VWD. Topics included the storage and release of von Willebrand factor (VWF), epidemiology and diagnostics in VWD, treatment of VWD, angiogenesis, and VWF inhibitors.

**Keywords:** Angiodysplasia, angiogenesis, factor VIII, genetics, pregnancy, von Willebrand disease, von Willebrand factor, surgery

## Introduction

In 1926, Erik von Willebrand published an article on a disorder 'Hereditär pseudohemofili', which later became known as von Willebrand disease (VWD) [1]. Over the next 90 years, there have been many advances in our understanding of the underlying mechanisms, pathogenesis and treatment of VWD. VWD has been reported to affect up to approximately 1% of the general population and is the most common bleeding disorder. VWD is clinically heterogeneous and is classified into three main categories based on distinct quantitative and qualitative abnormalities in von Willebrand factor (VWF) [2-4]. Type 1 VWD is the most common form (~70–75% of all cases) and is characterized by a partial quantitative deficiency in functional normal VWF. Type 3 VWD (~1–3% of cases) is characterized by a severe quantitative deficiency in VWF together with very low plasma factor VIII coagulant activity (FVIII:C). Type 2 VWD (~20% of cases) is characterized by qualitative deficiencies in VWD and is further subdivided into 2A, 2B, 2M and 2N subtypes.

The fifth Åland Islands 2016 meeting, which coincided with the 90$^{th}$ anniversary of the first VWD case description by Erik von Willebrand in a 5-year-old girl named Hjördis S from the Åland islands, brought together experts in VWD to discuss the latest scientific and clinical knowledge on VWD.

## VWF structure and function

### Nanoscopic view on VWF structure and Weibel-Palade bodies

Weibel-Palade bodies (WPBs) are storage organelles for VWF in endothelial cells (Fig. 1A) [5, 6]. After its synthesis in the endoplasmic reticulum, VWF pre-pro-proteins undergo cleavage and go on to form multimers [6]. WPBs are first formed in the trans-Golgi network and, as WPBs develop, the stored VWF is assembled into tubules [6, 7]. During maturation of the WPBs, VWF tubules grow and WPBs become elongated and more protein-dense [6, 8, 9]. At the end of this process, other co-stored proteins are also packaged into the WPB granules [6]. Upon stress stimulation, such as injury or inflammation, mature WPBs are transported to the plasma membrane [6]. VWF is then secreted into blood vessels via exocytosis where it structurally rearranges into strings to capture platelets and initiate primary haemostasis [6].

In order to elucidate the mechanistic details of the WPB life cycle, biogenesis of WPBs was followed using various ultrastructure imaging techniques [10-12] (Fig. 1B–D). WPBs were first depleted from human umbilical cord venous endothelial cells (HUVECs) by phorbol 12-myristate 13-acetate (PMA) treatment, which induces exocytosis of WPBs [11]. Synthesis of nascent WPBs over time was then followed by visualization of VWF. Confocal microscopy and transmission electron microscopy analyses of immunofluorescence- and immunogold-labelled VWF, respectively, showed the appearance of newly formed WPB-like structures containing VWF tubules after 4 hours of recovery from PMA treatment [11]. These findings were then confirmed using correlative light and electron microscopy (CLEM), which combines confocal microscopy with conventional transmission electron microscopy, using transfected HUVECs expressing enhanced green fluorescent protein (EGFP)-tagged VWF propeptide [10, 11] (Fig. 1B). The CLEM analysis revealed that 4 hours after transfection, early electron-lucent WPBs began to appear and that 6 and 8 hours after transfection they appeared denser and more elongated. The 3D morphology of nascent WPBs was also investigated by electron tomography as well as serial block face scanning electron microscopy (Fig. 1C–D), where a focussed ion beam removes layers of sample material in between cycles of scanning electron microscopy imaging [11, 12]. This showed that WPBs are tightly associated with the Golgi through numerous connections during their formation. As

WPBs develop, non-tubular VWFs are delivered from the Golgi to the lumen of the WPBs through these connections and once inside VWFs rearrange into tubules. Interestingly, the WPB-Golgi connections continue to be maintained 8 hours after transfection of the labelled propeptide when dense elongated WPBs are already visible [11]. Findings from this nanostructure imaging study, where WPBs remain closely Golgi-associated until maturation, are in line with results from a separate study using super resolution microscopy [13].

There are several modes of VWF secretion from mature, stimulated WPBs [14, 15]. Single WPBs can exocytose individually and several WPBs may fuse intracellularly to produce large secretory pods prior to release of VWF [16]. Live-cell imaging in HUVECs confirmed that large secretory pods are formed through coalescence of multiple WPBs shortly before secretion [17]. The analysis also revealed that VWF adopts a globular conformation immediately after exocytosis and remains anchored to the cell periphery for structural remodelling to form strings [17]. Under more physiological conditions using a flow chamber, the extension of VWF strings was predominantly observed at the edge of cells, which suggests this structural remodelling process may be facilitated by surface receptors such as integrins [17]. Another mode of secretion is known as the "lingering kiss" where smaller co-stored cargo in WPBs, such as interleukins, are selectively released while VWF is retained intracellularly [14].

Genetic mutations in a discrete region of the VWF gene have been associated with VWD patients who are non-responders or partial responders to desamino D-arginine vasopressin (DDAVP), which acts to induce VWF secretion from endothelial cells [18]. However, the mechanism underlying the response to DDAVP is likely to be very complex as similar mutations are found in both non-responders and responders [18]. In a cell-based study, these VWF mutations were studied in more detail by their transient expression in HEK293 cells [19]. Key mutant phenotypes included the inability to form mature elongated WPBs, retention of VWF in the endoplasmic reticulum and reduced VWF secretion upon PMA stimulation. These findings suggest that impairment at different stages of the WPB life cycle, including release of VWF and string formation, may play a role in the pathogenesis of VWD [19, 20].

Advances in cell imaging techniques have greatly improved our knowledge of the physiological and pathophysiological processes involved in the storage and release of VWF. Impaired VWF storage, release and string formation seem to be potential pathophysiological mechanisms in VWD.

## VWD epidemiology and diagnostics

### Diagnosing type 1 VWD

Although laboratory tests can generally distinguish type 1 VWD from other subtypes, the diagnosis of type 1 VWD remains challenging. Diagnosis of type 1 VWD is complicated by the high frequency of bleeding symptoms in the general population, the overlap of VWF level ranges in non-VWD individuals and VWD patients, and the lack of a strict relationship between VWF level and the severity and frequency of bleeding [21-23]. The continuous spectrum from severe VWD to non-affected individuals presents difficulties for setting decisional thresholds to distinguish those affected from those not affected. This is evident in the variation in reported prevalence estimates, which span 2 to 3 orders of magnitude. The prevalence is estimated to be 1 in 100 based on population-based studies and 1 in 1000 or 1 in 10,000 in specialized centres and most review articles [24-28].

The aim of diagnosis should be to achieve a positive clinical outcome after a diagnosis. The outcome of diagnosis can be viewed in terms of diagnostic utility (patient perspective) and diagnostic accuracy (physician perspective) [21]. As clinicians, we should pursue a clinically useful diagnosis, which means that the diagnosis should be of some benefit for the patient and not simply be accurate (i.e. a diagnosis not biased by high false positive or negative rates) [21]. However, this is a particularly difficult task in mild bleeding disorders such as type 1 VWD.

Collaborative studies on large cohorts of patients with mainly type 1 VWD have demonstrated the utility of a bleeding assessment tool (BAT) in diagnosis [29-32]. In the first of these studies, an international multicentre study (Vicenza BAT study) [29], the number of bleeding symptoms, especially the quantitative bleeding score, identified patients with type 1 VWD with a high discriminative power. Males and females with bleeding scores of >3 or >5, respectively, were 70 times more likely to have VWD compared with non-affected individuals [29]. Furthermore, bleeding score has been shown to

predict bleeding risk in patients with VWD [33] and to correlate with VWD severity [34]. Subsequent studies using modified versions of the Vicenza BAT have confirmed the usefulness of BATs as a screening tool for the diagnosis of type 1 VWD [30-32, 35]. In 2010, the International Society on Thrombosis and Haemostasis (ISTH) proposed a single BAT to standardize reporting of bleeding symptoms for use in adult and paediatric populations [36]. In addition, a self-administered BAT based on the ISTH-BAT has been developed [37].

Using the distribution of VWF in non-affected and affected individuals, the likelihood ratio of having VWD based on each class of VWD values can be calculated. This likelihood ratio was also useful in the diagnosis of type 1 VWD in the MCMDM-1VWD study [30]. There was a steep increment of the likelihood ratio of VWD when VWF antigen content (VWF:Ag) and VWF ristocetin cofactor activity (VWF:RCo) values were below 40 IU $dL^{-1}$ and a 5-fold decrease of the likelihood ratio of VWD for values above 60 IU $dL^{-1}$ [30]. Using a Bayes theorem approach, a combination of likelihood ratios from bleeding scores, VWF:Ag level, and number of family members with plasma VWF lower than the 2.5 percentile were shown to influence the final odds of VWD [38]. Based on these observations, minimum criteria of significantly elevated bleeding score (>3 or >5 in men and women, respectively) and VWF:Ag or VWF:RCo levels <40 IU $dL^{-1}$ (either confirmed or consistently reported in patient clinical records) have been proposed [25] (Fig. 2).

With these minimal criteria, the Bayesian calculation allows the estimation of the probability of having VWD as 80/100 (80%) [25]. Using this approach, 1 in 5 of patients would be incorrectly classified, which can be reduced to 1 in 16 if the minimum criteria are extended to include a family member with VWF:RCo <40 IU $dL^{-1}$ [38]. Once the minimal criteria for VWD diagnosis have been met, the next step would be to conduct laboratory analysis to characterize the type and severity of VWD, including a DDAVP test when appropriate, and determine the most suitable treatment.

In summary, 90 years after the discovery of VWD, the diagnosis of type 1 VWD is still intriguing. Using a 2-step process for diagnosis, in which minimal criteria are used to select most-likely affected individuals (step 1) followed by full laboratory evaluation (step 2), may improve diagnosis of VWD.

However, this approach needs to be validated in large cohorts of patients (adults and children) with various types of VWD.

Genetic diagnostics of VWD

Clinical diagnosis of VWD and differentiation between its subtypes can be difficult, particularly when the phenotype is ambiguous. Genetic diagnostics may help facilitate correct differential diagnosis and ensure appropriate treatment and can also be useful in genetic counselling and prenatal diagnostics [39].

The VWF gene spans 178 kb of genomic DNA in 52 exons that encodes an 8.8 kb mRNA and a 2813-amino acid protein [39, 40]. Over 160 normal variants of the VWF gene are known in the exons and closely flanking intronic sequences, and amino acid substitutions are reported at 30 residues [40]. Until recently, genetic analysis of the VWF gene has been limited to Sanger DNA sequencing, which, given the complexity of the VWF gene, has rendered genetic analysis and data interpretation complex and time intensive. However, the availability of next generation sequencing (NGS) now permits rapid simultaneous analysis of multiple genes. Additionally, large exon deletions and duplications can be detected by multiplex ligation-dependent probe amplification and micro-array analysis. The entire VWF gene coding sequence can now be readily analysed for phenotype/genotype correlation.

Overall, pathogenic variants have been identified in >90% type 3 alleles, 50–90% type 2 alleles and <65% type 1 alleles. The location of key VWF mutations in type 1, 2 and 3 VWD are shown in Fig. 3 [41, 42]. Type 1 VWD mutations can be found throughout the length of VWF, 75% being missense mutations and 25% other types of mutations [41]. A special form of type 1 VWD is "type 1C", which is characterized by a rapid clearance of VWF, for example caused by p.Arg1205His ("Vicenza mutation") [43, 44]. Type 3 VWD is the most severe form of VWD with mutations found throughout the entire coding region of the VWF gene. Over 80% are null mutations that result in complete lack of VWF production [41]. Type 2 VWD is subdivided into four subtypes, 2A, 2B, 2M and 2N. In most cases the multimer profile is normal, but loss or gain of specific cysteine residues in the D' and D3 assembly often lead to pleiotropic abnormalities where both reduced VWF levels and multimer abnormalities may be present. The key locations of VWF mutations in type 2 VWD are shown in Fig. 4 [41, 42]. Type 2A VWD is mostly caused by missense mutations predominantly in the A2 domain (exon 28) and the D3

domain (exons 25–27), which reduce binding to platelets. Type 2B VWD is limited to missense mutations in a discrete region of the A1 domain (exon 28), which enhance binding to platelets. Missense mutations in the A1 and A3 domains are found in type 2M VWD, leading to reduced binding of VWF to platelets or collagen. VWD type 2N missense mutations predominate in the D´ domain (exons 17–20) and D3 domain (exons 24–25), leading to reduced binding of VWF to FVIII. If no mutations are found in this region, type 2N VWD can be excluded and the F8 gene should be screened for haemophilia A.

Modern molecular genetic analysis has greatly enhanced our understanding of the molecular basis of VWD and its subtypes and continues to contribute considerably to improving differential diagnosis and management of patients with VWD.

Phenotype/genotype correlation of patients with VWD

The Centre de Référence de la Maladie de Willebrand (CRMW; French reference centre for VWD) was initiated in 2006 and is a collaboration of the main haemostasis departments in France (~50 university hospitals). The CRMW provides a unique national biologic platform for highly specialized analyses of phenotype, genotype and phenotype-genotype correlations in patients with VWD.

To investigate the impact of phenotype-genotype on the VWD classification, data have been analysed for 1167 patients enrolled between 2007 and 2012 [45]. Patients previously diagnosed locally with VWD were included in the study. VWD phenotype was classified as type 1: VWF levels <30 IU $dL^{-1}$ together with VWF:RCo (or VWF:CB)/VWF:Ag and FVIII:C/VWF:Ag ratios >0.6; type 2: decreased or normal VWF levels with a discrepancy between the antigenic and the functional levels (VWF:RCo (or VWF:CB)/VWF:Ag or FVIII:C/VWF:Ag ratios ≤0.6); or type 3: VWF levels <5 IU $dL^{-1}$. Upon entry into the study, first-level phenotypic assays of VWF:Ag, VWF:RCo, FVIII:C, platelet count, activated partial thromboplastin time, ristocetin-induced platelet aggregation (RIPA) and platelet function analyzer-100 occlusion time were performed locally. Second-level VWF assays were performed by the CRMW biologic platform and included VWF multimeric distribution, VWF binding to platelet glycoprotein Ib, collagen and FVIII, and, in some patients, analysis of proteolytic fragments. Genomic

DNA was screened for sequence variations by direct sequencing of the VWF gene and, in some cases, by multiplex ligation-dependent probe amplification. Human Genome Variation Society (HGVS) nomenclature (www.hgvs.org/mutnomen/) was used for the sequence variations. The final classification of VWD was established by a working group taking into account the detailed phenotype, genotype, response to DDAVP (if available) and familial pedigree. The classification could be revised to reflect newly acquired information.

Among the 1167 patients tested for both phenotype and genotype, there were 670 index cases (from 670 unrelated families) and 497 affected family members. The gender ratio (F/M) of the 1167 patients was 1.46 and age ranged from 6 months to 90 years (median 34 years). The percentage of O blood group was 56%, and in those patients, mean VWF:Ag levels were 19 IU $dL^{-1}$ lower than in patients with non-O blood group. The majority of patients were Caucasian (90%) and 10% were Afro-Caribbean.

Of the 670 index cases, 167 (25%) were classified as type 1 VWD, 442 (66%) as type 2 VWD and 54 (8%) as type 3 VWD (Fig. 5). The distribution of VWD subtypes was similar in the overall population. The percentage of patients with type 1 VWD (25%) was lower than previous estimates (~75%) [40]. This may be due to the stringent inclusion criteria (VWF < 30 IU $dL^{-1}$) and the extensive identification of type 2 VWD in the CMRW cohort, which could otherwise have been classified as type 1 VWD. This is consistent with a European study in patients with type 1 VWD in which one-third of patients could have been reclassified as having type 2 VWD [35]. Data from the CRMW are generally consistent with those from a multicentre Spanish study [46]. The percentage of patients with types 1 and 3 VWD were similar in the CRMW and Spanish studies, although there was some variation in the distribution of type 2 subtypes [45, 46].

Of the 167 index cases with type 1 VWD, 88 (53%) had type 1 mutations associated with a decreased synthesis/secretion of VWF, 33 (20%) exhibited mutations inducing an accelerated VWF clearance, 6 (3.5%) had propeptide cleavage site mutations, and 34 (20%) exhibited heterozygous mutations and were considered as type 3 carriers; no mutation was found in 6 type 1 VWD patients (3.5%) [45]. In the 54 type 3 VWD patients, 61 distinct sequence variations were identified that were novel mutations in two-thirds of cases. These sequence variations were spread over the VWF gene, with a predominance at

the N-terminal part of VWF (D domains), and consisted mainly of truncating mutations leading to silent alleles (82%) and a lower percentage of missense mutations (18%) [45]. The number of patients with type 3 VWD analysed from the CRMW database has now increased to 75 patients and 72 different mutations were identified throughout the VWF gene (25 (37%) located in the propeptide region and 47 (65%) were novel mutations). Among the 75 patients, 27 (36%) were homozygous and 48 (64%) were compound heterozygous. For 9 patients (12%), only one VWF mutant allele was identified. In the 442 patients with type 2 VWD, 118 distinct sequence variations were identified, including new mutations in one-third of cases [45]. These sequence variations were clustered in the A domains (types 2A, 2B, and 2M) or D'-D3 domains of VWF (type 2N) and were missense mutations in a large majority of cases (95%) [45]. A total of 102 patients (88 families) with type 2N have been diagnosed (2007 to 2014). In all cases, the VWF:FVIIIB assay was null or severely decreased, leading to low median FVIII:C levels of 19 (range 2–45) IU $dL^{-1}$.

The CRMW biologic platform has provided valuable data on the phenotype-genotype relationship in VWD. Studies are continuing to explore these relationships and further characterize VWD, particularly type 2 variants. The methodology is continually evolving to strengthen and broaden data capture. For example, bleeding score is now a mandatory inclusion criterion and NGS has been progressively introduced since 2013. The CRMW does not collect prospective data on clinical events as these are collected in the national registry FranceCoag Network, which is not currently connected to the CRMW database. Connection of these databases, if regulatory issues can be overcome, would be an important extension of the CRMW biologic platform.

Diagnosis in the USA

VWF testing is not standardized in the USA. For example, different centres use different VWF:RCo assays. In general, clinicians in the USA seem to be 'over-educated' in what the VWF:RCo should be and less well educated on the importance of FVIII levels. Few centres can perform all of the increasingly complex subtyping tests and most samples are sent to the Blood Center of Wisconsin for analysis. The use of standardized tests for diagnosis of VWD is not the standard of care because diagnosis has serious

implications for life and health insurance. Premiums are higher for people with pre-existing conditions and hospitalization and treatment of a pre-existing condition are not covered.

**Treatment of VWD**

*Towards pharmacokinetic population models for VWD: A lot "To WiN"?*

Inter-individual variation in the pharmacokinetic (PK) parameters of replacement FVIII in patients with haemophilia A is well documented [47, 48]. This variation has stimulated interest in developing PK-guided treatment regimens to optimize the treatment of patients with haemophilia A in the prophylactic setting [49, 50], although the use of this approach in the perioperative setting, beyond determination of pre-surgery FVIII in vivo recovery (IVR), has received less attention. PK-guided dosing of VWF/FVIII concentrates has been shown to be effective and safe in patients with VWD undergoing surgery [51, 52]. However, little is known about the PK parameters during surgery in patients with VWD and the correlation between pre- and post-surgery IVR is weak [53].

The "patient tailored pharmacokinetic-guided dosing of clotting factor concentrates in bleeding disorders (OPTI-CLOT)" study group is a multinational collaboration that aims to develop PK-guided perioperative treatment strategies for patients with bleeding disorders. The OPTI-CLOT studies are using perioperative data from clinical studies to construct a perioperative population PK model that can facilitate PK-guided iterative adaptive Bayesian dosing. During this procedure individual PK parameters are iteratively updated by combining PK information (e.g. dose, concentration, time) from the individual patient with a priori PK information from the population. To date, PK modelling has been applied to perioperative data from a Dutch retrospective study in patients with haemophilia A [54] and PK modelling of perioperative VWD data is currently underway.

In the retrospective analysis of 119 haemophilia A patients undergoing 198 surgical procedures in the Netherlands, 45% of FVIII plasma concentrations were below the target range during the first 24 hours after surgery and 75% of the plasma concentration were above the target range after six days of hospitalization during perioperative management with FVIII concentrates [54] (Fig. 6). In addition, a reduction of 44% in the use of factor concentrates could have been achieved if plasma concentrations

had been maintained within target levels in the perioperative setting [54]. Population PK parameters were subsequently estimated in 75 adults undergoing 140 surgeries (median age: 48 years; median weight: 80 kg) and 44 children undergoing 58 surgeries (median age: 4.3 years; median weight: 18.5 kg) [55] who participated in the Dutch study [54]. The model incorporated variations in PK parameters, for example, clearance decreased with increasing age ($P < 0.01$), increased in cases with blood group O (26%; $P < 0.01$), and there was a small decrease during major surgical procedures (7%; $P < 0.01$). The final PK model showed a good agreement between FVIII concentrations predicted by the model and those assessed by laboratory measurements [55]. An ongoing, randomized controlled trial is comparing iterative Bayesian PK-guided dosing with a standard dosing regimen in 60 patients with haemophilia A [56].

Iterative Bayesian PK modelling of perioperative data from patients with VWD treated with VWF/FVIII in the WiN study (ClinicalTrials.gov Identifier: NCT00510042) is currently being evaluated in the To WiN study. Data for 103 patients (148 procedures) are being evaluated. The majority of patients have type 1 (52%) or 2 (42%) VWD and 6% have type 3. The To WiN study will also include a similar evaluation of patients treated with DDAVP in the WiN study. In addition, a prospective study of iterative Bayesian PK-guided dosing in VWD and surgical and dental procedures (OPTI-CLOT: TARGET) is planned.

Iterative Bayesian PK-guided dosing may allow individualization of perioperative therapy in patients with bleeding disorders and minimize under- or over-dosing during surgery. This approach has shown promise in patients with haemophilia A and data from patients with VWD will soon be available.

### Surgery in VWD

Bleeding is a major surgical complication and is associated with mortality rates of 20% in cases of severe bleeding [57]. Approximately 75% to 90% of intraoperative and early postoperative bleeding can be attributed to technical factors [57]. However, acquired or congenital coagulopathies may favour, if not directly cause, surgical haemorrhage in some cases [57].

Bleeding rates during surgery in patients with VWD have been shown to be high in the absence [58] and the presence [59] of perioperative haemostatic therapy. Bleeding rates during surgery, in the absence of perioperative haemostatic treatment, have been assessed in 311 patients (498 surgical procedures) who were diagnosed after surgery as definitive type 1 VWD (VWF:RCo 15–30 IU $dL^{-1}$) and possible type 1 VWD (VWF:RCo 31–49 IU $dL^{-1}$) [58]. Major haemorrhages occurred in 81 patients (26%) and 87 surgical procedures (17.5%) and there was no statistically significant difference between the percentage of type 1 and possible type 1 VWD patients who had major haemorrhages (32.6% and 24.8%, respectively) [58]. In a multicentre, cross-sectional study in patients with VWF:RCo ≤30 IU $dL^{-1}$, bleeding complications were reported by 19 of 79 (24%) patients in 23 of 126 (18%) large joint procedures despite the use of perioperative haemostatic therapy in the majority of cases (81%) [59]. Possible causes of excessive bleeding in VWD patients during surgery include: anatomic changes due to prior bleeds, including neovascularization; insufficient haemostatic therapy; lack of stringent perioperative coagulation factor (VWF and FVIII) monitoring; lack of experience of surgeons/haematologists; and unnecessary use of anticoagulants in patients with bleeding disorder.

The main treatment options for the perioperative management of VWD patients are DDAVP and replacement factor concentrates. European Haemophilia Treatment Standardisation Board (EHTSB) recommendations for the practical use of DDAVP [60] and factor concentrates [61] in VWD patients undergoing invasive procedures have recently been published. The suitability of DDAVP should be determined by a test infusion in candidate patients [60]. DDAVP is ineffective in type 3 VWD and its use in type 2B remains controversial due to the possibility of thrombocytopenia [60]. It can, however, be used effectively to cover minor surgery and dental procedures in most other VWD patients, although factor concentrate is generally preferred for major surgery [60]. Concomitant use of antifibrinolytics should be considered in most surgical interventions covered by DDAVP, particularly those involving the mucous membranes [60]. DDAVP is not recommended in patients with ischaemic heart disease; a history of stroke and/or peripheral vascular disease; myocardial infarction; heart failure; and hyponatremia [60]. Due to the risk of tachyphylaxis, DDAVP should not be given for more than 3–5 days unless the patient can be monitored closely and switched to a concentrate if this occurs [60].

Factor concentrates (VWF/FVIII or VWF) are the treatment of choice in patients in whom DDAVP is ineffective or contraindicated [61]. EHTSB recommendations on the perioperative dosing and monitoring of factor concentrates in VWD patients undergoing surgery, together with other published guidelines/recommendations, are summarized in Table 1. In the most recent publication, the EHTSB highlighted a number of important issues [61]. For example, the ratio of VWF:RCo/FVIII:C in the concentrates varies significantly and doses of concentrate expressed in IU $dL^{-1}$ are not interchangeable. Therefore, the recommended dose of a given concentrate should be specific to that concentrate as the required dose can be completely different when another concentrate is used. Similarly, because various types of VWD are characterized by great variability in VWF:RCo and FVIII:C, patients should have baseline values of VWF:RCo and FVIII:C measured before undergoing major surgery to plan treatment [61]. For monitoring, FVIII:C seems to be more important than VWF:RCo [61]. The parallel decay of VWF:RCo and FVIII:C curves with wilate® (Fig. 7), a VWF/FVIII with VWF:RCo/FVIII:C ratio of 1:1, may facilitate dosing and monitoring via either VWF:RCo or FVIII:C [62, 63], while differing half-lives for VWF:RCo and FVIII:C in other concentrates might require monitoring of both factor levels. Tranexamic acid was also recommended by the EHTSB as a valuable adjunctive agent that may be used concomitantly with concentrates in patients with VWD undergoing surgery, particularly those involving mucous membranes [61].

The perioperative management of patients with VWD can be challenging. Current treatment strategies are effective and will often include perioperative treatment with a factor concentrate. However, the dosing and monitoring of a given factor concentrate should be based on the properties of that concentrate and in accordance with its prescribing information, achieving critical target levels, but avoiding sustained excessive FVIII:C levels.

Surgery in the USA

PK analysis to guide dosing is not always done and is generally considered to be of academic interest. DDAVP is often used if the patient is responsive and combined with factor concentrate as required. When DDAVP is used it is important to monitor sodium levels to avoid water intoxication, particularly when fluid replacement during surgery is high. However, combination therapy is not the standard of

care in the US and most institutions only use factor concentrates. Achieving haemostasis during surgery is the most crucial factor and should take precedence over the current 'over-anxiety' about VWF and FVIII levels during surgery and potential thrombosis.

Treatment in the USA

Available treatments in the USA are adjunctive therapies (DDAVP and antifibrinolytics) and factor replacement concentrates. Adjunctive therapies should not be overlooked. DDAVP is effective in many patients and may be particularly useful for the pre-operative reversal of aspirin effects or in patients with coexisting renal insufficiency. Antifibrinolytics may be particularly useful in the treatment of GI bleeds and in oral surgery. There are many different indications for concentrates. It is important to recognize that the US Food and Drug Administration (FDA) license a product for a specific indication, yet clinicians have the right to use any product any time for any reason. This raises the issue of potential litigation and, to protect themselves, institutions may not permit the use of products that are not specifically indicated.

**Angiogenesis in VWD**

VWF and angiodysplasia

The prevention and treatment of gastrointestinal (GI) bleeding due to angiodysplasia is an important unresolved problem in the management of VWD patients. Angiodysplasia is characterized by degenerative lesions in the mucosal venules and capillaries in the GI tract associated with abnormal development of new blood vessels (angiogenesis) [64-68].

GI bleeding due to angiodysplasia is common in the elderly, with incidences ranging from 2.6% to 6.2% in the general population [69, 70]. Angiodysplasia-related GI bleeding is also a well-documented complication of VWD, although it occurs more frequently at a younger age and almost exclusively in patients lacking high molecular weight multimers (HMWMs) [65, 71, 72]. In a 2-year, prospective study of 107 patients, GI bleeding was much more frequent in patients with VWD characterized by a lack of HMWMs (type 2A) compared with type 2M VWD (Fig. 8).

Indirect evidence implicating a lack of HMWMs in angiodysplasia-related GI bleeding in VWD has come from studies in acquired von Willebrand syndrome, especially in patients with aortic stenosis or lymphoproliferative disorders [67, 68, 73, 74]. Aortic stenosis is often accompanied by a lack of HMWMs due to the increased shear stress exerted by the stenotic valve, which stretches the VWF molecule and increases susceptibility to proteolysis by ADAMTS-13 [75, 76]. Surgical correction of valve stenosis restores a normal multimeric pattern and abolishes bleeding complications [74]. The precise pathophysiological mechanisms underlying angiodysplasia-related GI bleeding in VWD have not yet been fully elucidated. However, in vitro data [77] have shown that low VWF in Weibel-Palade bodies of endothelial cells can promote angiogenesis and decrease vessel stability, which could lead to the vascular malformations typical of angiodysplasia [67].

The treatment of GI bleeding in patients with VWD and angiodysplasia is challenging due to frequent recurrence and the severity of bleeding, which usually increases with age [67, 68]. Replacement therapy with plasma-derived VWF/FVIII concentrates is the mainstay for the episodic treatment of acute GI bleeding in VWD, although the clinical effectiveness of VWF/FVIII concentrates in treating acute GI bleeds appears lower than for other bleeding sites [67, 68]. For example, a prospective study reported that a higher average daily dose (44 vs. 29 IU $kg^{-1}$) and a longer duration of treatment (4.23 vs. 1.93 days) were required for the treatment of GI bleeds compared with bleeds at other sites [78]. A similar picture has emerged in studies evaluating VWF/FVIII concentrates in secondary prophylaxis of recurrent GI bleeding, which have demonstrated the efficacy of VWF/FVIII concentrates but with lower efficacy than at other bleeding sites [67, 68]. In the largest published prophylaxis study, the VWD Prophylaxis Network retrospectively evaluated the efficacy of secondary prophylaxis with VWF/FVIII concentrates in 59 patients with clinically severe VWD that were unresponsive to other treatments [79]. Recurrent GI bleeding and epistaxis were the most common reasons to initiate prophylaxis, with each accounting for 23.6% (13/55) of cases [79]. Although the number of GI bleeds occurring during prophylaxis was significantly reduced, the percentage reduction in bleeding frequency (49%) was lower than that achieved for joint bleeding (86%) [67, 79].

A possible explanation for the lower efficacy of VWF/FVIII concentrates in treating GI may be related to the fact that replacement therapy does not replenish VWF within endothelial cells and platelets (only plasmatic VWF) and the HMWM composition of concentrates may be less efficient in treating GI bleeds compared with bleeds at other sites [68, 80].

A number of alternative therapeutic approaches for the management of recurrent GI bleeds have been explored [67, 68]. These include oestrogen, progesterone, octreotide, tranexamic acid and DDAVP, although these treatments do not appear to be effective based on the limited evidence currently available. Drugs with anti-angiogenic properties have also been used, such as thalidomide and very large doses of atorvastatin (up to 80 mg daily). Although a few cases have reported positive effects on the frequency of GI bleeding, larger controlled studies are needed to establish the clinical usefulness of these therapies. Surgical approaches have not been shown to be consistently effective because the angiodysplastic lesions are usually multiple and diffuse in the GI tract.

In summary, the prevention and treatment of GI bleeding due to angiodysplasia is an unresolved problem in the management of VWD patients. VWF/FVIII concentrates remain the mainstay therapy for the treatment of GI bleeds. Other strategies, such as rVWF and anti-angiogenic drugs, may have a future role in the prevention and treatment of GI bleeding due to angiodysplasia, but need further exploration.

Reproductive tissue alterations in VWD

Women with VWD are at increased risk of bleeds during the menstrual cycle and during pregnancy [81]. The most common symptom is menorrhagia (heavy menstrual bleeding), which is estimated to affect 32–100% of women with VWD [23, 81]. Although VWF levels are known to rise in pregnancy, levels in women with VWD remain significantly lower than in their healthy counterparts [81]. Thus, women with VWD are at increased risk of bleeding complications associated with pregnancy and childbirth.

A study conducted in pregnant women with type 1 or type 2 VWD investigated management of VWD during pregnancy. For inclusion in the study, patients had to have had at least two previous bleeding-associated miscarriages or were experiencing bleeding during an ongoing pregnancy. Data are available for 61 patients (mean age of 32.7 (±11.3)) with type 1 VWD who received replacement therapy as

secondary prophylaxis or treatment during pregnancy. Live birth rate in treated patients was 80.3% (49 of 61) compared with a live birth rate of 19.2% (28 of 146) prior to treatment ($P < 0.0001$). All 12 pregnancy losses during replacement therapy occurred in the first trimester. Of the 118 historical pregnancy losses, 98 (83.1%) occurred in the first trimester, 9 (7.6%) occurred in the second trimester and 11 (9.3%) occurred in the third trimester.

Complex and dynamic changes in vasculature occur during the menstrual cycle and during pregnancy and angiogenesis plays a vital role [81-83]. However, little is known about histological changes in women with VWD. In order to gain more insights into VWD during pregnancy, an animal study was initiated in 2016 and is due for completion in 2019. Several Meishan pigs [84] are being studied, suffering from type 3 VWD as well as type 1 VWD, and healthy controls. Collection of tissue from reproductive organs is planned for different stages of the menstrual cycle in non-pregnant animals of reproductive age, implantation, and placentation, respectively. Parameters being assessed include formation of blood vessels by haematoxylin and eosin staining, localization of angiogenesis mediators and receptors by immunohistochemistry, presence of causal mutations, and functional gene analysis using geneMANIA. Various angiogenic factors are being investigated in the study, including angiopoietin-1 (ANG-1), which promotes vascular maturation [83], vascular endothelial growth factor (VEGF), which is vital for vascular growth [83], and the αVβ3 integrin, which is a cell adhesion receptor [85].

Preliminary results from three reproductive organs – uterus (endometrium), ovary, and oviduct- showed significantly fewer blood vessels with small lumens, whereas type 3 VWD animals have more thin walled vessels which appear dilated and filled with red blood cells (data not shown). Compared with control animals, there is decreased or even absent VWF staining in the uterine glands and endometrium of VWD animals. While type 3 samples show strong αVβ3 staining, this is in clear contrast to type 1 samples. Ongoing studies include comparative quantification of the protein expression by real-time polymerase chain reaction (RT-PCR) to further elucidate the role of VWF in female reproduction and angiogenesis.

In conclusion, study results in VWD patients during pregnancy demonstrated a dramatic decrease in rate of pregnancy loss under VWD replacement therapy. Detailed analysis of the expression and localization of angiogenic factors in an animal model suggests that disrupted formation of vessels in several female organs may be an important pathogenic factor.

**VWF inhibitors**

Detecting inhibitors in VWD: an academic question?

Anti-VWF inhibitors are a rare complication of replacement therapy that negates the haemostatic response to infused VWF concentrates [86]. Inhibitors develop in an estimated 5.8% to 9.5% of patients with type 3 VWD and have very rarely been reported in patients with other VWD types [86, 87]. In a retrospective analysis of the Italian Association of Hemophilia Centers (AICE), 96 of 1650 VWD patients had type 3 VWD (5.8%). Anti-VWF inhibitors were identified in 6 type 3 VWD patients, which corresponds to 6.2% (6/96) of type 3 patients and 0.4% (6/1650) of the total VWD population [88]. In contrast, FVIII inhibitors developed in 19.2% (316/1688) of patients with severe haemophilia A [88].

These anti-VWF inhibitors are allo-antibodies and might be related to deletions of the VWF gene, although it is important to recognize that not all gene deletions are associated with inhibitor development. In the late 1980s, gene defects associated with inhibitor development were identified using the Southern blot technique [89, 90]. In one study, homozygous complete VWF gene deletions were identified in the 2 of the 19 type 3 VWD patients who developed inhibitors [89]. In another study, complete homozygous and heterozygous deletions were found in 6 of 10 type 3 VWD patients [90]. In the 1990s, one complete homozygous and one partial heterozygous deletion among 28 type 3 VWD German patients from 32 families were identified [91], whereas one complete heterozygous VWF gene deletion was identified among 5 type 3 VWD Italian patients [92]. A common 253-kb deletion involving VWF and TMEM16B (ANO2) was subsequently identified in German and Italian patients with severe type 3 VWD [93]. In addition, partial homozygous VWF gene deletions including exons 1–3, 6–16, 42, 33–38, 22–43, 23–52 and 17–18 have been described in 7 type 3 VWD patients; among these defects only the Alu-mediated deletion of exons 1–3 was found in several patients, constituting the most common defect in type 3 VWD patients in Hungary [94].

Whereas the Bethesda method with the Nijmegen modification is well established in the laboratory measurement of inhibitors in patients with haemophilia A, no general consensus has been reached for diagnosing anti-VWF inhibitors. Mix test assays, which are available in a small number of expert laboratories, mimic the Bethesda assays used in haemophilia A by measuring VWF/FVIII activities in patient-normal pool plasma after 2–4 hours incubation at 37°C. The titre of anti-VWF inhibitor is calculated by the actual dilution of VWD plasma inhibiting 50% of normal plasma pool diluted 1:2 compared with control mixture (buffer instead of patient plasma). Several solid phase tests have been proposed, but these are not frequently used. The inhibitory activity of VWF inhibitors should be assessed by a range of measures, such as RIPA in normal platelet rich plasma, anti-VWF:Ag, anti-VWF:RCo, anti-VWF:CB and anti-FVIII. However, antibodies might also occur against 'mute' regions of VWF molecules that cannot be identified with functional assays. An assay to test anti-VWF antibodies by using a sandwich ELISA (Ex-VWF-Plasma-HRP goat anti-human IgG-IgM) appears to be more sensitive than functional assays [95]. The outcome of an ongoing initiative of an ISTH subcommittee on VWF may lead to a more standardized approach to laboratory measurement of VWF inhibitors.

In most reported cases, antibody development is heralded by poor clinical response to replacement therapy accompanied by lower than expected recovery of VWF with absence of the delayed and sustained raise of FVIII (secondary response to VWF). In cases where inhibitor titre is relatively low, it is not difficult to treat soft-tissue bleeds and to prevent bleeding in surgery. In patients with high-titre inhibitors, replacement therapy is not only ineffective, but it may also trigger life-threatening anaphylactic reactions associated with activation of the complement system [96]. A rise in antibody levels is usually seen 5–10 days after replacement therapy with VWF concentrates, with features typical of a secondary response to a foreign antigen. Recombinant FVIII (no VWF) was effective in a type 3 patient with inhibitors undergoing emergency abdominal surgery [97]. Other possible therapeutic approaches are recombinant activated factor VII (rFVIIa) or a combination of rFVIII and rFVIIa [98-100]. However, the use of rFVIII and rFVIIa in combination should take into account the possibility of thrombosis [100].

The 3WINTERS–IPS study – Type 3 VWD International Registries and Inhibitor Prospective Study (3WINTERS–IPS, 2011–2018) has been set up to record clinical and laboratory data on a large cohort of at least 250 type 3 VWD patients at a network of European and Iranian centres [101]. The study comprises three phases (Fig. 9). In the first phase, ~250 type 3 patients were retrospectively identified. In the second phase, the diagnosis will be confirmed by centralized laboratory evaluation of the patients identified in the retrospective phase. Those patients with confirmed diagnosis of type 3 VWD (≥150 expected) will then enter a 2-year, prospective third phase of the study. So far, a total of 266 patients (146 Europeans and 120 Iranians) have been identified with type 3 VWD in the retrospective phase and laboratory samples are undergoing confirmatory centralized testing.

Even though the development of VWF inhibitors against replacement therapy is rare in patients with VWD, it is an important limitation of current therapy. 3WINTERS-IPS will provide important information on VWF inhibitors in type 3 patients and is expected to improve our understanding of the diagnosis and treatment of patients with type 3 VWD who develop inhibitors.

Case report of immune tolerance induction in two brothers with VWF inhibitors

Brother 1: Presented as a 7.5-year-old diagnosed with VWD at the age of 2.5 years after presenting with a foot contusion. At the age of 4 years he was hospitalized due to prolonged epistaxis and treated with VWF/FVIII concentrate every 12 hours. During this intensive treatment he developed mild shortness of breath responsive to albuterol, but subsequently did well with on-demand therapy.

Brother 2: Presented as a 20-month-old diagnosed with VWD at birth (asymptomatic) due to the family history of VWD. He experienced frequent epistaxis and iron deficiency anaemia, which was treated on-demand. He received about 15 prior VWF/FVIII concentrate infusions. Vague allergic symptoms were reported initially.

Genetic testing confirmed that both brothers had a large homozygous deletion, VWF c.658_7887del, consistent with type 3 VWD. Prophylaxis was initiated for each with VWF/FVIII concentrate, but brothers manifested allergic symptoms, which worsened with subsequent doses. The older brother had mild shortness of breath; the younger experienced progressive symptoms of shortness of breath, urticaria,

tachycardia, and mild hypotension. Symptoms were responsive to IV diphenhydramine. Despite trialling a different VWF/FVIII concentrate, similar symptoms were observed, so further use of VWF/FVIII concentrate was avoided.

While abroad, both were treated with a highly purified VWF concentrate. Although they did not develop allergic symptoms, bleed treatment was increasingly less effective. A mixing study confirmed the presence of neutralizing allo-antibodies.

Skin pick and intradermal testing to VWF/FVIII concentrates and excipients were not suggestive of an IgE mediated allergic response. There is currently no standard approach to immune tolerance induction (ITI) in patients with VWD inhibitors. Following a graded challenge for the older brother and a 12-step desensitization protocol for the younger brother, ITI with 50 VWF:RCo IU $kg^{-1}$ daily of a 1:1 VWF/FVIII concentrate and mycophenolate mofetil 600 $mg/m^2$ twice daily was initiated in both brothers.

During approximately 8 months of treatment, the brothers received rFVIIa and aminocaproic acid to treat ongoing epistaxis, gingival bleeding and occasional injury. For procedures FVIII infusion and platelets were also used. Due to the ongoing symptoms and presence of VWF inhibitors, the ITI regimen was changed to rituximab 375 $mg/m^2$ (4 doses) with continued VWF/FVIII concentrate treatment. Subsequent single rituximab doses were administered with B-cell recovery. This resulted in modest reduction of VWF inhibitor titres in both brothers and an improvement in clinical symptoms (Fig. 10).

**Memories of Margareta Blombäck on research on/about Åland and VWD**

I have had the great privilege of being involved in three research trips to the Åland Islands in 1957, 1977 and 1992 [102].

1957: Prior to the first trip in 1957, Inga Marie Nilsson and I had investigated many Swedish families with ‘pseudohemophilia’ and found low FVIII and a prolonged bleeding time [103]. In addition, Birger Blombäck and I had purified a plasma fraction (Fraction I-0) that contained fibrinogen and FVIII, which stopped bleeding in patients with VWD, normalized FVIII and corrected the prolonged bleeding time [102]. On the 1957 trip to the Åland Islands, I was joined by colleagues from Stockholm (Professor Erik

Jorpes and Inga Marie Nilsson) in collaboration with Birger Blombäck and Stig-Arne Johansson. We wanted to investigate whether the 'pseudohemophilia' in Åland and Sweden was the same. We analysed data from Family S described by Erik von Willebrand in 1926 and some other families. Investigations of 15 living patients showed that FVIII levels were 30–60% of normal, platelet activity was normal and there were no capillary defects. Infusion of Fraction I-0 to one patient resulted in normalization of FVIII activity and the prolonged bleeding time as we had previously seen in Swedish patients [104].

1977: I returned to the Åland Islands together with Dag Nyman (then working in Stockholm). By this time, VWF:RCo and VWF:Ag assays were available. We found that the families could be divided into four categories [102, 105]: the survivors of family S, the original family, from Föglö, had the characteristics of type 1 VWD, i.e. they had similarly decreased levels of VWF:Ag and VWF:RCo in addition to normal or decreased levels of FVIII. One family had a "pure" platelet dysfunction (cyclooxygenase defect), one family had a mixture of type 1 VWD and a cyclooxygenase defect, and one family had an "aspirin" type platelet dysfunction.

1992: On my final research visit to the Åland Islands, I was joined by Dag Nyman (now again in Åland) and Zhiping Zhang from Stockholm. We collected DNA in VWD families and found exon 18 mutation in those with bleeding symptoms in Family S and 2 other families as well as in a 2-year-old boy with severe VWD (homozygote) and his parents (heterozygotes) [102, 106]. One cytosine deletion in exon 18 was detected in all the families. Linkage analysis and genealogical studies suggest that the deletion in these families probably has a common origin also with the Swedish patients.

Pier Mannucci concluded by paying tribute to the enormous contribution that Margareta had made to the scientific and clinical understanding of VWD. This sentiment was shared by all present, including Gerda S, the non-affected sister of the index case, Hjördis S.

## Conclusions

The Fifth Åland Island Meeting 2016 was a great success as were the previous meetings on these beautiful islands. The soul of the meeting is to convene a nice blend of international leaders in the field of VWD and younger, interested and motivated physicians. The scientific agenda is translational going from molecular genetics and molecules via laboratory issues and pharmacokinetics to epidemiology and clinical challenges, and of course, history. The meeting has a high scientific standard with plenty of room for discussions and networking. However, there is also another dimension that is unique: a visit to Hjördis´ house and graveyard gave a fundament for those who contribute to future research and development in the understanding and treatment of VWD.

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## TABLE AND FIGURE LEGENDS

**Table 1.** Guidelines on the dosing and monitoring of VWD patients undergoing surgery.

**Figure 1.** Visualizing the life cycle of WPBs at the ultrastructural level [10-12]. (A) The life cycle of WPBs. (B) Correlative light and electron microscopy of nascent WPB formation. (C) Serial block face scanning electron microscopy imaging of WPBs adjacent to (yellow), and connected to (red) the Golgi (green). The nucleus is shown in blue. (D) Electron tomography of WPB (green) with numerous connections to the Golgi (yellow). WPB, Weibel-Palade bodies.

**Figure 2.** Clinical spectrum of VWD: implications for management. DDAVP, desamino D-arginine vasopressin; FVIII, coagulation factor VIII; IU, international units; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:RCo, von Willebrand factor ristocetin cofactor activity.

**Figure 3.** Location of key VWF mutations in type 1, 2 and 3 VWD. VWD, von Willebrand disease; VWF, von Willebrand factor.

**Figure 4.** Key locations of VWF mutations in type 2 VWD. VWD, von Willebrand disease; VWF, von Willebrand factor.

**Figure 5.** Distribution of VWD types in 670 index cases [45]. VWD, von Willebrand disease.

**Figure 6.** Achieved perioperative FVIII levels in a Dutch study in patients receiving clotting factor replacement therapy [54]. FVIII, coagulation factor VIII; IU, international units.

**Figure 7.** wilate® is characterized by parallel VWF and FVIII decay curves [63]. FVIII:C, coagulation factor VIII activity; IU, international units; VWF:Ag, von Willebrand factor antigen content; VWF:RCo, von Willebrand factor ristocetin cofactor activity.

**Figure 8.** Incidence rate/100 patient-years of spontaneous bleeding requiring treatment occurring in patients with type 2A or type 2M VWD [72]. VWD, von Willebrand disease.

**Figure 9.** Overview of the 3WINTERS–IPS study. BAT, bleeding assessment tool; FVIII, coagulation factor VIII; VWD, von Willebrand disease; VWF, von Willebrand factor.

**Figure 10**. Anti-VWF antibodies measured by ELISA during ITI. ELISA, enzyme-linked immunosorbent assay; FVIII, coagulation factor VIII; ITI, immune tolerance induction; MMF, mycophenolate mofetil; VWF, von Willebrand factor.

**Table 1.** Guidelines on the dosing and monitoring of VWD patients undergoing surgery.

| **Guideline/ recommendation** | **Dose, dosing frequency and monitoring** | |
|---|---|---|
| | **Minor surgery** | **Major surgery** |
| European Haemophilia Treatment Standardisation Board [61] | Pre-operative dose should target FVIII:C ≥80–100 IU $dL^{-1}$ and VWF:RCo ≥50 IU $dL^{-1}$<br>Postoperative doses should target trough levels of FVIII:C and VWF:RCo should be >50 and 30 IU $dL^{-1}$, respectively, on the day of surgery and for 3–5 days or until wound healing is complete. Simple dental extractions may require only one infusion immediately before the procedure, usually combined with tranexamic acid given orally for 7–10 days; the recommended peak target levels of both FVIII:C and VWF:RCo for this indication is >50 IU $dL^{-1}$ | Pre-operative dose should target FVIII:C ≥80–100 IU $dL^{-1}$ and VWF:RCo ≥50 IU $dL^{-1}$<br>Postoperative doses should target trough levels of FVIII:C of 80–100 IU $dL^{-1}$ on the day of surgery, decreasing to 50–80 IU $dL^{-1}$ on days 1–7 and 30–40 IU $dL^{-1}$ by day 14 or until wound healing has been completed. For VWF:RCo, these levels are >50 IU $dL^{-1}$ on the day of surgery, >30 IU $dL^{-1}$ until day 14 or until wound healing is complete |
| Evidence-based recommendations on the treatment of VWD in Italy [107] | Daily or every other day doses of 30–60 IU $kg^{-1}$[a] of VWF/FVIII to maintain FVIII:C levels >30 U $dL^{-1}$ until healing is complete (usually 2–4 days) | Daily doses of 50 IU $kg^{-1}$[a] of VWF/FVIII to maintain FVIII:C levels >50 U $dL^{-1}$ until healing is complete (usually 5–10 days) |
| Consensus Statement on the treatment of VWD in Spain [108] | 40–60 IU $kg^{-1}$ every 24–48 hours to maintain FVIII:C >80–100 IU $dL^{-1}$ on the first 2 days and >50 IU $dL^{-1}$ on the following days | 40–60 IU $kg^{-1}$ every 24–48 hours to maintain FVIII:C >30 IU $dL^{-1}$, from 5 to 7 days |
| United Kingdom Haemophilia Centre Doctors Organization [109] | FVIII:C should be monitored regularly in all major and most minor surgical procedures<br>FVIII:C should be ≥1.0 IU $mL^{-1}$ to cover major surgery and sustained above 0.5 IU $mL^{-1}$ in the postoperative period<br>The VWF:RCo should be monitored in major surgical procedures, particularly in the perioperative period. The VWF:RCo should be maintained above 0.5 IU $mL^{-1}$ in the perioperative period | |
| USA National Heart, Lung, and Blood Institute [23] | 30–60 U $kg^{-1}$ (loading dose[b]) and 20–40 U $kg^{-1}$ (maintenance dose) every 12–48 hours<br>Trough VWF:RCo and FVIII >50 IU/dL for 3–5 days | 40–60 U $kg^{-1}$ (loading dose[b]) and 20–40 U $kg^{-1}$ (maintenance dose) every 8–24 hours<br>Trough VWF:RCo and FVIII >50 IU/dL for 7–14 days |

[a]Dosage indicated for VWD patients with FVIII:C/VWF:RCo levels <10 U $dL^{-1}$.

[b]Loading dose is in VWF:RCo IU $dL^{-1}$.

FVIII:C, factor VIII coagulant activity; IU, international units; VWF:RCo, von Willebrand factor ristocetin cofactor activity; U, units.