

From DEPARTMENT OF WOMEN'S AND CHILDREN'S
HEALTH

Karolinska Institutet, Stockholm, Sweden

**VON WILLEBRAND DISEASE IN WOMEN:
HEAVY MENSTRUAL BLEEDING AND
OBSTETRICAL BLEEDING**

IGOR GOVOROV



**Karolinska
Institutet**

Stockholm 2018

All previously published papers were reproduced with permission from the publisher.

Cover illustration by [iStock.com/Bernie_photo](https://www.iStock.com/Bernie_photo)

Published by Karolinska Institutet.

Printed by E-Print AB 2018

© Igor Govorov, 2018

ISBN 978-91-7831-146-0

**VON WILLEBRAND DISEASE IN WOMEN:
HEAVY MENSTRUAL BLEEDING AND OBSTETRICAL
BLEEDING**
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Igor Govorov

Principal Supervisor:

Associate Professor Miriam Mints
Karolinska Institutet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Co-supervisor(s):

Associate Professor Margareta Holmström
Karolinska Institutet
Department of Medicine

MD, Ph.D. Roza Chairati
Karolinska Institutet
Department of Molecular Medicine and Surgery

Associate Professor Katarina Bremme
Karolinska Institutet
Department of Women's and Children's Health

Opponent:

Associate Professor Susanna Ranta
Karolinska Institutet
Department of Women's and Children's Health
Division of Pediatric Oncology

Examination Board:

Assistant Professor Anna-Elina Lehtinen
Helsinki University
Department of Hematology
Coagulation Disorders Unit

Associate Professor Karolina Kublickiene
Karolinska Institutet
Department of Clinical Science, Intervention and
Technology

Professor Angelica Lindén Hirschberg
Karolinska Institutet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

To my family

ABSTRACT

Von Willebrand disease is the most common inherited bleeding disorder worldwide with a prevalence, reaching 1% of general population. vWD is equally distributed between genders. However, vWD-affected females experience specific hemostatic challenges during menstruation and childbirth. Despite intensive research within the field, there are still many unsolved issues, related to the fundamental mechanisms of the increased bleeding tendency. This thesis was divided into clinical part (Study I and Study II) and fundamental part (Study III and Study IV). The former has explored current management options for vWD-patients, suffering from excessive menstrual bleeding and postpartum hemorrhage (PPH), respectively. Fundamental part aimed to enhance our understanding of the dynamics of hemostasis contributing to excessive menstrual bleeding in women with VWD.

Study I explored the prevalence of heavy menstrual bleeding (HMB) among vWD affected females, its impact on everyday life activities and overall health-related quality of life. Information was obtained using self-administered forms and medical records. Of the 30 women (18-52 years) included in the study, a half (50%) suffered from HMB. This occurred despite the fact, that the majority of women received treatment for HMB. Almost all women had limitations in their everyday life activities, caused by HMB. The overall health-related quality of life was lower with regards to 'bodily pain' in women with HMB, compared to general Swedish population. Close interaction between hematologists and gynecologists is necessary in order to prevent limitations and improve quality of life in women with vWD.

Study II investigated the incidence of PPH in women with vWD, its correlation with i) type of vWD ii) levels of vWF and FVIII iii) treatment options. 34 women (59 deliveries) occurred in 14 different clinics (years 1995-2012) were included in the study. The incidence of primary PPH, severe primary PPH and secondary PPH was 44%, 20% and 12%, respectively, which is greater than in general population. Women with type 3 vWD was at greater risk of experiencing severe primary PPH, compared to other types. Another risk factors were instrumental assisted delivery and undiagnosed vWD at the time of delivery. FVIII levels during late pregnancy was inversely correlated to blood loss during delivery. Therefore, in order to decrease morbidity, we should identify women with yet unknown vWD more actively, probably through providing them with the validated self-

administered blood questionnaires in the antenatal settings. Once being diagnosed, vWD require comprehended approach, so that PPH could be prevented.

Study III assessed the changes in hemostatic variables in women with vWD during a regular menstrual cycle and compared them with healthy controls. 12 vWD affected females (NOT pregnant/breastfeeding/on hormonal treatment) were compared with 102 healthy controls, matched for age and BMI. In women with vWD, thrombin generation profiles were altered, with prolonged lag-time, time to peak and decreased peak thrombin concentration, compared to controls. AT was also higher in the study group, which may potentially contribute to the excessive bleeding in this cohort. Within the vWD group, FVIII and FX were significantly lower during the luteal phase, than in follicular phase. The decrease in procoagulant agents prior to menstruation may predispose women with vWD to the development of HMB. Therefore, altered thrombin generation dynamics, together with increased AT and decline in FVIII and FX prior to menstruation, can potentially play role in the excessive blood loss during menstruation in women with vWD.

Study IV explored how do inflammatory and endothelial markers change during the menstrual cycle in women with vWD. 12 vWD affected females (NOT pregnant/breastfeeding) were compared with 102 healthy controls, matched for age and BMI. Within the study group (vWD), endostatin levels were higher during the follicular phase, than in luteal phase. Since endostatin inhibits angiogenesis and coagulation, its rise during early follicular phase (which corresponds to menstrual phase of the uterine cycle) may affect the hemostasis within the uterine cavity and therefore contribute to the development of HMB. Women with vWD were different from healthy controls in terms of balance between pro- and anti-angiogenic markers, with sICAM-1 and IL-6 being higher in women with vWD, compared to controls, while sVCAM-1, cathepsin S and sP-selectin were lower. The pattern was constant across the menstrual cycle. Hypothetically, this imbalance could lead to the formation of angiodysplasia - common complication of vWD. The above statement, however, requires verification in larger well-designed studies.

In conclusion; this thesis reports, that the current approaches are still insufficient in terms of preventing common vWD complications - HMB and PPH. It emphasizes the importance of early diagnosis, providing to the patients the reliable information on drugs, proactive management and close collaboration between hematologists and ob&gyn specialists. Furthermore, the thesis provides additional knowledge on how do coagulation and inflammatory systems change in response to the sex steroids fluctuations during the

menstrual cycle. It reaffirms intimate interactions between coagulation and inflammatory systems. Our work suggests, that multiple factors with versatile effects, rather than simple decrease in vWF, are responsible for the development of HMB in women with vWD.

LIST OF SCIENTIFIC PAPERS

- I. GOVOROV I, Ekelund L, Chaireti R, Elfvinge P, Holmström M, Bremme K, Mints M
Heavy menstrual bleeding and health-associated quality of life in women with von Willebrand's disease.
Experimental and therapeutic medicine 11.5 (2016): 1923-1929.
- II. GOVOROV I, Löfgren S, Chaireti R, Holmström M, Bremme K, Mints M
Postpartum Hemorrhage in Women with Von Willebrand Disease–A Retrospective Observational Study
PloS one. – 2016. – T. 11. – No. 10. – C. e0164683.
- III. GOVOROV I, Bremme K, Lindahl TL, Holmström M, Komlichenko E, Chaireti R, Mints M
Thrombin generation during a regular menstrual cycle in women with von Willebrand disease
In manuscript, to be submitted
- IV. GOVOROV I, Bremme K, Larsson A, Holmström M, Komlichenko E, Chaireti R, Mints M
Blood inflammatory and endothelial markers in women with von Willebrand disease
In manuscript, to be submitted

CONTENTS

1	Introduction	1
1.1	von Willebrand factor.....	4
1.2	Classification of von Willebrand disease.....	6
1.2.1	Type 1.....	6
1.2.2	Type 2.....	7
1.2.2.1	Type 2A.....	7
1.2.2.2	Type 2B.....	8
1.2.2.3	Type 2M.....	8
1.2.2.4	Type 2N.....	8
1.2.3	Type 3.....	9
1.2.4	Acquired vWS.....	10
1.3	Epidemiology.....	11
1.3.1	Animals.....	12
1.4	Clinical presentation.....	13
1.4.1	Heavy menstrual bleeding.....	14
1.4.2	Postpartum hemorrhage.....	16
1.5	Diagnostics.....	16
1.6	Management.....	18
1.6.1	Antifibrinolytic drugs.....	18
1.6.2	Desmopressin.....	18
1.6.3	Plasma-derived vWF/FVIII factor concentrates.....	19
1.6.4	Recombinant vWF.....	19
1.6.5	Treatment for gynecological complications.....	20
2	Aims of the thesis.....	21
2.1	Specific aims.....	21
2.1.1	Study I.....	21
2.1.2	Study II.....	21
2.1.3	Study III.....	21
2.1.4	Study IV.....	21
3	Materials and methods.....	23
3.1	Study I.....	23
3.1.1	Questionnaire.....	23
3.1.2	PBAC.....	23
3.1.3	SF-36.....	25
3.2	Study II.....	26
3.2.1	Laboratory measurements.....	26
3.3	Study III.....	27
3.3.1	Measurement of hemostatic variables.....	27

3.4	Study IV.....	28
3.4.1	Measurement of inflammatory and endothelial markers.....	28
3.5	Statistical analysis	29
3.5.1	Study I.....	29
3.5.2	Study II.....	29
3.5.3	Study III and IV	29
3.6	Ethical approval.....	30
4	Results	31
4.1	Study I	31
4.1.1	Menstruation pattern in women with vWD.....	31
4.1.2	Impact of menstruation on everyday activities.....	32
4.1.3	Health-related quality of life	33
4.1.4	Treatment for HMB.....	34
4.2	Study II	35
4.2.1	Incidence of PPH	36
4.2.2	PPH and type of vWD.....	36
4.2.2.1	PPH in undiagnosed vWD.....	37
4.2.3	PPH and levels of coagulation factors	37
4.2.4	PPH and hemostatic treatment	38
4.3	Study III.....	40
4.4	Study IV.....	43
5	Discussion.....	45
5.1	Study I	45
5.2	Study II	48
5.3	Study III.....	51
5.4	Study IV.....	54
6	General conclusions.....	57
7	Future perspectives	59
8	Acknowledgements	61
9	References.....	65

LIST OF ABBREVIATIONS

ADAMTS13	a disintegrin and m etalloproteinase with t hrombospondin type 1 motif, member 13
AHF	antihemophilic factor
AT	antithrombin
AUC	area under the curve
avWS	acquired von Willebrand syndrome
BT	bleeding time
CAT [®]	Calibrated Automated Thrombogram
CATHL	cathepsin L
CATHS	cathepsin S
COCs	combined oral contraceptives
cd	cycle day
CFC	clotting factor concentrate containing vWF and FVIII
CS	caesarean section
DDAVP	D-amino D-arginine vasopressin
ETP	endogenous thrombin potential
FII	coagulation factor II
FVII	coagulation factor VII
FVIII	coagulation factor VIII
FX	coagulation factor X
FVIII:C	coagulation factor VIII activity
HMB	heavy menstrual bleeding
HRQOL	health-related quality of life
HRT	hormone replacement therapy
hs-CRP	high sensitivity C reactive protein
IL-6	interleukin 6
IUD	intrauterine devices
Lag-time	time point at which thrombin generation begins
LNG-IUS	levonorgestrel-releasing intrauterine system
PBAC	pictorial blood loss assessment chart

PPH	postpartum hemorrhage
PPP	platelet poor plasma
PRP	platelet rich plasma
PT	prothrombin time
PT-INR	prothrombin time – international normalized ratio
rvWF	recombinant vWF
SF-36	short form-36
sE-selectin	soluble E-selectin
sICAM-1	soluble intercellular cell adhesion molecule 1
sP-selectin	soluble P-selectin
sVCAM-1	soluble vascular cell adhesion molecule 1
ttpeak	time to reach peak thrombin concentration
TA	tranexamic acid
vWD	von Willebrand disease
vWF	von Willebrand factor
vWF:RCo	von Willebrand factor ristocetin cofactor assay

1 INTRODUCTION

Just then a woman who had been subject to bleeding for twelve years came up behind him and touched the edge of his cloak. She said to herself, “If I only touch his cloak, I will be healed.”

Jesus turned and saw her. “Take heart, daughter,” he said, “your faith has healed you.” And the woman was healed at that moment.

Matthew 9:20-22

Throughout the history of mankind, blood has always been considered as an entity, which sustains and preserves life. Blood was used in rituals and sacrifices, was a subject of controversy, both between medieval doctors and philosophers. Of all body fluids, blood is the most sacred one. What Mephistopheles called ‘Blut ist ein ganz besonderer Saft’¹. The equating of life with blood is natural and inevitable, as life leaves a person when he bleeds to death.

Despite significant progress in the development of medical science, blood still conceals many secrets. And among the most unexplored ones is the enigma of von Willebrand disease.

It is traditionally stated that the first description of this disease was given by the Finnish doctor Erik von Willebrand in the second quarter of the twentieth century (1). Worth mentioning, that there are earlier reports, with Minot&Lee (2) were probably the first to describe this disease in 1920, followed by reports from four independent groups (3, 4). However, today it is impossible to say exactly what kind of bleeding disorder was described in every single case, back 100 years ago. Diagnostic tools were not perfect and many bleeding disorders shared the same clinical features. For example, nosebleeds, that are common in patients with von Willebrand disease, were previously attributed solely to hereditary telangiectasia (Osler–Weber–Rendu disease), to that extent, that “the possibility of epistaxis being a cardinal symptom of another bleeding disease was given little consideration” (5).

Nevertheless, today this disease deservedly bears the name of Erik Adolf von Willebrand, because he was the one who brought the attention to this condition. While reviewing medical

¹ ‘Blood is a juice of very special kind’

journals, he marked a peculiar fact. There were 19 reported cases of extensive familial bleedings. Despite the fact that both women and men were affected, all cases were treated as hemophilia. This was in opposite to Nasse's law formulated in 1820: hemophilia affects only boys but is transmitted through females. Was it possible that under the guise of hemophilia there was another completely unknown disease? A family from Åland Islands helped him to find the answer to this conundrum.

It all started with 5-years old girl – Hjördis, who was admitted to the hospital with rather severe bleeding. Subsequently, she exsanguinated to death during her fourth menstrual cycle at the age of 13. Erik von Willebrand discovered that many of her relatives also suffered and even died from mysterious bleedings (Figure 1). Surprisingly, bleeding trait was not only more common among females (16/35 in women vs. only 7/31 in men), but also manifested in graver forms. Year 1925, Federley concluded that in contrast with classical hemophilia, both genders are equally affected and the mode of inheritance is dominant. Despite the predominance of female bleeders, von Willebrand cautiously described it under the name "hereditary pseudohaemophilia" (1). Interestingly, when Rendu for the first time (1896) described telangiectasia, a condition that now bears his name, he also called it pseudohaemophilia (6). It is understandable, as hemophilia was widely known and thus usually served as the reference for other bleeding conditions.

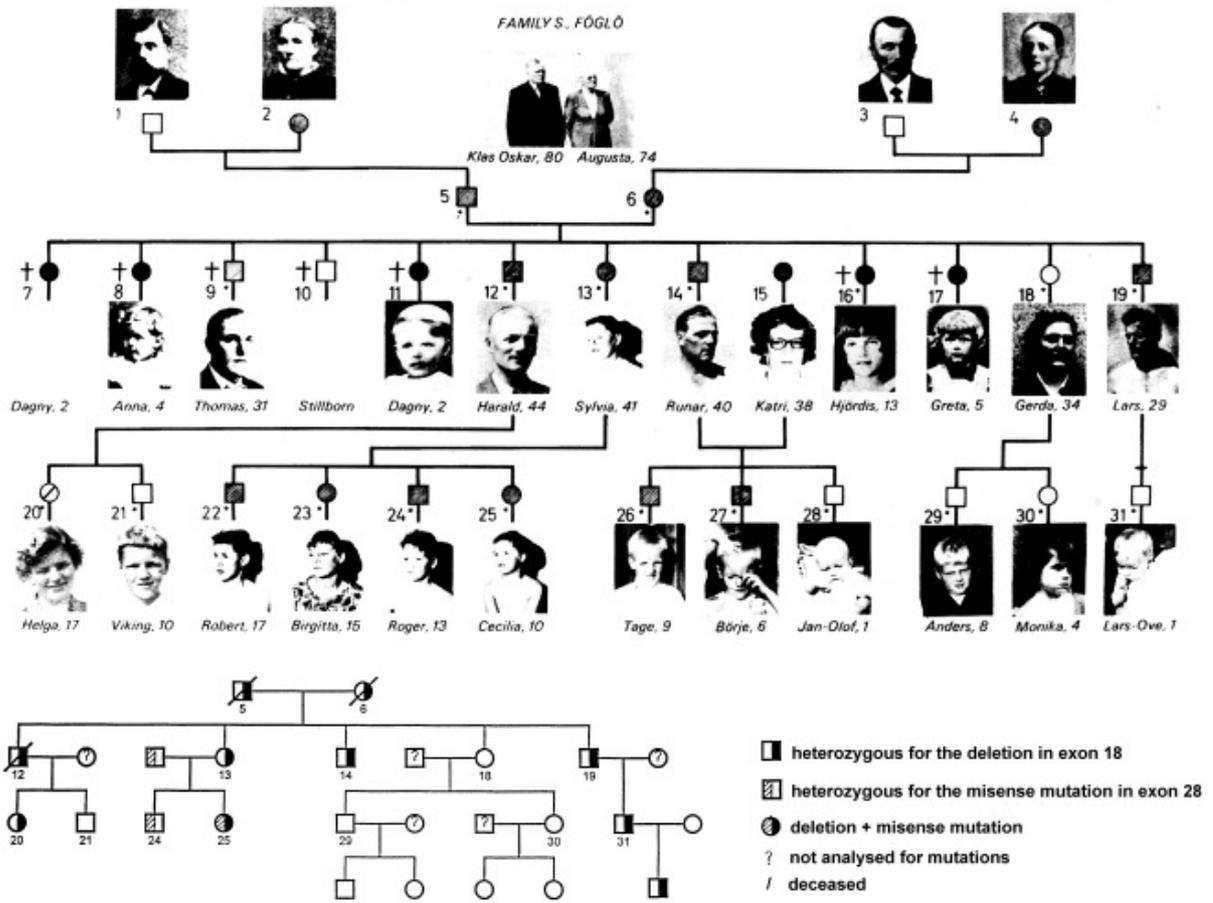


Figure 1. Pedigree of the family described by Erik von Willebrand (so called Family S). The index case (Hjördis) is in the second row, #16. Reproduced with permission from Thieme Group from D. Nyman *et al.* Recent Investigations of the First Bleeder Family in Åland (Finland) Described by von Willebrand. *Thromb Haemost* 1981; 45(01): 073-076.

Rigorous analysis of the clinical features and blood tests helped Erik von Willebrand to distinguish the new disease from thrombocytopenic and anaphylactoid purpurae and also from thrombasthenia, described by Glanzmann (7).

During next decades, there were several scientific trips to Åland, usually when a new laboratory method became available. Unfortunately, most of these investigations did not result in any clarification. New theories emerged, so did the names of the disease: pseudo-haemophilia (8-10), vascular haemophilia (11-14), angiohaemophilia (15-18). Willebrand-Jurgens syndrome (19, 20), Minot-von Willebrand syndrome (5).

In the 1950s new methods contributed to determination of the antihemophilic factor (AHF), its low level was subsequently associated with prolonged bleeding time (8, 10, 13, 17, 21, 22). At the same time, half of the vWD patients also had positive tourniquet test and/or morphological abnormalities in capillaries, suggesting a defect in the vessel wall (13, 23).

The next step was taken by Blombäck&Blombäck, scientist couple from Sweden, who succeeded in purifying and concentrating AHF in so called plasma fraction I-0 (24). Together with Nilsson they went to Åland and discovered that out of sixteen patients with von Willebrand disease all but one had decreased levels of AHF - a defect, which, however, could be corrected by I-0 fraction (25). Surprisingly, they also found that even if I-0 fraction was prepared from hemophiliac's blood (and therefore contained almost no AHF), it could still correct bleeding time in patients with von Willebrand disease. It became obvious, that there was a specific plasma factor, different from AHF, that is lacking in patients with von Willebrand disease. It took another 30 years to purify and establish the ultimate sequence of the lacking plasma protein (26-29). In memory of the great scientist, the protein was called – von Willebrand factor.

1.1 VON WILLEBRAND FACTOR

von Willebrand factor (vWF) is a large multimeric blood glycoprotein with versatile functions both within and beyond the hemostasis.

vWF gene is located on the short arm of chromosome 12 and composed of 178 kilobases (52 exons) (26, 27, 30). Megakaryocytes and endothelial cells are the primary sites of the vWF production.

vWF is initially produced as a large precursor protein, containing 2813 amino acids, which subsequently goes through extensive posttranslational modification. The signal sequence (aa 1-22) and the propeptide (aa 23-763) detach sequentially from the molecule. Remaining subunit (aa 764-2813) corresponds to mature vWF monomer. These monomers then merge into pairs within the endoplasmic reticulum, through binding the C-terminals with disulfide bonds (31). Further modifications take place in Golgi and post-Golgi compartments and include fusion of the dimers into multimers of different sizes. The latter may range from 500 000 to more than 20 000 000 daltons. Observations testify, that the larger multimer is, the more binding sites for collagen and platelets it provides, *i.e.* the more active it is. Multimers of smaller size are constitutively secreted into the plasma, while the bigger ones are stored in platelets and endothelial cells, waiting to be released in response to external stimulation (epinephrine, histamine, thrombin, estrogen or vasopressin analogue D-amino D-arginine vasopressin - DDAVP) (32-34).

vWF is not only stored within α -granules in platelets and so called Weibel-Palade bodies in endothelial cells, but rather actively interplays with them. It was shown that proper compaction of otherwise massive vWF multimers not only sustains typical rod-shape of Weibel-Palade

bodies, but also plays an important role in fast exocytosis. Long filaments of vWF are stored densely packed into tubules, which prevents them from tangling and also promotes rapid unfolding at the time of exocytosis (35). The actin filaments that make up the cytoskeleton, in turn, take an active part both in preventing and augmenting vWF exocytosis (36). Single Weibel-Palade bodies can exocytose individually or several organelles can fuse within the cell in order to produce large secretory pods prior to release of vWF (37).

Role distribution between the vWF derived from platelets and endothelial cells is currently debatable (38, 39).

vWF structurally consists of several domains (Figure 2), each of them has a specific function:

- D'D3 assembly provides the binding site for FVIII (coagulation factor VIII) (40);
- A1 - binding sites for platelet GPIb, collagen, heparin, and ristocetin (41-43);
- A2 – binding site for cleaving protease ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) (43, 44);
- A3 – primary binding site for collagen (43);
- C4 – binding site for platelet receptor GpIIb/IIIa (45);
- CK – dimerization interface (45).

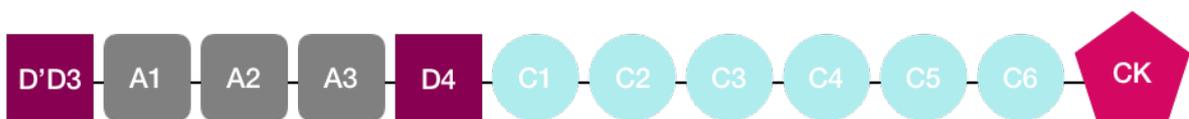


Figure 2. Schematic illustration of vWF domains

Such a complicated structure plays though an important physiological role, as conformational changes in vWF domains can modulate the ultimate blood clotting functions (46).

In addition to participating in primary hemostasis, through binding to the exposed subendothelial matrix, vWF is a major player within the secondary hemostasis. This activity is due to vWF ability to bind FVIII. It is important to note, that this binding is size-independent, *i.e.* vWF multimers of different size may participate (47). FVIII is highly dependent on vWF, since the latter serves as the carrier. In the absence of vWF, FVIII undergoes proteolytic cleavage five times faster (48, 49).

Obviously, vWF also plays a significant role in pathogenesis of the thrombotic diseases, such as thrombotic thrombocytopenic purpura, ischemic stroke and coronary heart disease (50-53).

New therapies based on inhibiting vWF activity are currently under development (54). Overall, being an acute phase reactant, vWF may fluctuate due to many pathological and physiological conditions. These determinants include, but is not limited to gender, nation and race, phase of menstrual cycle, stress and exercise.

vWF spatial conformation, and hence its functional activity, depends on rheological characteristics of blood, and primarily, the shear force. Interestingly, vWF is the only plasma protein able to operate in high shear stress environment (more than $50,000\text{s}^{-1}$) (55). While under low shear stress conditions vWF remains in globular form, with its active binding sites concealed. When the shear force increases, vWF elongates, revealing its receptors for platelets and fibrinogen. Moreover, binding site for the cleaving protease - ADAMTS13 becomes accessible, which is essential in vWF down-regulation (56). ADAMTS13 cleaves large vWF multimers (cleavage site - A2 domain) into smaller, less active ones (57, 58). Wu *et al.* had cleaved vWF into smaller peptides and identified 24 amino acid sequence (pro1645 – lys1668) as the shortest peptide that can competitively bind to ADAMTS13 (57). This suggests existence of complementary sequence at ADAMTS13. Average half-life of normal vWF in plasma is 8-12 hours. However, it is usually shortened in people with blood group 0 (see Epidemiology section for possible explanation).

Animal studies indicate, that vWF inactivation occurs in the macrophages of the spleen and liver (59). However, spleen contribution to the process is limited, as evidenced by monitoring patients after splenectomy. In asplenic patients DDAVP administration results in vWF rise and subsequent decline, comparable to that of healthy individuals' (60).

The complex structure and versatile functions of the vWF not only ensure its participation in many physiological processes, but, unfortunately, also contributes to the development of the versatile diseases and first of all - von Willebrand disease.

1.2 CLASSIFICATION OF VON WILLEBRAND DISEASE

Impairment in vWF function arises either from quantitative deficiency or qualitative alterations. Based on that all cases of vWD are classified into one of three types.

1.2.1 Type 1

Type 1 is the most common form of vWD, accounting for 75-80% of all cases. It arises from partial quantitative deficiency of vWF, which may result in bleeding tendency of various degree, from mild to severe.

vWF level strongly correlates with bleeding risk. vWF levels <30 IU/dL are associated with clinical severity and presence of mutations in vWF gene (61, 62).

Cases clinically consistent with type 1 vWF are heterogeneous in relation with causative mutations. For instance, R760H mutation results in concordant decrease in vWF:Ag, vWF:RCO and vWF:CB, with preserved multimer pattern (63). Several mutations (C1149R, Y1584C) result in intracellular retention of vWF and its consequent decreased plasma levels (64, 65). Y1584C also makes vWF susceptible to proteolysis (66).

A very specific subtype, Vicenza, arises from the replacement of Arg-1205 by His and is characterized by increased vWF clearance and presence of ultra-large multimer, similar to those occurring after DDAVP infusion (67, 68). This was confirmed in animal studies, where recombinant R1205H vWF was infused in vWF-deficient mice and had shortened survival compared with wild-type vWF (0.3h vs. 2.8h respectively) (69).

Mutations may have different penetrance and in some cases are exacerbated by O-blood group (70). At the same time, specialists currently agree that there is no need to conduct routine mutation analysis among patients with clinical symptoms corresponding to type 1. However, this approach could be helpful in establishing accurate diagnosis in unclear cases.

1.2.2 Type 2

Type 2 vWD embraces four subtypes that are all characterized by qualitative changes in the structure of the vWF.

In contrast to type 1, in which vWF levels confidently predict bleeding, other variables such as platelet count, absence of large multimers or exact mutation determine bleeding tendency in type 2 vWD. Clinical and familial history is also of a great value (71).

1.2.2.1 Type 2A

Type 2A is primarily characterized by loss of the most active large and ultra-large multimers. Experiments on cell cultures transfected with mutant vWF allowed to detect two possible mechanisms of “large multimers loss”. Several mutations cause defect in intracellular transport of vWF, which becomes retained within the cell, even upon secretagogue stimulation (72). It is assumed, that larger multimers, containing more defective monomers are more likely to linger within the cell. It results in relatively more efficient secretion of smaller multimers – typical laboratory pattern of the subtype 2A. An alternative mechanism may be due to the increased cleavage of predominantly large multimers. In latter case synthesis and secretion of

larger multimers remain unaffected, but they undergo rapid proteolysis moderated by specific plasma protease, possibly a calpain (73-75). These assumptions are confirmed by preservation of multimer structure in platelet vWF (stored in α -granules) and secretion of intact multimers in transfection cell studies (72).

It is not unusual that vWF-platelet interaction is also disrupted. With several exceptions, desmopressin administration is usually ineffective in patients with type 2A, as it results in secretion of hemostatically dysfunctional units of vWF (76, 77).

1.2.2.2 Type 2B

Type 2B accounts for 5-8% of all vWD cases. It is characterized by gain-of-function in A1 domain, *viz.* increased affinity of vWF to platelet receptor complex GpIb/IX/V (78, 79). It results in spontaneous vWF/platelet binding and subsequent clearance from circulation, at that larger multimers, being more active, bind vigorously to the platelets. Ultimately, it leads to a very specific laboratory pattern and clinical manifestations. For instance, desmopressin administration in patients with type 2B may result in increased bleeding tendency due to accrescent thrombocytopenia and thus DDAVP is contraindicated in this cohort (80). Thrombocytopenia is also usually aggravated during conditions associated with short or long-term rise in vWF, such as pregnancy, surgeries or infections (81-83). Worth mentioning that thrombocytopenia represents an independent bleeding risk in type 2B patients (84).

The identification of vWF gene mutations in suspected type 2B cases allows its distinction from so-called platelet-type vWD, characterized by a similar phenotype but with mutations located in the platelet GpIb receptor (85).

1.2.2.3 Type 2M

In type 2M alterations in vWF structure are similar to those in type 2B in the sense of affected domain – A1. However, phenotype is completely the opposite, manifesting with loss-of-function abnormalities. vWF exhibits decreased ability to bind with platelets, whereas its multimer distribution usually remains unaffected.

DDAVP administration is usually of no effect, albeit test dose may be prescribed to evaluate individual response.

1.2.2.4 Type 2N

Unlike other subtypes, type 2N is inherited as autosomal recessive trait.

Type 2N represents qualitative defect in vWF causing altered binding to FVIII. The latter becomes vulnerable to rapid proteolysis.

The identification of type 2N mutations, which is suspected in the presence of a marked reduction in FVIII:C in comparison to vWF and is confirmed by the FVIII-vWF binding test (vWF:FVIII:B), is important for genetic counseling to exclude the presence of the state of carrier for hemophilia A. It is not unusual that clinical picture in type 2N vWD patients resemble to hemophilia, primarily due to significantly decreased levels of FVIII and, as a result, soft-tissue bleeding. However, in opposite to X-linked hemophilia, type 2N vWD show autosomal recessive inheritance.

Some patients with type 2N may benefit from DDAVP administration, though altered binding to FVIII may sometimes counteract rise in plasma vWF levels (86). In other cases vWF-containing concentrates are preferable.

1.2.3 Type 3

Type 3 is the most rare type of vWD, with only 0.1-5.3 cases per million population, varying significantly between countries (87-89).

At the same time, it is the most severe type manifesting with life-threatening bleeding. It is characterized by dramatical decrease in vWF levels (almost ultimate absence in some cases). Thus, patients with type 3 vWD usually become symptomatic early during infancy or when they become mobile. Consanguinity is a frequent finding among patients with type 3 vWD (90, 91).

In conditions of severe vWF paucity FVIII, left unprotected, undergoes rapid cleavage. Thus, clinical picture in type 3 vWD patients may mimic hemophilia (92). However, vWD usually manifests with mucocutaneous bleedings in contrast to hemophilia, in which soft-tissue bleedings are more common. Lak *et al.*(91) performed large comparative study between 385 patients with type 3 vWD and 100 age-matched hemophiliacs and reported certain differences. Menorrhagia and epistaxis were more common, as expected, among vWD patients than in patients with hemophilia ($p < 0.0001$), while hemarthrosis and muscular hematomas were more frequent among patients with hemophilia ($p < 0.0001$).

DDAVP is ineffective in the vast majority of cases, due to critical lack of vWF reserve in depot. Plasma derived factor concentrates or recombinant coagulation factors normally become a method of choice. Nevertheless, this approach occasionally leads to the unwanted sequelae to be described in the "Treatment" section.

1.2.4 Acquired vWS

Year 1968, Simone *et al.* (93) reported an intriguing clinical case. A young boy was admitted to the hospital with complaints and clinical picture consistent with von Willebrand disease. However, his increased bleeding tendency was recent in onset and none of his relatives experienced prolonged bleeding episodes. Coagulation tests were also normal in his relatives. Later he was diagnosed with systemic lupus erythematosus, which required administration of corticosteroids. Bleeding symptoms disappeared shortly after initiation of therapy and did not return even after cessation. It all led to belief that bleeding syndrome was acquired rather than inherited and it was named “acquired von Willebrand syndrome (avWS)”.

avWS is an uncommon condition with slightly more than 700 cases reported (94). However, real numbers can hardly be established, due to the fact that not all patients are diagnosed and not all cases are reported. avWS occurs in any age, though is more common in elder, with median age of onset being 62 years (95).

AvWS is always secondary to the main disease. Federici *et al.* (95) analyzed data from an international register (186 cases) and discovered that avWS was related to:

- lymphoproliferative/myeloproliferative disorders in 63%,
- cardiovascular disorders – 21%,
- solid neoplasms - 5%,
- autoimmune diseases – 2%,
- miscellaneous disorders – 9%.

Another common primary condition giving rise to avWS is hypothyroidism. Bleeding tendency and related laboratory abnormalities normalize with rare exceptions (96) after substitution therapy with levothyroxine (96-98). Several drugs, such as ciprofloxacin (99), valproic acid (100) and griseofulvin (101) were also reported being the possible provoking agents.

Several possible mechanisms of pathogenesis were proposed:

- High shear-stress induced disintegration. vWF is extremely dependent on hemodynamical conditions in blood flow. In high shear stress milieu vWF unfolds and elongates, making its otherwise cryptic cleavage sites susceptible for ADAMTS13. It usually occurs in the settings of pronounced vessel obstruction, due to atherosclerosis, malformations or valvular heart disease (102, 103). Several iatrogenic conditions are also responsible for avWS – placement of left ventricular assist devices or artificial valves, as they also may cause high shear forces (104, 105).

- Formation of antibodies against vWF or vWF/FVIII complex. This is potentially applicable to the autoimmune diseases, monoclonal gammopathies and lymphoproliferative disorders (106-108). Certain antibodies may be more directed towards larger multimers, explaining typical “large multimer loss” pattern. Interestingly, it seems like antibodies may cross the placenta, causing transient avWS in baby (109).
- Adsorption onto cells. Certain type of cells are able to precipitate vWF on their surfaces and in this way take it out of circulation. Possible candidates are malignant cells or abnormal lymphocytic clones in multiple myeloma, Wilms tumor or non-Hodgkin lymphoma (110).
- Other causes are relatively rare and may include reduced synthesis, *e.g.* in hypothyroidism (97, 111), or proteolytic cleavage of vWF by proteases other than ADAMTS13 (112).
- Multicausality is also possible.

1.3 EPIDEMIOLOGY

Von Willebrand disease is considered to be the most common inherited bleeding disorder with 1% of general population having decreased levels of vWF upon laboratory investigation. However, numbers vary significantly depending on calculation method. Clinically evident vWD occurs 100 times less often with prevalence of 3-10 cases per 100000. In Nordic countries prevalence is 8 per 100000 inhabitants (113). However, even in this proportion, vWD cause significant effects, both at individual and socio-economic levels. Recent Swedish study reported that patients with vWD are twice more frequently hospitalized (inpatient and outpatient) than controls (114).

In severe cases equal male:female ratio is obvious, in contrast to milder forms, which are more often diagnosed in women, probably due to hemostatic challenges during menstruation and childbirth (115).

vWD is present everywhere across the globe. However, it may be of a clinical importance to keep in mind, that Caucasians generally have lower vWF levels compared to African Americans (116, 117).

Interestingly, people with blood-group O have lower levels of vWF in plasma (up to 35% less). The reason for that probably lies in the structural features of vWF. ABO determinants link to vWF during glycosylation process. It is believed that these carbohydrates play an important role in vWF polymerization and also determine clearance of vWF. ABO significantly

accelerates vWF clearance from plasma, but does not influence its production or secretion. It is confirmed by increased ppvWF/vWF ratio and rapid decrease in vWF levels after DDAVP administration (118). High heterogeneity of vWF levels in different blood groups makes ABO-specific reference ranges a reasonable approach (119, 120). When comparing with standard donor plasma, vWF levels may fluctuate from 75% for blood group O to 123% - for AB (119).

Overall, type 1 vWD is the most common type, accounting for up to 70% of all vWD cases. Type 2 is responsible for 25% of cases, while subtypes prevalence distributes in following order – 2A>2N>2M>2B (121). Type 3 is the most rare one with less than 5% affected.

1.3.1 Animals

vWD-like conditions is encountered not only in humans, but also in many animal species. While mice are used as vWD models, other animals, such as dogs, cats, horses and pigs develop vWD naturally (122-128). vWD was described in dozens of dog breeds, with extreme high prevalence in certain ones – 43% of Corgi and up to 73% of Doberman Pinschers are affected (Figure 3) (127, 129).

Interestingly, animal studies suggested that vWD might provide some resistance against atherosclerosis and bacterial endocarditis (130, 131). Unfortunately, it has never been demonstrated in humans.



Figure 3. Corgi and Doberman Pinschers are often affected with vWD

Picture of Welsh Corgi by Bensbro at Wikimedia Commons, licensed CC BY-SA 4.0
<https://commons.wikimedia.org/wiki/File:WelshCorgi.jpeg>

Picture of European Dobermann by Ilicivan at English Wikipedia, public domain
https://commons.wikimedia.org/wiki/File:European_Dobermann.jpg

1.4 CLINICAL PRESENTATION

Von Willebrand disease usually manifests with mucocutaneous bleedings. Soft tissue hemorrhages similar to those in hemophilia are rare and occur in case of significant decrease in vWF and, as a consequence, FVIII. However, clinical symptoms may vary considerably due to the heterogenous nature of vWD. The most frequent symptoms with corresponding prevalence among patients with vWD are shown at Figure 4 (132).

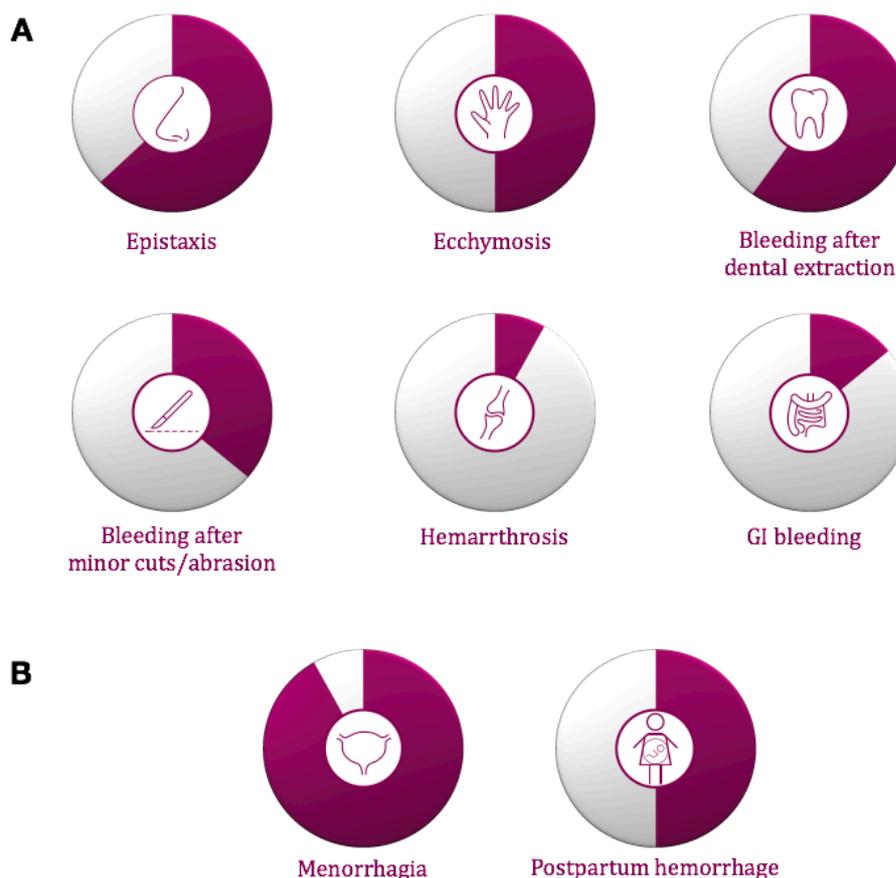


Figure 4. **A.** Most frequent clinical symptoms of vWD in both genders. **B.** Prevalence of heavy menstrual bleeding and postpartum hemorrhage in female vWD patients. Colored segments illustrate the percentage of vWD-affected patients experiencing the exact symptom.

However, clinical symptoms may be rather extraordinary. Argyris *et al.* (133) and Ozhan *et al.* (134) reported two cases of maxillary pseudotumors as a manifest of vWD (type 2N and type 3, respectively). According to the histological examination, lesions were characterized by cystic spaces filled with organizing hematoma. Previously, maxillary pseudotumor was considered a rare complication of hemophilia A or B, with less than 30 cases reported.

It was established that vWF levels are inversely correlated with angiogenic cytokines and circulating endothelial cells, which results in increased angiogenesis in vWD patients (135, 136). Microscopical evaluation of the capillaries in vWD patients testifies the presence of

structural alterations – capillaries are tangled, with irregular contour and fail to constrict properly (23, 137).

Decreased clotting potential together with alterations in angiogenesis may act jointly, predisposing individual to vascular abnormalities, such as Dieulafoy's lesions or pseudoaneurysms (138). Pseudoaneurysms of different localizations (*aa. supraorbitalis, glutea superior, hepatica, temporalis superficialis, uterina*) were previously reported in patients with vWD (139-143). Another possible complication is the formation of arteriovenous malformations in vWD patients. However, no comparative studies between vWD and non-vWD patients in regards to prevalence of arteriovenous malformations were performed to date. Nevertheless, reported cases emphasize the necessity to suspect structural lesions in vWD patients with intracranial hemorrhage and resist the temptation to attribute it only to the bleeding tendency and previous trauma, as it may significantly affect management and prognosis (144).

Angiodysplasias are also a common findings in patients with vWD, especially when gastrointestinal bleeding is present. It was reported that angiodysplastic lesions occur in up to 10% of vWD affected patients (145). Angiodysplasia may affect any part of the gastrointestinal tract, from tongue to colon (146, 147). One of the hypothesis links frequent occurrence of angiodysplasia in vWD patients to angiogenesis enhancement (148). Interestingly, it was shown that FVIII expression is reduced in endothelial cells within the affected areas, while remaining normal in non-ectatic regions (149). This hypothetically implies a close relationship between alterations in angiogenesis and coagulation. However, it is still debatable whether vWF deficiency predisposes to angiodysplasia formation or barely instigates bleeding onset from preexisting vascular anomalies. Dysplastic vessel regions are vulnerable and may cause recurrent gastrointestinal bleedings. Current treatment options are thalidomide and its derivative lenalidomide, although administered off-label (150).

In addition, there were reports of a greater spread of mitral valve prolapse among patients with vWD disease than in age, sex-matched controls (60% vs. 13.3%, $p < 0.01$) (151). Overall, co-existence of vWF deficiency and disorders, accompanied with mesenchymal dysplasia (skeletal abnormalities, dysplastic vessels, Ehlers-Danlos syndrome) suggests, that vWF may play a major role in the development and functioning of mesenchyme (152).

1.4.1 Heavy menstrual bleeding

The very first patient described by Erik von Willebrand was a young girl – Hjördis, who unfortunately bled to death during her fourth menstruation. Excessive cyclic bleedings still

remain a significant problem in vWD-affected females. The situation is complicated by the fact that in the modern world, reproductive behavior has dramatically changed. Today, women experience 10 times more menstrual periods than their ancestors (153).

There are obvious difficulties with the precise assessment of menstrual blood loss. Furthermore, classification approaches may differ and therefore it is not unusual that the term heavy menstrual bleeding (HMB) is defined differently. However, an accepted boundary is the blood loss exceeding 80 ml per menstrual cycle (154).

HMB is usually reported as a major complication of vWD. According to the systematic review performed by Shankar *et al.* (155) up to 13% of HMB cases have vWD as the underlying cause. Conversely, 73% of vWD-affected women were found to have objectively confirmed HMB (156). Depending on the method of calculation, this figure can reach horrendous 93%, which significantly exceeds the prevalence of HMB in the general population (10% in Sweden) (157-159). It is still not uncommon in modern Sweden, where heavy menstrual bleeding is in top three most frequent causes of hospitalization among vWD patients (together with gastrointestinal bleedings and epistaxis) (114).

Heavy menstrual bleeding may significantly affect woman's life. In Sweden, more than half of patients with HMB reported avoiding social activities during their periods (160). Sometimes, menstrual blood loss is so devastating that the only way towards the ultimate cure is hysterectomy. In 1968 Taylor stated: "When menorrhagia is so severe as to require repeated transfusions consideration might be given to the induction of an artificial menopause, but in most cases hysterectomy would appear to be the treatment of choice" (161). Unfortunately, 50 years later we still cannot avoid removing the uterus in some cases. Today, half of women undergoing hysterectomy for HMB have no organic pathology and some of them may have unrecognized vWD (90).

In a recent survey, which involved ob&gyn specialists, it was found that bleeding disorders, *inter alia* von Willebrand disease, were included in the differential diagnosis of heavy menstrual bleedings in 77% of adolescents and only in 39% of women of reproductive age (162). Ten years earlier, a similar survey reported even lower figures – 16% and 4%, respectively (163). Alertness regarding bleeding disorders has obviously grown among medical doctors, but is it sufficient? According to the recent study by Jacobson *et al.* it is not, because most of the adolescents, experiencing HMB do not undergo screening for vWD (164).

The situation is so emerging that it has been proposed to test for underlying vWD in any case of menorrhagia and no obvious pelvic pathology (132).

It was stated that HMB has a larger impact on women with bleeding disorders than on women with unaffected hemostasis (165). Excessive bleeding during menstruation is a well-known risk factor for developing iron-deficiency anemia and there are implications for women's social activities. Barr *et al.* (166) reported that women with vWD were less inclined to engage in post-secondary education, probably due to the anemic state.

Today, several management options (described in detail in "Treatment" section) are available to help women with vWD to normalize their menstrual blood loss, and, as a consequence, prevent unwanted complications. However, it is not yet well established whether provided treatment options are good enough to improve overall quality of life.

1.4.2 Postpartum hemorrhage

Another significant problem, which women with vWD face, is the bleedings that occur during or soon after childbirth. In late pregnancy, 15% of cardiac output falls on the uterine circulation with minute blood flow of 500-700mL. If coagulation system fails to establish effective hemostasis shortly after delivery, severe bleeding occurs.

Postpartum hemorrhage (PPH) is the obstetrical emergency affecting 14 millions women worldwide every year (167). It is responsible for more than 100,000 maternal deaths per year, or 1 death in 4 minutes. Prevalence of PPH varies significantly across the world, ranging 2-11% (168). Worldwide, rate of PPH increased from 1.5% in 1999 to 4.1% in 2009 (169). Overall, PPH is the leading cause of maternal death worldwide and the problem becomes even more pronounced in women with bleeding tendency.

PPH is traditionally classified as primary and secondary. Primary PPH corresponds to blood loss of 500ml or more, while severe primary PPH - 1000ml or more within first 24 hours from delivery. Secondary PPH relates to any excessive bleeding, occurring up to six weeks postpartum, regardless of volume (170, 171).

Pregnancy is considered to be the prothrombotic state with range of coagulation factors (including FVIII and vWF) rising during gestation (172, 173). However, in patients with bleeding disorders this increase is less pronounced (174, 175). After delivery, levels of coagulation factors decrease rapidly, predisposing women, and especially those with bleeding disorders, to PPH.

1.5 DIAGNOSTICS

A recent report from USA (>32,000 patients) announced frequent misrecognition of vWD, with quarter of patients visiting the same doctor at least twice with complaints about bleeding

episodes. Within a year from the first visit to the specialist, 37% of vWD patients had no diagnostic test (176).

The classical diagnostic triad necessary for the diagnosis of vWD includes bleeding episodes previously in life, positive family history and low levels of plasma vWF. Nevertheless, there are justifiable doubts that these criteria are exhaustive. Having studied clinical data from 139 vWD patients, Alamin and Satti (177) concluded that only 65.5% of patients meet minimum criteria.

Of these three components, bleeding history is probably the most important both for diagnostics and prognosis. Tosetto *et al.* (61) compared the efficacy of integral bleeding score (calculated by summing up spontaneous bleedings) with laboratory measurement of FVIII and vWF levels. Bleeding score was equally effective for the prediction of bleeding after tooth extraction, while it turned out to be superior to coagulation tests in predicting bleeding after surgery. Today, a number of validated bleeding questionnaires are available, including self-administered and pediatric versions (178). Unfortunately, despite undeniable utility of the bleeding scores they still have certain limitations (179).

At the same time, no bedside test specific for vWD is currently available. Bleeding time (BT) is considered an imperfect diagnostic tool, since many patients remain within the reference ranges. For instance, Fressinaud *et al.* (180) reported that almost half of patients with type 1 and one fifth of those with type 2A vWD had normal BT. Global hemostatic testing, such as thrombin generation assay, is considered to be superior to classic tests, as the former could provide comprehensive knowledge on the entire coagulation system.

Laboratory testing for bleeding disorders is often challenging due to the fact, that both preanalytical and analytical conditions may substantially affect the ultimate results. For instance, Favaloro *et al.* emphasized the importance of proper mixing of thawed plasma samples. Non-mixing resulted in 25% decrease in coagulation factors, which may lead to false positive diagnosis of vWD (181). Another example is the potential loss of FVIII and vWF (predominantly high-molecular weight multimers) if using serum instead of citrated plasma. Even minor errors, such as under-filling the tubes, may lead to excess in citrate concentration and subsequent false results. Taking together, it all makes an accurate diagnosis of vWD extremely challenging, especially in the resource-limited conditions.

1.6 MANAGEMENT

1.6.1 Antifibrinolytic drugs

Antifibrinolytics, such as ϵ -aminocaproic and tranexamic acid (TA) are often used in vWD patients to control bleedings. The basis for their use is the rich fibrinolytic activity of mucosal tracts – the source of most bleedings in vWD. Antifibrinolytics alone may be sufficient to control less severe forms of bleedings or can be used as adjuncts to desmopressin or factor concentrates.

Both women suffering from HMB or PPH may benefit from using antifibrinolytic drugs. A recent report from an international, randomized, double blind, placebo-controlled trial named WOMAN assessed the efficacy of TA in reducing postpartum morbidity, mortality and hysterectomy rates (182). The trial lasted 6 years (2010-2016) and included more than twenty thousand women. Authors concluded that TA significantly reduces bleeding. They also stressed the importance of giving TA as soon as possible after bleeding onset.

1.6.2 Desmopressin

Desmopressin (trade name DDAVP[®]) is a type 2-vasopressin receptor agonist and it causes release of vWF from depot. Desmopressin has no uterotonic effect and can be safely used in pregnant women before invasive procedures (amniocentesis or villus sampling) with no bleeding complications (183). Recent systematic review also confirmed its safety and efficacy in PPH prophylaxis among women with bleeding disorders (184). Desmopressin may cause hyponatremia if combined with abundant fluid intake, and thus electrolytes should be monitored carefully, especially in infants and elderly people.

Patients are more likely to respond adequately to desmopressin stimulation, if they have normal vWF structure, *e.g.* if they belong to type 1 (76, 77). Moreover, one may expect satisfactory response if preinfusion levels of FVIII and vWF are at least 10-20% of normal (185). Therefore, desmopressin is contraindicated in patients with type 2B, as it results in secretion of dysfunctional vWF, which rapidly binds to platelets causing thrombocytopenia (80). In addition, the drug should be used with caution in patients with severe atherosclerosis. Upon desmopressin stimulation larger vWF multimers release and they may become highly thrombogenic under high shear stress conditions (atherosclerotic plaques) (186-188).

Individual response to desmopressin is usually constant over time and therefore giving a test dose is probably a suitable approach to estimate how does exact patient respond (189). Rise in

vWF levels lasts for 8-10 hours, which determines the mode of administration – every 12-24 hours (190).

1.6.3 Plasma-derived vWF/FVIII factor concentrates

Initially, plasma-derived vWF/FVIII factor concentrates were developed for the treatment of hemophilia. Today, they prove to be the treatment of choice in vWD patients, unresponsive to desmopressin (185). However, there are several concerns related to plasma-derived vWF/FVIII factor concentrates. Despite many of the measures taken, such as viral screening and attenuation, there is still a risk of the pathogen persistence in these products (191, 192). In addition, due to their plasma origin, factor concentrates contain extraneous proteins that may provoke severe allergic reactions (193). Secondly, different products vary significantly in regards to precise coagulation factor levels, vWF/FVIII ratio and presence of the most active large vWF multimers (194-197). All the above mentioned parameters certainly affect the efficacy and safety of the exact product. For instance, an unbalanced vWF/FVIII ratio can lead to a significant increase in plasma FVIII levels, which multiplies the risk of thromboembolic complications (198). Therefore, it is recommended to maintain FVIII levels at a maximum of 250-300 IU/dl during replacement therapy (199).

One should take into account, that vWF/FVIII concentrates predominantly sustain the plasma pool of vWF, without being able to replenish its depot within the endothelial cells and platelets. This is a probable reason for lower efficacy of the concentrates in vWD patients, suffering from gastrointestinal bleedings due to angiodysplasia, compared with bleeds at other sites (200).

Another drawback is related to the antibody formation. Up to 15% of patients who have received multiple transfusions develop antibodies against vWF. vWF containing products are contraindicated after this complication has occurred, since it may lead to the life-threatening anaphylactic reactions (201, 202). Important to notice, that being the polyclonal IgG they may cross the placenta and cause transient vWD in fetus. In patients with anti-vWF antibodies FVIII-only products should be used.

1.6.4 Recombinant vWF

Baxalta Inc. has recently introduced recombinant vWF (rvWF) under the name Vonvendi®. In contrast to plasma derived vWF/FVIII concentrates, rvWF does not contain FVIII, which allows obviating FVIII related risk of thrombosis (203). Another advantage of rvWF is the presence of large and ultra large multimers – the most beneficial ones for substitution in patients with type 2A, 2B and 3. At the same time, rvWF remains available for ADAMTS13 cleavage, ensuring its proper lifecycle (204).

Recombinant FVIII contains no vWF and thus is unsuitable in case of severe vWF deficiency. In the absence of its carrier FVIII undergoes rapid cleavage in the bloodstream. However, recombinant FVIII remains an option for those patients who have developed anti-vWF antibodies.

1.6.5 Treatment for gynecological complications

In treating gynecological bleedings several treatment options are available in addition to conventional management. This includes predominantly hormonal treatment, although surgical approaches are also present.

Combined oral contraceptives (COCs) are both estrogen and progesterone containing medications. COCs is currently accepted as the initial treatment for menorrhagia in the general population. The positive effect in the treatment of vWD-related excessive menstrual bleedings predominantly builds up from two mechanisms. Firstly, it is a reduction in the amount of blood loss typical for many modern COCs, as well as the possibility to control the timing of menstruation by changing the regimen. Secondly, estrogen is known to increase blood vWF levels, which leads to amelioration of the bleeding symptoms (205). However, the individual satisfaction rate for COCs, varies significantly depending on the vWD type and COCs' compound (206, 207).

Levonorgestrel-releasing intrauterine system (LNG-IUS) is a tiny hormone-containing device, placed inside the uterus, which provides contraceptive effects and reduces significantly monthly blood loss. This effect is also present in vWD cohort with the majority of women reporting decrease in the amount of bleeding and more than half become amenorrhoeic (208).

Surgical options are chosen when conservative treatment fails to provide effective hemostasis or in order to stop an acute severe bleeding. Endometrial ablation, which implies destroy of endometrial lining through cryo/thermal ablation or endomyometrial resection, is currently employed as an alternative to hysterectomy (209). However, the overall efficacy of ablation does not reach 100%, which necessitates repeat procedure or ultimate removal of the uterus. Hysterectomy rates remain high among vWD patients, somewhat varying between the vWD types: 8-18% in type 1 patients and 23% in type 2 and 3 vWD patients (156, 206, 207).

2 AIMS OF THE THESIS

During the project we aimed to evaluate the impact of von Willebrand disease on women's everyday life, with special attention to the most common bleeding complications: heavy menstrual bleeding and postpartum hemorrhage. We intended to assess current management in terms of its rationality and sufficiency for prevention of bleeding episodes. An important goal was to scrutinize the possible reasons for developing bleeding complications, which would help to ameliorate clinical management of the patients and ultimately improve their quality of life.

Furthermore, we took aim to assess how do regular fluctuations of the sex hormones during menstrual cycle influence hemostasis and inflammatory system. The goal was to elucidate fundamental mechanisms, underlying common bleeding complications in vWD-affected females.

2.1 SPECIFIC AIMS

2.1.1 Study I

To investigate whether Swedish women diagnosed with von Willebrand disease receive appropriate treatment, and to assess its reliability in terms of preventing heavy menstrual bleeding. In addition, to evaluate the impact of excessive menstrual blood loss on routine activities and overall health-related quality of life.

2.1.2 Study II

To estimate the prevalence of postpartum hemorrhage in women with von Willebrand disease. In addition, we intended to find possible correlation between postpartum blood loss and (1) type of von Willebrand disease (2) hemostatic drug treatment (3) levels of coagulation factors – vWF and FVIII.

2.1.3 Study III

To measure thrombin generation during two phases of menstrual cycle in women with von Willebrand disease. To associate the results with a range of hemostatic parameters. To expand knowledge on mechanisms underlying heavy menstrual bleeding in women with vWD.

2.1.4 Study IV

To analyze fluctuations of the blood inflammatory and endothelial markers during a regular menstrual cycle and therefore to enlighten interactions between the inflammatory and hemostatic systems in vWD patients.

3 MATERIALS AND METHODS

3.1 STUDY I

Study participants were recruited from a register at Coagulation Unit, Karolinska University Hospital. This register aims to accumulate clinical data about people with bleeding disorders from entire Sweden. The inclusion in the register is voluntary for patients, though everyone is asked to participate.

The register was filtered using the inclusion criteria: diagnosis of vWD, female gender and age limits 18-52 years (the latter is the average age for menopause in Sweden (210)). Invitation letters were sent to the eligible patients. After excluding the dropouts, 30 women were enrolled in the study.

We retrieved background clinical information about each participant from the register – type of vWD and any pharmacological treatment. At the same time, in order to collect data related to menstruation and its impact we send several forms to each participant. This included specially designed questionnaire, pictorial blood-loss assessment chart (PBAC) and a health survey form (SF-36).

3.1.1 Questionnaire

A research team from Coagulation Unit, Karolinska University Hospital initially designed the questionnaire to assess impact of heavy menstrual bleeding (HMB) on everyday life activities (unpublished study). Originally, the questionnaire consisted of 102 questions on 11 themes, but in order to limit the scope of the study to relevant data only first 3 themes were included in the analyzed pool.

Questions 1-12 relate to overall life conditions and are routinely used in clinical practice in Coagulation Unit.

Questions 13-17, designed by the abovementioned research team, embraced menstruation features: duration, amount of blood loss, need for advice.

Questions 18-29, engendered from studies by Ruta *et al.*(211) and Coulter *et al.*(212) assessed the impact of menstruation on everyday life activities.

3.1.2 PBAC

There is a well-known problem of estimating the exact volume of blood loss during menstruation. Being estimated by women themselves, figures may differ significantly from

reality. At the same time, using the preweighed tampons and pads presents obvious practical inconvenience.

Pictorial blood loss assessment chart (PBAC) is a semiobjective method, developed by Higham *et al.* (213), which makes possible to approximate accuracy in assessing volume of the menstrual blood loss. In this method woman quantify the number of tampons/pads used in each day of menstrual cycle. Depending on the degree of soaking the corresponding coefficient (multiplying factor) is used. At the end of menstrual cycle woman calculates the sum and if the latter exceeds 100 points (equals 80mL), then heavy menstrual bleeding is diagnosed (Table 1).

Tampons	Multiplying factor	Days							
		1	2	3	4	5	6	7	8
	x1								
	x5								
	x10								
Pads									
	x1								
	x5								
	x20								
Small blood clots	x1								
Large blood clots	x5								
Menstrual accidents	x5								
Daily points									

Table 1. Pictorial blood loss assessment chart

3.1.3 SF-36

Short Form-36 (SF-36) is a well-recognized health survey, designed to evaluate health-related quality of life (HRQOL) with 36 questions in 8 dimensions (Table 2). Answers to these 36 questions were rated with accordance to SF-36 algorithms. SF-36 scale fluctuates from min=0 to max=100 points (214). Results were compared with those obtained from Swedish general population (215). Reference group included 4582 Swedish women aged 15-93 years (mean age=42,7 years).

Median SF-36 scores were calculated separately for each of 8 dimensions for entire study population and following subgroups: vWD type 1, type 2 and type 3; women with HMB, with normal menstrual blood loss, with no menstruation at all.

Dimension	Lowest possible score	Highest possible score
Physical functioning	Very limited, including daily activities of personal hygiene and dressing	Not limited, can perform all types of physical activities
Physical health	Limited performance at work or regular activities due to poor physical health	Not limited, can perform
Bodily pain	Severe pain	No pain
General health	Perceives general health status as poor and believes it will deteriorate	Perceives general health status as excellent
Vitality	Feels constantly tired and worn out	Feels constantly alert and energetic
Social functioning	Limited in social activities due to physical or mental health status	Not limited, can maintain normal social activities
Emotional impact	Limited in work and regular activities due to emotional status	Not limited, can perform work and regular activities
Mental health	Feels constantly depressed or nervous	Feels constantly calm, in harmony and happy

Table 2. Short Form-36. 8 dimensions with theirs lowest and highest scores.

3.2 STUDY II

Study design was observational and retrospective.

We analyzed data stored in the previously described register at Coagulation Unit, Karolinska University Hospital. Primarily, we sorted the register in consistency with inclusion criteria: female gender, vWD diagnosis, age 18-50 years and the history of at least one delivery. All the eligible women (n=47) were invited to participate with 34 of them included in the study group.

In total, 34 women who had 59 deliveries formed the final cohort. Their age varied from 19 to 42 years (median 32). Each delivery was supplemented with following information: hospital of delivery, maternal age, mode of delivery and complications, if any. Blood loss estimation was executed through weighing soaked materials and blood clots. Diagnosis of vWD was established if classical triad of bleeding episodes, family history and low vWF was fulfilled. Laboratory tests for vWF:Ag, ristocetin cofactor activity of von Willebrand factor (vWF:RCo) and their ratio helped to distinguish between subtypes of vWD.

Finally, we also obtained characteristics of hemostatic treatment: (1) type of drug – tranexamic acid, desmopressin or clotting factor concentrate (2) duration of treatment (3) dose prior to delivery (4) total dose.

3.2.1 Laboratory measurements

In addition to the abovementioned data, we also collected available information regarding levels and activity of coagulation factors in third trimester.

vWF was measured with either of two methods – vWF:RCo or vWF:GpIb. Methods resemble each other and they have similar reference ranges, which makes them comparable.

vWF:RCo is based on the ability of antibiotic currently withdrawn from the market – ristocetin to increase agglutination, via enhancing vWF binding to the platelets. These aggregates drown to the bottom of the tube, thereby changing the optical density of the plasma. Increase of absorbance is then measured by photometry (216). vWF:GpIb does not need ristocetin and is currently used at the Karolinska University Laboratory. In this method plasma is mixed with reagent containing GpIb receptors (recombinant), the latter binds to vWF. Agglutination degree is then measured with photometry (217).

FVIII activity (0,06 – 2,10 kIU/L) was measured using well-established method (218). This is an indirect enzymatic method, which is based on following process - FVIII binds to FIX and this complex catalyzes the activation of FX. The amount of generated FXa is proportional to FVIII activity.

3.3 STUDY III

This was an observational study.

The inclusion criteria were female gender, established vWD, age 18-52 and regular menstrual cycle (21-35 days). All the participants met conventional criteria for vWD diagnosis, though in some of them additional tests were previously performed, such as vWD multimer assessment. None of the participants were prescribed with medications that may affect hemostasis. This included predominantly hormonal treatment (combined oral contraceptives, contraceptive implants, IUDs, HRT). The exclusion criteria were irregular menstrual cycle, pregnancy or breastfeeding at the time of blood sampling.

Blood samples were taken twice in each participant: first during early follicular phase (cycle day, cd 2-5) and second during luteal phase (cd 22-25). Blood were drawn in the morning, from an antecubital vein after 15 min in supine position. We used standard vacuum citrated tubes, that were centrifuged at 2000g for 15 min immediately after blood collection. After removal of the cells, plasma was centrifuged once again for another 15 min at 2000g. Platelet poor plasma was stored at -70°C until analyzed.

Afterwards following components were assessed: antithrombin, fibrinogen, D-dimer, coagulation factors II, VII, VIII, X and vWF, together with global hemostatic assay - thrombin generation.

The results from the study group were then compared with those from healthy controls (n=102), coming from the recent study by one of the co-supervisors of the current project - Dr. Roza Chaireti (219).

3.3.1 Measurement of hemostatic variables

Thrombin generation was measured by the calibrated automated thrombogram method as described in the Thrombogram Guide by Thrombinoscope BV (Maastricht, the Netherlands). We computed following thrombin generation parameters: lag-time (time point at which thrombin generation starts, in minutes), time to peak (time to reach max thrombin concentration, in minutes), endogenous thrombin potential (ETP, total amount of generated thrombin, in nM*min), peak (max thrombin concentration, in nM). All samples were measured in triplicate. The final mixture of PPP reagent (trigger) and PPP used in the assay contained 5 pM tissue factor (TF) and 4mN phospholipids. All reagents were obtained from Thrombinoscope BV, Masstricht, The Netherlands. 96-well plates used were obtained from Ninolab, Stockholm, Sweden.

Clauss method was employed to measure fibrinogen. Hemostatic variables were measured by means of the Sysmex CS 2000i from Siemens Healthcare Diagnostics (Stockholm, Sweden). Antithrombin (AT) and factor VIII (FVIII) were assessed by a chromogenic methods; factors II, VII and X (FII, FVII and FX) - clotting methods, von Willebrand factor (vWF:Ag) - immunochemical method. All reagents were produced by Siemens Healthcare Diagnostics (Stockholm, Sweden). D-dimer was measured by a latex-enhanced immunochemical method, using reagents from Medirox (Studsvik, Sweden)

3.4 STUDY IV

Study group was the same in studies III and IV and therefore inclusion criteria and preanalytical preparations of the blood samples were similar.

Following inflammatory and endothelial markers were analyzed (interleukin-6 (IL-6), endostatin, high sensitivity C-reactive protein (hs-CRP), soluble E-selectin and P-selectin (sE-selectin and sP-selectin), intracellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) and cathepsins L and S). The results were compared with corresponding data from the recent study by Chaireti *et al.* (220).

3.4.1 Measurement of inflammatory and endothelial markers

High sensitivity C-reactive protein was analyzed on a BS380 instrument (Mindray, Shenzhen, China) with CRP reagents (CRP-6K26) from Abbott Laboratories (Abbott Park, IL, US). The total coefficient of variation (CV) for the CRP method was 6.9% at 1.30 mg/L. Cathepsin L (DY952), Cathepsin S (DY1183), Endostatin (DY1098), sE-selectin (DY724), ICAM-1 (DY720), IL6 (DY206), sP-selectin (DY137), and VCAM-1 (DY809) were analyzed by the means of commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer. The total CVs of the ELISAs were approximately 7%.

3.5 STATISTICAL ANALYSIS

In all studies statistical analysis was performed using IBM SPSS statistics software for Mac OS version 21 (Study I and II) and 24 (Study III and IV).

3.5.1 Study I

The study population was arranged into subgroups according to the type of vWD and the amount of the menstrual bleeding. Non-parametric Kruskal-Wallis test was employed to compare variables, that could be ranked according to an ordinal scale.

The median SF-36 health profiles for study participants were plotted and subsequently compared with those from Swedish female general population. In order to detect statistically significant differences between the median SF-36 values of the study population and the Swedish women of the general population, approximate confidence intervals with $P < 0.10$ were constructed for the median values of the total study population and the subgroups with $n \geq 15$. The approximate confidence intervals for the median values were obtained through ranking of the SF-36 values in each dimension, and the results obtained were compared to corresponding tabulated ranks (28) for a 90% confidence interval. A 90% confidence interval was selected due to the size of the study population and the relatively low number of ranks obtained.

3.5.2 Study II

To compare the incidence of postpartum hemorrhage between the groups we used Fisher's exact test, due to the small sample sizes. To compare blood loss (mL) between three types of vWD the Kruskal-Wallis test was used. We used Spearman's correlation test to investigate the correlation blood loss during delivery and levels of coagulation factors, hemostatic drug treatment (dose prior to delivery). In order to compare hemostatic treatment between the groups with regard to its duration and total dose we used Mann-Whitney U test.

3.5.3 Study III and IV

Since there are no studies with a design similar to ours, i.e. measuring hemostatic (Study III) or inflammatory (Study IV) markers in vWD patients during the menstrual cycle, we calculated the required power for the cohort by using results from the studies by Chaireti et al.(219) and Rugeri et al. (221), where thrombin generation was measured in healthy women and patients with von Willebrand disease, respectively. The required cohort size in order to achieve a power of 0,8 with a type I-error of 5% was 12 patients.

Due to predominant non-normal distribution and relatively small number of participants in the study group, non-parametric tests were chosen for calculations. We used Wilcoxon signed-rank

test to compare changes in variables during menstrual cycle within study group. Mann-Whitney U test was employed to assess differences between control and study groups. In all cases an exact p-value was calculated, while statistical significance was set at $p < 0,05$.

3.6 ETHICAL APPROVAL

All participants were informed about voluntary nature of the studies, as well as their characteristics. Written informed consent was obtained from all participants and personal data was made anonymous directly after collection.

Central Ethical Review Board of Karolinska Institute, Stockholm, Sweden granted the ethical permission for Study I and Study II under registration №2007/1373-31/4. Studies III and IV were approved by Stockholm Regional Ethics Committee (№2016/503-31) and was subsequently supplemented with the local permission from Almazov National Medical Research Centre in Saint-Petersburg, Russia (№ 17/III).

4 RESULTS

4.1 STUDY I

Study cohort included 30 women with vWD aged 19-51 (mean age \pm SD=35.1 \pm 8.1 years). vWD type distribution is shown in Figure 5. Worth mentioning, that the distribution is different from what is observed in general population, probably due to a small sample size.

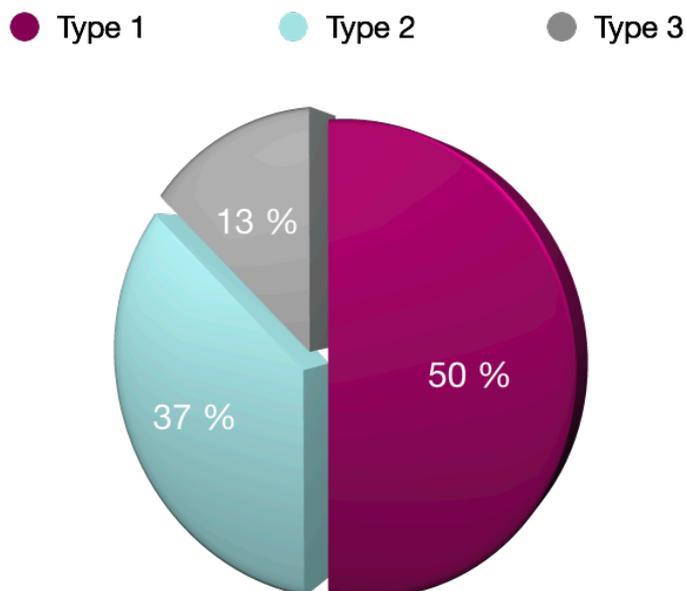


Figure 5. vWD types distribution in the study cohort

4.1.1 Menstruation pattern in women with vWD

Half of study cohort reported presence of heavy menstrual bleeding, based on personal perceptions. However, based on clinical measurements the real incidence of HMB was shown to be 53.5%. The difference arises from the discrepancy between subjective and objective evaluations. For instance, of the 15 women who reported having HMB, only 11 (73.3%) met the clinical criteria for this. In contrast, 5 out of 7 women (71.4%), who reported normal menstrual pattern had excessive menstruations in reality. The latter illustrates the well-known underestimation of menstrual blood loss by women affected with bleeding disorders. This could happen for several reasons. Women may consider their menstruation normal just because they habituate themselves to this pattern. Otherwise, they may compare their bleedings with mother's or sister's, which is not a good approach, taking into account hereditary nature of vWD. It is worth noting, that a *vice versa* situation is possible (and was also present in our study group) with women overestimating their blood loss. All this testifies the importance of using more objective methods to assess menstrual bleedings.

Menstruation characteristics in different types of vWD are shown below (Table 3).

Menstrual bleeding	vWD type 1 (n=15), n(%)	vWD type 2 (n=11), n(%)	vWD type 3 (n=4), n(%)
None	3 (20.0)	3 (27.2)	2 (50.0)
Normal	2 (13.3)	4 (36.4)	1 (25.0)
HMB	10 (66.7)	4 (36.4)	1 (25.0)

Table 3. Menstruation characteristics in women with different types of von Willebrand disease. vWD, von Willebrand disease; HMB, heavy menstrual bleeding.

7 women had amenorrhea due to pregnancy or breastfeeding (2 women), combined oral contraceptives (COCs)(1 woman), using levonorgestrel-releasing intrauterine system (LNG-IUS)(4 women) or previous hysterectomy (1 woman). Differences in incidence of HMB between different types of vWD were not statistically significant, likely due to the small size of the subgroups.

4.1.2 Impact of menstruation on everyday activities

After excluding 8 women with amenorrhea, we analyzed the impact of menstruation on overall life activities. It was found, that menstruations dramatically influenced women’s lives. Results are shown in Figure 6.

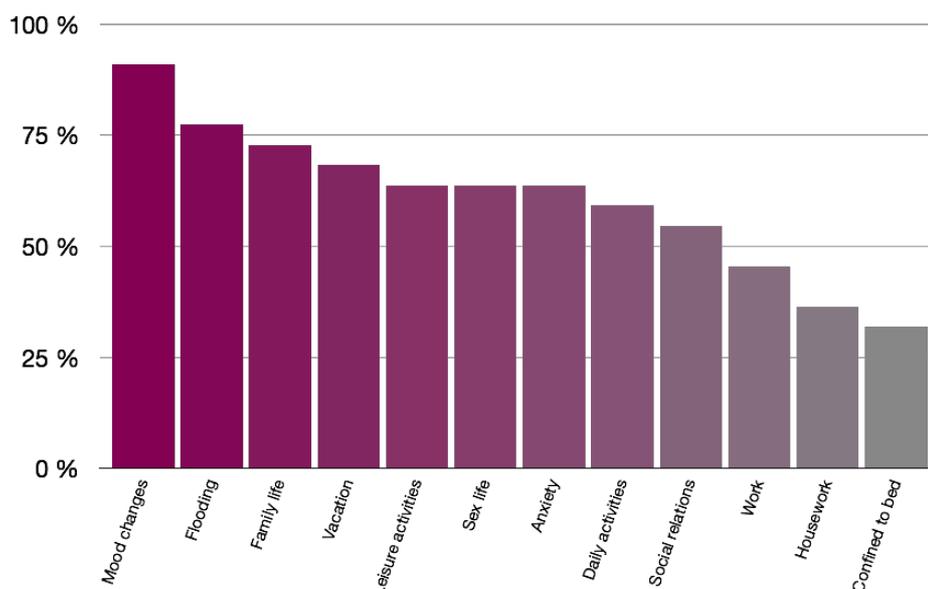


Figure 6. Impact of menstruation on overall life activities

The most affected sides were mood changes (90.9%), family life (72.7%) and vacations (68.2%). More than half of study cohort also reported alterations in leisure activities, sex life and increase in anxiety (63.6% in each category). Daily activities and social relations were also appreciably affected (59.1% and 54.5% respectively). To a slightly less degree women reported negative changes in their ability to work (45.5%) or to carry out housework (36.4%). However, nearly one third of women was literally confined to bed during the menstrual period.

4.1.3 Health-related quality of life

Median SF-36 scores were compared between women with different types of vWD, patterns of menstruation and Swedish women in general. The results are illustrated in Figure 7.

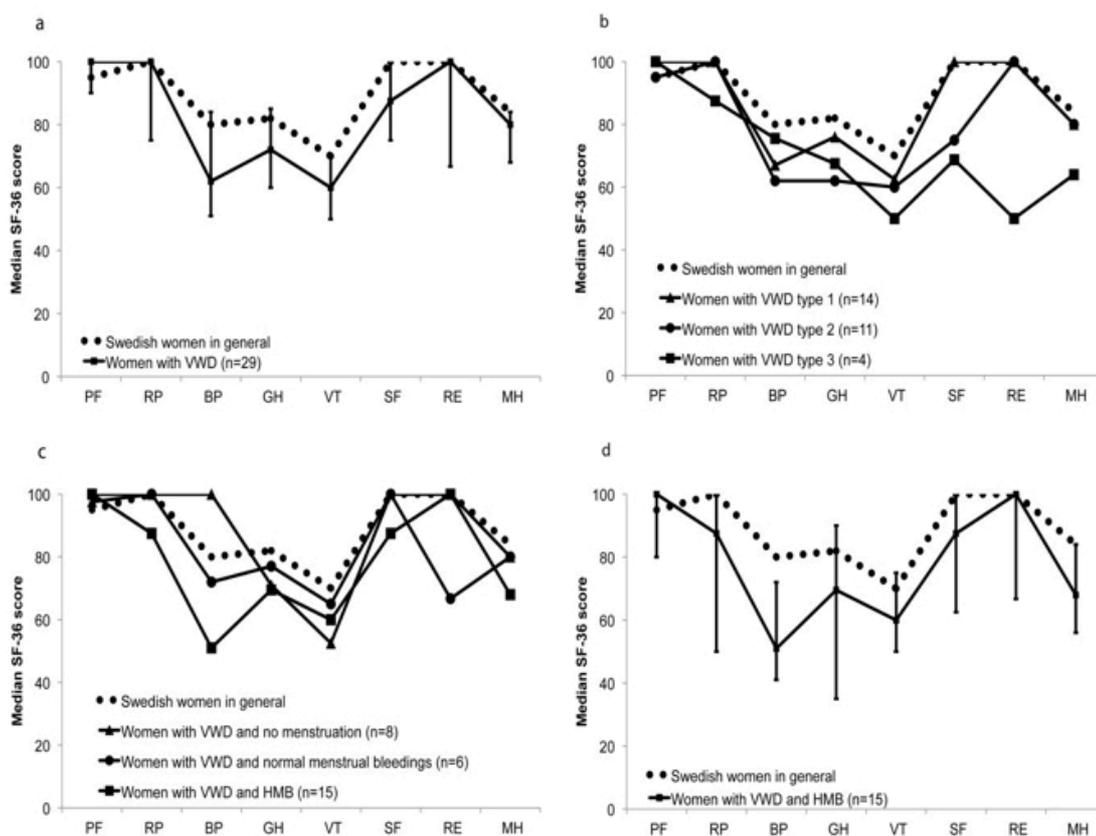


Figure 7. SF-36 profiles for different subgroups. Each point represents a median for exact dimension, while error bars, if present, stand for confidence intervals ($p < 0.10$). Dotted line is for control group – Swedish women in general. **A.** The health profile for women with vWD (all types) **B.** The health profiles for different types of vWD differentially **C.** The health profiles of women with amenorrhea, normal menstruation pattern and HMB **D.** The health profiles of women with HMB (an excerpt from Figure 7C). vWD, von Willebrand disease; HMB, heavy menstrual bleeding; PF, Physical Functioning; RP, role-physical; BP, bodily pain; GH, general health; VT, vitality; SF, social functioning; RE, role-emotional; MH, mental health.

When compared median SF-36 scores of women with vWD (all types) and Swedish women in general, no significant differences were observed. Nevertheless, in the dimension “Vitality” upper limit of CI for women with vWD was equal to Swedish general population. Median scores for different types of vWD were also lower, though did not reach the significant differences (Figure 7B). It may imply the necessity to enlarge the study group in order to reach the level of statistical significance. Comparing different patterns of menstruation, it was observed that in the dimension “Bodily pain” the subgroup of women with HMB showed significantly lower scores (Figure 7D).

4.1.4 Treatment for HMB

The vast majority of women received therapy for excessive bleedings (24/30; 80%). However, treatment options distributed unevenly between women with different menstruation patterns (Figure 8).

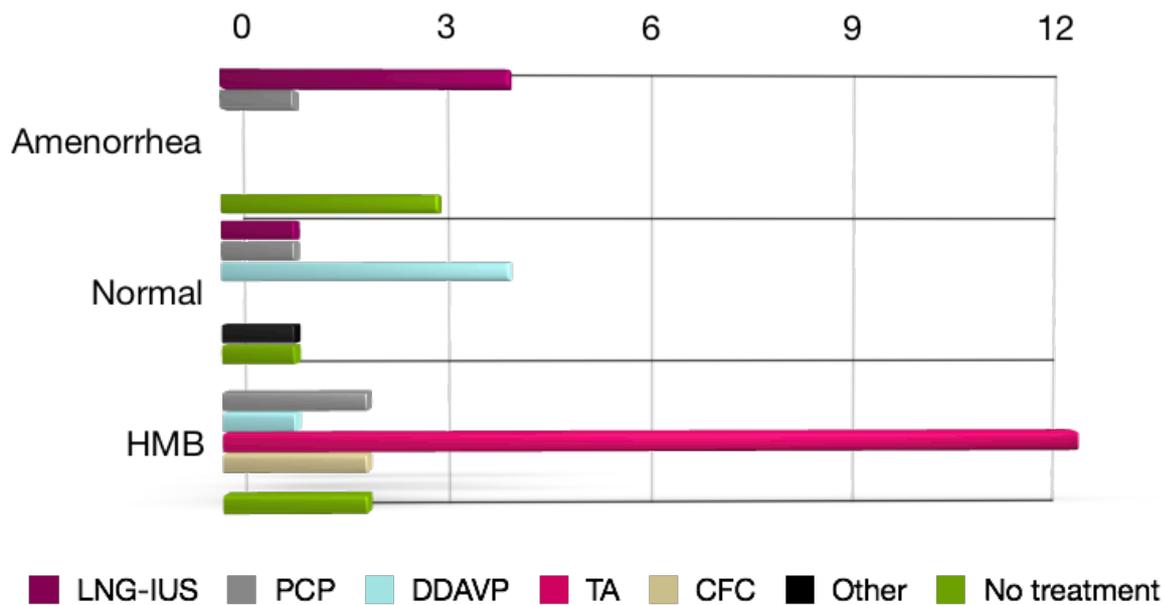


Figure 8. Pharmacological treatment in women with vWD and resulting menstrual bleeding pattern. HMB, heavy menstrual bleeding; LNG-IUS, levonorgestrel-releasing intrauterine system; PCP, progesterone contraceptive pills; DDAVP, desmopressin D-arginine vasopressin; TA, tranexamic acid; CFC, clotting factor concentrates.

Treatment was not equally effective in all women. Based on women’s personal evaluation, treatment was considered to be effective in 15/24 (62.5%) of the cases, while according to the clinical measurements it was effective even in less number of women – 10/24 (41.7%). Important to notice, that 3 women with preserved menstruations received no therapy, and two of them met the clinical criteria for HMB.

4.2 STUDY II

Study group included 34 women and 59 deliveries, occurred in 14 different obstetric clinics in Sweden during 1995-2012 (Table 4).

		Type 1	Type 2	Type 3	Unknown	All
Participants		21	9	3	1	34
Deliveries		39	14	4	2	59
Mode of delivery (%)	Vaginal	29 (74.4)	9 (64.3)	1(25)	2 (100)	41 (69.5)
	Instrumental vaginal	4 (10.3)	2 (14.3)	1 (25)	-	7 (11.9)
	Caesarean	6 (15.4)	3 (21.4)	2 (50)	-	11 (18.7)
Induced labour (%)		7 (17.9)	5 (35.7)	1 (25)	-	13 (22.0)
Maternal age (%)	≤23	4 (10.3)	-	-	-	4 (6.8)
	24-30	13 (33.3)	3 (21.4)	1 (25)	1 (50)	18 (30.5)
	31-37	18 (46.2)	8 (57.1)	3 (75)	1 (50)	30 (50.8)
	≥38	4 (10.3)	3 (21.4)	-	-	7 (11.9)
Parity (%)	1	20 (51.3)	7 (50)	3 (75)	1 (50)	31 (52.5)
	2	13 (33.3)	4 (28.6)	1 (25)	1 (50)	19 (32.2)
	3 or more	6 (15.4)	3 (21.4)	-	-	9 (15.2)
Birth weight (%)	<2499	2 (5.1)	1 (7.1)	-	-	3 (5.1)
	2500-3999	31 (79.5)	11 (78.6)	4 (100)	2 (100)	48 (81.4)
	>4000	6 (15.4)	2 (14.3)	-	-	8 (13.6)
Gestational age (%)	<36	2 (5.1)	-	1 (25)	-	3 (5.1)
	36-41	30 (76.9)	12 (85.7)	3 (75)	2 (100)	47 (79.7)
	>41	7 (17.9)	2 (14.3)	-	-	9 (15.2)
Obstetric unit in close connection with a coagulation unit (%)		21 (53.8)	8 (57.2)	3 (75)	-	32 (54.3)
Plasma levels checked in pregnancy (%)		26 (66.7)	13 (92.9)	4 (100)	-	43 (72.9)
Median VWF:RCo/vWF:GpIb, kIU/L (range)		0.55 (0.08-0.86)	0.21 (0.08-0.68)	0.08 (0.08-0.24)	-	0.25 (0.08-0.86)
Median FVIII:C, kIU/L (range)		1.07 (0.32-2.10)	0.84 (0.63-1.86)	0.70 (0.06-1.17)	-	0.94 (0.06-2.10)
No haemostatic treatment (%)		13 (33.3)	1 (7.1)	-	2 (100)	16 (27.1)
TA (%)		7 (17.9)	2 (14.3)	-	-	9 (15.3)
TA and DDAVP (%)		11 (28.2)	1 (7.1)	-	-	12 (20.3)
TA and CFC (%)		8 (20.5)	10 (71.4)	4 (100)	-	22 (37.3)

Table 4. Characteristics of the study group. vWD, von Willebrand disease. vWF:RCo, Ristocetin cofactor activity of Von Willebrand factor; vWF:GpIb, von Willebrand factor activity by glycoprotein Ib; FVIII:C, factor VIII activity; TA, tranexamic acid; DDAVP, D-amino D-arginine vasopressin; CFC, clotting factor concentrate.

Important to notice, that in 28 women (43 deliveries) the diagnosis of vWD was established prior to delivery, while in 11 women (16 deliveries) vWD was diagnosed after delivery. In the remaining 5 women vWD had been determined in between two pregnancies, thus they were included in both groups.

4.2.1 Incidence of PPH

The overall incidence of primary PPH was 44% (n=26), at the same time varying significantly between different delivery modes: postpartum blood loss exceeding 500mL occurred in 37% of vaginal deliveries, 57% of instrumental deliveries and 64% of caesarean sections (CS). Severe primary PPH (>1000mL) complicated 17% of vaginal deliveries, 43% of deliveries, in which forceps or vacuum were used and 18% of CS. Overall incidence of severe primary PPH was 20%. Secondary PPH occurred in 12% of cases.

Vaginal hematoma developed in three patients and it was one severe bleeding complication from the trachea following intubation. Three patients (5%) received blood transfusions.

4.2.2 PPH and type of vWD

The median blood loss was higher in type 3 vWD patients compared to non-type 3, but the difference was not statistically significant. All types of PPH occurred more frequently in women with type 3 vWD (Figure 9). However, only for severe primary PPH the difference proved to be statistically significant (p=0.02).

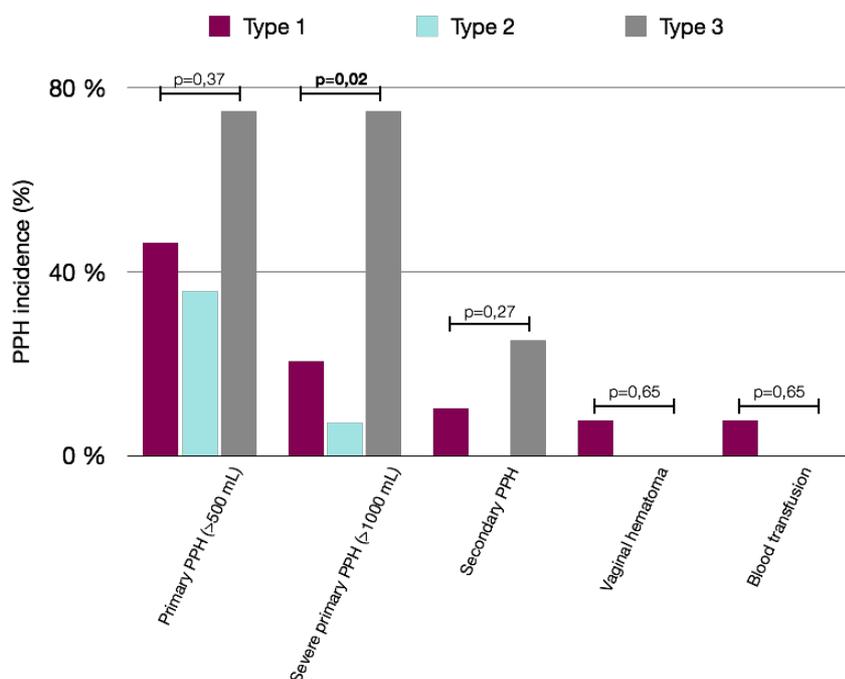


Figure 9. Incidence of PPH in different types of vWD. vWD, von Willebrand disease; PPH, postpartum hemorrhage.

4.2.2.1 PPH in undiagnosed vWD

vWD was unknown at the time of 16 deliveries (11 women). Therefore, there were no indications for both assessment of the coagulation factors levels and prescription of the hemostatic drugs. The majority was subsequently diagnosed with type 1 vWD (81%), 6% - with type 2 and 13% - unspecified.

The incidence of PPH was higher in women in whom diagnosis of vWD had not been established at the time of delivery. This was true for all types of PPH (Figure 10), though statistical significance was achieved only in secondary PPH ($p=0.013$). Blood transfusions were needed in three cases, where the diagnosis of vWD was unknown, compared with no blood transfusions in the group of known vWD ($p=0.017$).

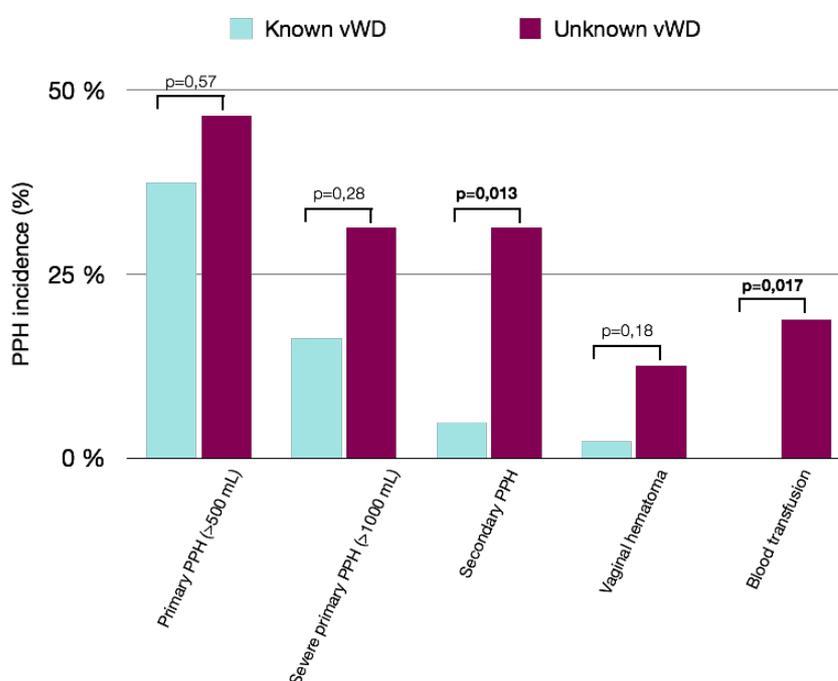


Figure 10. PPH incidence in known vs. unknown vWD at the time of delivery. vWD, von Willebrand disease; PPH, postpartum hemorrhage.

4.2.3 PPH and levels of coagulation factors

The plasma levels of FVIII and vWF were analyzed in all women, in whom diagnosis of vWD was established before delivery. Analyzes always took place in third trimester.

FVIII levels were subnormal in half of pregnancies in type 3 vWD – 2/4 (50%), no pregnancies in type 2 vWD and in 5/26 (19.2%) of pregnancies in type 1 vWD. vWF levels were below the reference range in all type 3 vWD pregnancies ($n=4$) and almost all pregnancies in type 2 vWD (12/13, 92.3%). At the same time, in type 1 vWD group only 10/26 (38.5%) women had subnormal levels of vWF in the late pregnancy.

The blood loss associated with the delivery inversely correlated with levels of FVIII:C in third trimester ($r = -0.428$, $p = 0.01$), but not with the levels of vWF ($r = -0.31$, $p = 0.6$). In opposite, vWF levels showed moderate inverse correlation with the duration of treatment with CFC postpartum ($r = -0.56$; $p = 0.01$), but not FVIII ($r = -0.31$; $p = 0.17$)

4.2.4 PPH and hemostatic treatment

Exact treatment in every case was consistent with contemporary clinical recommendations (222, 223).

In all women with known vWD at the time of delivery tranexamic acid (TA) was prescribed. TA was administered either orally or intravenously every 8 hours at the start of labor and continues for a median of 10 days (range 2 – 14). Desmopressin (DDAVP[®], Sanofi-Aventis U.S. LLC) or plasma derived factor concentrates (Haemate-P[®], CSL Behring GmbH) was always given on top of TA. All but one woman who received desmopressin were diagnosed with type 1 vWD, as it considered to be more efficient in these patients. CFC was used in all type 3 vWD affected patients or if a trial dose of desmopressin failed to provide therapeutic effect. The prophylactic median dose of CFC given prior to delivery was 2000 IU (range 1000-4000 IU). It was then prescribed as daily *i/v* bolus injections for a median of 9 days (range 1-18). The total CFC dose ranged from 2000 – 35000 IU.

The incidence of primary PPH was higher when CFC or desmopressin was given on top of TA, compared with no treatment. However, in regard to severe primary PPH and secondary PPH the ratio turned out to be the opposite, with “No treatment” group showing the higher incidence of excessive blood loss. Sample size, alas, was insufficient to provide significant differences between different treatment subgroups (Figure 11).

We were unable to find significant correlation between CFC given prior to and blood loss after delivery ($r = 0.03$; $p = 0.84$). There was also no association found between incidence of secondary PPH and duration of TA treatment ($p = 0.56$), duration of CFC treatment ($p = 0.58$) or total dose of CFC ($p = 0.64$).

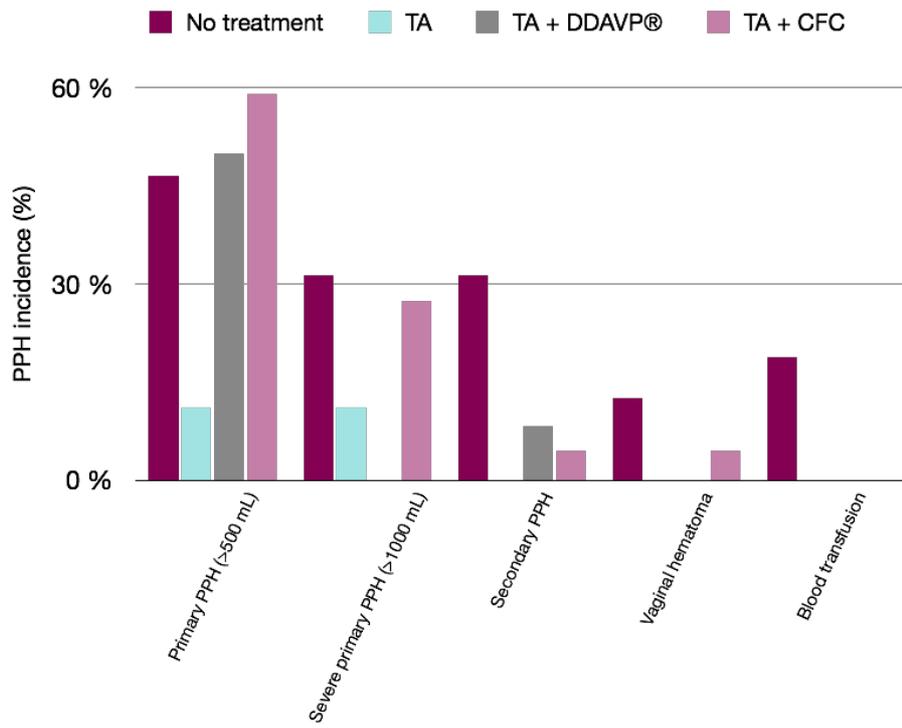


Figure 11. PPH incidence and mode of hemostatic treatment.

vWD, von Willebrand disease; TA, tranexamic acid; DDAVP®, desmopressin; CFC, clotting factor concentrate; PPH, postpartum hemorrhage.

4.3 STUDY III

12 female patients were included in the study group. Their median age was 35.0 (33.0-41.0)(hereinafter (Median (25-75 percentiles)) and BMI=23.1 (20.2-29.4). The majority of patients had vWD type 1 (7 patients), type 2 was diagnosed in 3 patients (with 2 of them having subtype 2M), unspecified - 2 patients.

While assessing levels of the hemostatic variables during different phases of the menstrual cycle in women with vWD, following differences were observed (Table 5). During follicular phase FVIII and FX were significantly higher, than in luteal phase (p=0.013 and p=0.033, respectively). Thrombin generation profiles were similar across the menstrual cycle within the vWD group.

	Follicular phase (cd 2-5)	Luteal phase (cd 22-25)	P
Fibrinogen, g/l	2.74 (2.37-2.92)	2.63 (2.28 - 3.02)	0.260
D-dimer, mg/l	0.06 (0.03-0.11)	0.04 (0.02-0.08)	0.124
Antithrombin, kIU/l	1.07 (1.02-1.12)	1.07 (1.01-1.17)	0.970
FII, kIU/l	1.13 (1.06-1.21)	1.15 (1.12-1.20)	0.875
FVII, kIU/l	1.11 (0.90-1.36)	1.12 (0.87-1.20)	0.240
FVIII,kIU/l	0.87 (0.52-1.14)	0.76 (0.49-1.12)	0.013
FX, kIU/l	1.08 (1.03-1.30)	1.04 (0.98-1.29)	0.033
vWF, kIU/l	0.68 (0.21-1.04)	0.65 (0.20-1.02)	0.577
Thrombin generation parameters			
Lag-time, min	3.22 (2.87-3.33)	3.00 (2.71-3.30)	0.679
ETP, nM*min	1698.36 (1450.22 - 2021.40)	1863.57 (1417.09 - 2113.60)	0.083
Peak, nM	215.00 (170.61 - 300.16)	242.02 (194.88 - 295.91)	0.240
Ttpeak, min	7.33 (6.44 - 7.89)	6.95 (6.33 - 7.22)	0.520

Table 5. Hemostatic variables during menstrual cycle in women with von Willebrand disease

p<0,05 (statistical significance) is marked in **bold**

kIU/l, kilo International Units;

We proceeded with comparing hemostatic variables between vWD patients and healthy controls, separately for the follicular and luteal phase. The ultimate results are summarized in Table 6.

	Follicular phase			Luteal phase		
	vWD patients	Healthy controls	P	vWD patients	Healthy controls	P
Fibrinogen,g/l	2.74 (2.37-2.92)	2.58 (2.34-2.84)	0.522	2.63 (2.28-3.02)	2.67 (2.42-2.97)	0.552
D-dimer, mg/l	0.06 (0.03-0.11)	0.06 (0.04-0.09)	0.545	0.04 (0.02-0.08)	0.06 (0.04-0.09)	0.032
Antithrombin, kIU/l	1.07 (1.02-1.12)	0.98 (0.93-1.04)	<0.0005	1.07 (1.01-1.17)	0.97 (0.93-1.03)	<0.0005
FII, kIU/l	1.13 (1.06-1.21)	0.99 (0.94-1.04)	<0.0005	1.15 (1.12-1.20)	0.99 (0.94-1.04)	<0.0005
FVII, kIU/l	1.11 (0.90-1.36)	0.87 (0.76-0.99)	0.001	1.12 (0.87-1.20)	0.82 (0.73-0.93)	0.001
FVIII, kIU/l	0.87 (0.52-1.14)	0.92 (0.78-1.09)	0.446	0.76 (0.49-1.12)	0.97 (0.83-1.08)	0.074
FX, kIU/l	1.08 (1.03-1.30)	0.95 (0.86-1.02)	<0.0005	1.04 (0.98-1.29)	0.91 (0.85-1.01)	<0.0005
vWF, kIU/l	0.68 (0.21-1.04)	0.80 (0.63-1.17)	0.059	0.65 (0.20-1.02)	0.86 (0.62-1.05)	0.044

Table 6. Comparison of hemostatic variables during follicular (cd 2-5) and luteal (cd 22-25) phases between vWD-affected females and healthy controls.

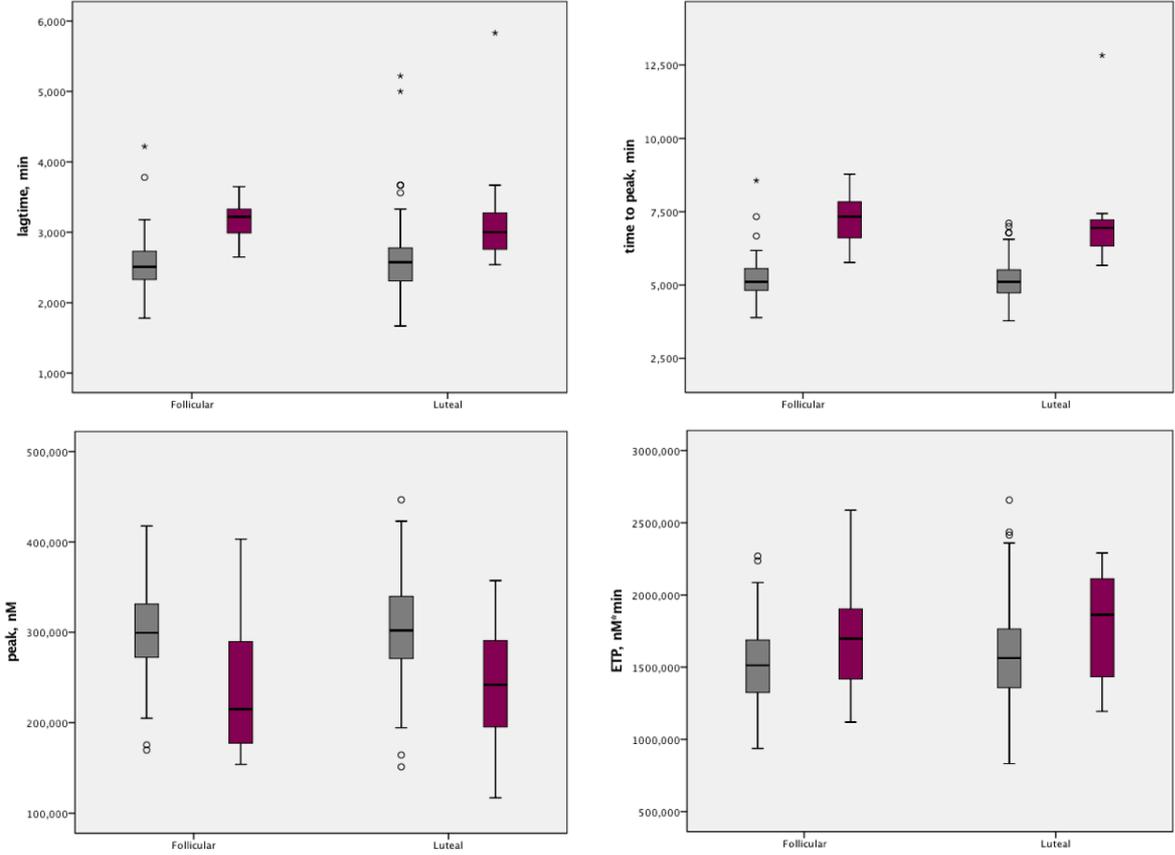
p<0,05 (statistical significance) is marked in **bold**

vWF levels were, as expected, lower in vWD group, although the p-value reached established level of significance only during the luteal phase (p=0.044 vs. P=0.059, during the luteal and follicular phase respectively). D-dimer was significantly lower also only during the luteal phase (p=0.032), while being similar in follicular phase. Surprisingly, AT, FII, FVII and FX were all significantly higher in vWD-patients, than in controls, irrespective of the phase.

We also found significant differences in thrombin generation parameters between vWD patients and controls. Lag-time and time to peak were significantly prolonged in vWD patients compared to controls (in all cases p<0.0005), while peak thrombin concentration was decreased (p<0.003). In contrast, the total amount of generated thrombin, ETP, remained the same between the groups. The observed differences in the parameters of thrombin generation are graphically depicted in Figure 12.

Figure 12. Comparison of the thrombin generation parameters between the groups during two phases of the menstrual cycle.

vWD patients are marked in plum, while healthy controls in grey.



4.4 STUDY IV

The background information on the control group is similar to Study III.

First, we compared levels of chosen variables between different phases of the menstrual cycle within the vWD group. The results are presented hereinafter as Median (25-75 percentiles) and summarized in Table 7.

	Follicular phase (cd 2-5)	Luteal phase (cd 22-25)	p-value
CATHL, pg/mL	6475.05 (3363.98 - 16656.98)	3444.47 (2091.28 - 16175.62)	0.875
CATHS, pg/mL	4137.74 (2950.11-5524.64)	4686.90 (3743.88-6143.64)	0.239
Endostatin, pg/mL	98066.45 (55628.48 - 132801.84)	71986.73 (52809.96 - 106878.43)	0.062
hs-CRP, mg/L	1.34 (0.38 - 2.23)	0.47 (0.27 - 1.41)	0.155
IL-6, pg/mL	3.99 (2.39 - 12.41)	3.64 (1.11-8.88)	0.508
sE-selectin, pg/mL	20686.28 (16741.31 - 31344.94)	23751.68 (15575.59 - 30806.03)	0.433
sICAM-1, pg/mL	193119.66 (177125.86 - 217278.64)	173952.76 (140408.86 - 196667.53)	0.209
sP-selectin, pg/mL	17945.70 (12700.38 - 27885.64)	19067.49 (13170.77 - 29360.94)	0.638
sVCAM-1, pg/mL	278598.80 (259183.24 - 328763.73)	276624.05 (248947.58 - 303147.40)	0.754

Table 7. Inflammatory and endothelial markers during follicular and luteal phase of menstrual cycle in patients with vWD.

As one may see from the above table, none of the inflammatory or endothelial parameters differed across the menstrual cycle, with the lowest p-value was found when comparing endostatin levels (although still above the established boundary, $p=0.062$).

Afterwards, we compared two groups - vWD patients and healthy controls - in regard to the differences of the inflammatory and endothelial markers during separate phase of the menstrual cycle. Several parameters were found to be different. They are presented on the boxplots (Figure 13).

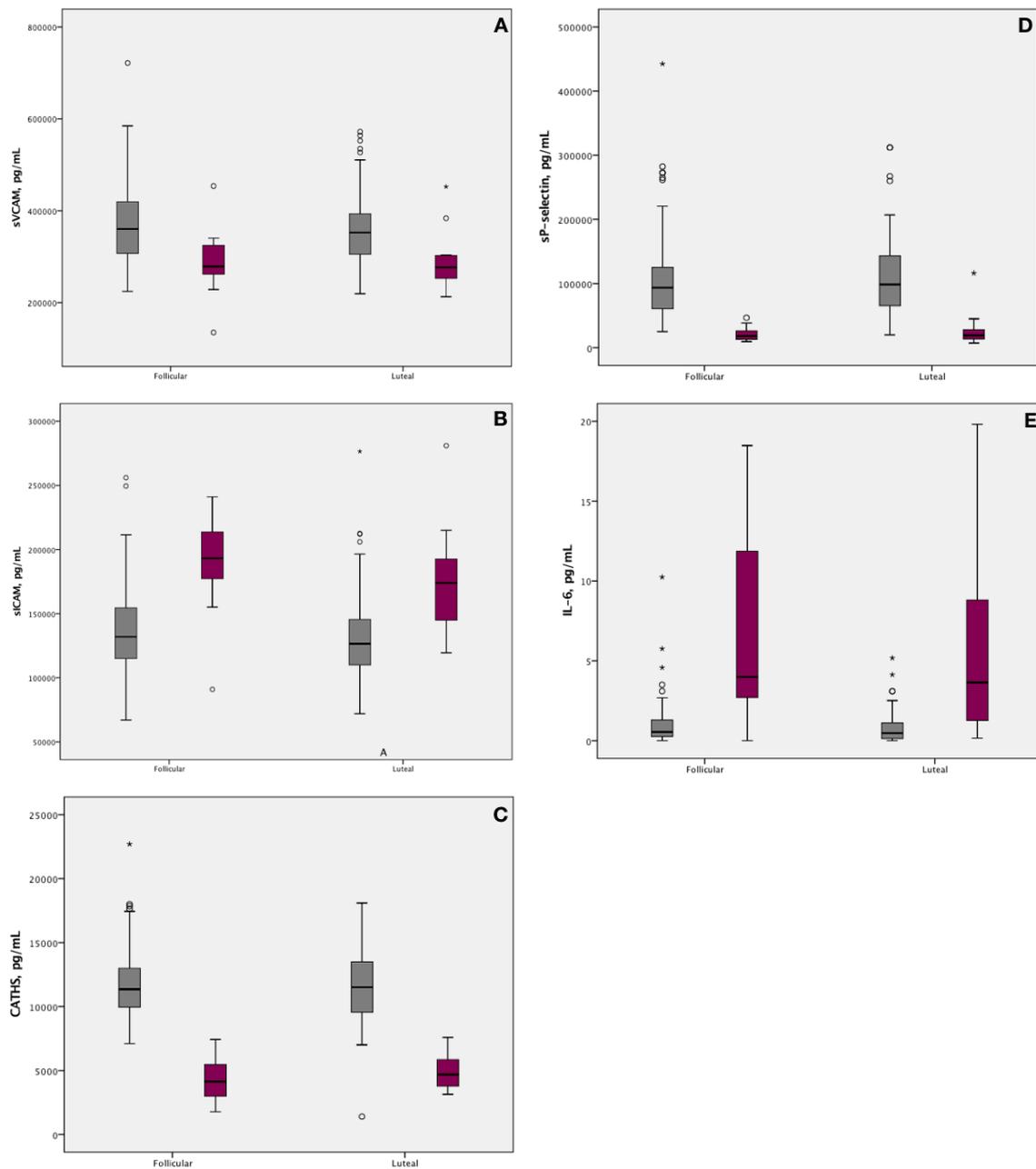


Figure 13. Comparison of those inflammatory and endothelial markers that differ significantly between the groups. vWD patients are marked in grey, while healthy controls in white.

CATHS, cathepsin S; IL-6, interleukin 6; sICAM-1, soluble intracellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; sP-selectin, soluble P-selectin.

In all cases p-value was <0.0005 , except for sVCAM-1 (Fig.13A), where it equaled 0.002 and 0.001 during follicular and luteal phase respectively.

5 DISCUSSION

5.1 STUDY I

The first study focused on heavy menstrual bleeding and its impact on everyday life activities and health-related quality of life (HRQOL) in women with vWD, in Sweden. Two major findings were made.

Firstly, women with vWD in their majority receive a variety of treatments aimed at reducing blood loss. However, despite wide treatment coverage, more than half of the women still experience heavy menstrual bleeding. This indicates the insufficiency of current pharmacological approach, as well as the demand for its optimization.

Secondly, excessive menstrual blood loss has a substantial impact on everyday life activities in women with vWD, with the vast majority of them being limited in several areas. Furthermore, HRQOL is lower in women with vWD, compared to Swedish general population. The most significant difference between the groups was observed in dimension ‘bodily pain’.

Heavy menstrual bleedings (HMB) was present in more than half of women with vWD (50.0% according to self-estimation and 53.3% according to objective measurements). This finding is consonant with the previous studies by Kirtava *et al.*(224), Rae *et al.*(225) and Byams *et al.*(226).

The discrepancy between subjective and objective assessment of menstrual blood loss is well-known and was present in our study. For instance, 5 women reported normal menstrual bleeding, albeit their PBAC score was consistent with HMB. One explanation may lie in hereditary nature of vWD. A woman with vWD may consider her menstrual blood flow normal just for the reason that it matches that of their mother or sister. Another reason might be related to the false subjective improvement. A woman with HMB may experience some degree of improvement following hemostatic treatment. However, real amount of menstrual blood loss sometimes remains beyond the normal values, although it somewhat decreases after the start of treatment. Worth noticing, that the opposite situation was also present, with 4 women reporting HMB, while in fact they lacked clinical criteria for HMB.

It testifies the necessity of using validated objective or at least semi-objective bleeding scores in order to approximate the results to reality. In our study we used both self-reported assessment and pictorial blood loss assessment chart (PBAC). The accuracy of the latter was debated. However, the PBAC is routinely employed both in clinical practice and scientific research with reliable outcomes.

It should be mentioned, that in certain cases the size of the study group was insufficient to reach the chosen level of statistical significance. Nonetheless, a meticulous selection process ensured the reliable results, comparable with other studies. All patients were connected to hematology centers and had a documented diagnosis of vWD.

Pharmacological treatment was versatile and covered the vast majority of patients (80%). Women who were prescribed with levonorgestrel-releasing intrauterine system (LNG-IUS) had normal or even no menstrual bleeding, while those receiving tranexamic acid (TA) continued to have HMB. This is in accordance with previous studies. Leminen *et al.*(227) reported that LNG-IUS is superior to TA in regard to reducing menstrual bleeding (83% vs. 47%, respectively). Chi *et al.* (228) performed a long-term follow up study on efficacy of LNG-IUS for the treatment of HMB in women with bleeding disorders. PBAC score was measured before starting the treatment and was 255 in its median (range 134–683). LNG-IUS was then prescribed for a median of 33 months (range 14-103) and during that period the median PBAC scores in women reduced to 35 (range 0–89), indicating normal menstrual bleeding. Furthermore, 42% of women had amenorrhea at the time of the follow-up. Authors also reported improvement in HRQOL and hemoglobin rise, associated with LNG-IUS use.

In the current study, the number of vWD-affected women using LNG-IUS was low. This may originate from women's reluctance towards hormonal treatment or lack of knowledge about LNG-IUS. Moreover, the reason for the rare use of LNG-IUS may arise from lack of medical counseling.

We reported considerable limitations in everyday life activities in women with vWD, due to menstruation. This is consistent with recent studies (224, 225). Kirtava *et al.*(224) published a case-control study in which they had compared the impact of menstruation on overall life activities in women with vWD (n=62) and control group (n=70). Menstruation caused disturbances in life activities of 23 (32.1%) of women with vWD, compared with only 7 (10.0%) in the control group. Rae *et al.*(225) examined 84 women with HMB and underlying inherited bleeding disorders, the majority of whom (77.5%) reported alterations in daily activities due to menstrual bleeding.

The HRQOL in women with vWD appeared to be lower in comparison with general Swedish population. Despite the fact that statistical significance has not been achieved in every dimension (most likely due to small sample size), the trends are obvious and consistent with recent research (225, 229). In current study women with vWD had significantly lower score in the dimension 'bodily pain', compared to general population. This conforms to the study by

Rae *et al.*(225), reporting lower scores in the dimensions ‘pain’ and ‘cognition’ among women with bleeding disorders and HMB. Worth noticing, that according to Rae *et al.* women with vWD experienced more pain, compared with men or women with other bleeding disorders. At the same time, other researchers stated that dimension ‘vitality’ is negatively affected in women with vWD (230, 231). This should not be considered as mismatching results, but rather as a reflection of substantial impact of HMB on life quality.

Summarizing the results, we reported that women with vWD in their majority receive pharmacological treatment for preventing bleeding episodes. However, more than half of women still experience HMB, which has significant negative influence on women’s everyday life activities and overall health-related quality of life. Therefore, the current situation reflects the inadequacy of current therapeutic approaches. The latter should possibly be optimized through intensive collaboration between gynecologists and hematologists.

5.2 STUDY II

The second study aimed to evaluate the incidence of postpartum hemorrhage (PPH) in women with von Willebrand disease (vWD) in regard to the different types of vWD, levels of coagulation factors and pharmacological management. The study embraced 18 years period and included 14 different obstetrical units.

The incidence of PPH was expectedly higher in women with vWD, compared with general population in high resource countries (232, 233). It is important to notice, that reported incidence of PPH varies significantly across the studies, primarily because of different study design. In studies, where questionnaires and self-reports were employed, the incidence of PPH appeared to be 31-59% (91, 158, 224, 234), while in those studies that used medical records and objective criteria for PPH incidence was 15-34% (235-237). This discrepancy complicates the comparison between studies and usually originates from differences in vWD types distribution within study cohort, but also unequal diagnostic approaches and management options. In our study group, the incidence of primary PPH was 44%, which is comparable to the studies where patient recall was employed, but higher than in studies with similar techniques of data collection.

Median blood loss was higher in women with type 3 vWD. They also experienced PPH (of all kinds) more frequently than women with other types of vWD. Surprisingly, women with vWD type 2 were at lower risk of developing PPH than women with type 1 ($p=0.02$), probably due to overrepresentation of the latter in the group that did not receive hemostatic treatment.

In the current study, PPH encountered frequently in all delivery modes, but especially in the instrumental assisted vaginal labors, where 3 out of 7 deliveries (43%) resulted in severe primary PPH (blood loss $> 1000\text{mL}$). This is substantially higher than in general population, where severe primary PPH occurs in 3.5% of vaginal deliveries, 8% of instrumental vaginal deliveries and 13% for CS (238). Our data strengthens the current recommendations to cautiously perform forceps/vacuum-assisted deliveries or even abstain from using these techniques in women with vWD in order to prevent lacerations of the maternal genital tract and subsequent bleeding (239).

There are conflicting results regarding whether women with undiagnosed bleeding disorder are at higher risk of experiencing hemorrhages. Chee *et al.*(240) have analyzed data from 33 women with vWD and concluded that the risk of PPH is higher if the diagnosis of vWD is established at the time of delivery. Authors have interpreted this fact as follows: women with apparent vWD are more likely to be diagnosed and to develop bleeding complications during

postpartum period. We obtained the opposite results with higher incidence of PPH in women with yet undiagnosed and therefore untreated vWD. The possible explanation for the controversy might arise from differences in prophylactic approaches. In current study all pregnant women with diagnosed vWD received at least antifibrinolytics before delivery, which is in accordance to recommendations by The Nordic Hemophilia Council (241). However, other routines are adapted in different countries. For instance, some authors recommend using TA only in case of significant decrease of vWF levels during third trimester (242, 243).

No significant differences in PPH incidence were observed between subgroups with different treatment approaches: TA, TA + DDAVP[®], TA + CFC. One may speculate that this is due to the fact that women differed in the severity of vWD, initial levels of coagulation factors, *etc.* In addition, subgroups with different treatment options were not large enough to provide statistically significant differences.

There are well-recognized difficulties in estimating exact blood loss after delivery (244). Routines also differ distinctly between hospitals. In current study, blood loss was assessed by weighing the soaked materials.

An important finding is the potential utility of FVIII level in third trimester as a predictor of postpartum bleeding. It has been reported that low FVIII levels may forecast surgical bleedings (244-246). Our results support this theory and testify similar association between obstetrical bleedings and low levels of FVIII (236). Important, that this remains true, even when CFC is prescribed preventively before labor onset, indicating the insufficiency of the therapy, at least in the most severe cases. The latter is further reinforced with the fact that the incidence of both primary and secondary PPH was higher in the study group, compared with the general population, even when the diagnosis of vWD was known and managed in accordance with the guidelines.

Despite the fact that the obtained results require confirmation in larger cohorts, it is necessary to note the strengths of the current study. All patients were recruited from Coagulation Unit and therefore had confirmed diagnosis of vWD. Sample size of 59 deliveries is similar to previous reports from United States and United Kingdom (158, 236, 240). An important advantage of the current study is the inclusion of 11 patients with undiagnosed vWD at the time of the delivery. This allowed us to compare the outcomes between vWD-affected women who received hemostatic treatment and those who did not.

To conclude, women with vWD in Sweden are at higher risk of developing bleeding after delivery. The most prominent risk factors are type 3 vWD and instrumental assisted delivery.

Furthermore, women with undiagnosed and thus untreated vWD have a greater risk of suffering from PPH. This indicates the importance of early diagnosis through rigorous assessment of family history with a special attention towards previous bleeding episodes. Validated bleeding scores, especially those that can be easily employed in antepartum clinics are also of a great importance. The promising significance of FVIII levels as a predictor of PPH in women with vWD should be further assessed in the larger studies.

5.3 STUDY III

To the best of our knowledge changes in hemostatic variables during regular menstrual cycle has never been assessed previously in women with vWD. We observed lower levels of FVIII and FX during the luteal phase, compared to the follicular phase in vWD affected females. When comparing vWD group and controls, we found higher levels of several coagulation factors in the study group - FII, FVII and FX. Thrombin generation profiles were also different and characterized by prolonged lag-time, time to peak and reduced peak thrombin concentration. At the same time, total amount of generated thrombin was similar between the groups.

Coagulation system is known to respond sensitively to the changes both in external and internal environment. In women, cyclic changes of sex steroids during the menstrual cycle also modulate functioning of hemostasis, although ultimate effect is still yet to be clarified, despite intensive research within the field (247-251). At the same time, understanding of how does exactly menstrual cycle affect coagulation is extremely important both for fundamental science and clinical routine. It may potentially help to enlighten basic mechanisms of coagulation and to choose a proper timing for coagulation analyses during the menstrual cycle.

We found lower levels of FVIII and FX during the luteal phase, compared to the follicular phase in women with vWD. The latter was also demonstrated in the recent study on healthy volunteers by Chaireti *et al.* (219). FVIII was a subject to a number of researches, with the majority of them reported no cyclic variation in its levels (252-258), while others found its lowest levels during menstruation or early follicular phase (259, 260). The decrease in FVIII and FX, active procoagulant factors, during the luteal phase may predispose women to excessive bleeding during menstruation - the common complication of vWD (261).

Thrombin generation profiles did not differ across the menstrual cycle in women with vWD, which is in contrast to the control group, where ETP increased during the luteal phase. However, it is difficult to establish whether there is actually no difference or the sample size is not large enough to provide reliable results.

When comparing vWD patients and healthy controls, more pronounced differences were found.

Thrombin generation profiles were significantly different between the groups, irrespective of the phase. Lag-time and time to reach peak thrombin concentration were prolonged in women with vWD compared to controls ($p < 0.0005$ for all cases), while maximum thrombin concentration was lower ($p = 0.003$ and $p = 0.002$ during follicular and luteal phase respectively).

The total amount of the generated thrombin was similar between the groups in either phase. Therefore, the thrombin generation curve became flattened, with a gentle rise and decreased peak. This observation is consistent with previous studies (221). Based on that, one may speculate that in order to achieve effective hemostasis it is more important to reach peak thrombin concentration faster, than to generate somewhat total thrombin amount over a longer period. Obviously, this idea should be corroborated by exact clinical data. Summarizing, thrombin generation assay, which is often used today to evaluate hemostasis, has confirmed its usefulness in differentiating patients with the vWD and healthy controls.

We also observed higher levels of FII, FVII and FX in vWD group, compared to controls ($p < 0.0005$; $p = 0.01$; $p < 0.0005$ respectively). It could be hard to draw robust conclusion from this observation. Probably, some uncontrolled confounders might be involved. This may include but not be limited to the blood group, smoking status or chronic inflammation. Important to mention, that increase in procoagulant markers might reflect the activation of hemostasis in order to compensate for the bleeding.

During luteal phase, D-dimer was lower in vWD group, compared to controls ($p = 0.032$). The previous studies on D-dimer levels during menstrual cycle reported conflicting results (255, 262). It is possible, that relatively higher levels of D-dimer during early follicular phase (which corresponds to the menstrual phase of the uterine cycle) may reflect activation of fibrinolysis within the uterine cavity.

Another interesting finding was higher AT levels in women with vWD, compared to controls, both during follicular and luteal phase ($p < 0.0005$). AT expresses its anticoagulant properties through inhibiting serine proteases (*i.a.* thrombin). Therefore, we speculate that increase in AT among women with vWD might contribute to diminished thrombin generation and subsequently development of bleeding complications.

The current study has several limitations. We performed blood tests twice during the menstrual cycle, which might be considered insufficient, although remains comparable to previous studies. Recent systematic review by Knol *et al.* (251) reported that studies differ significantly in regard to number of sampling and exact day within the menstrual cycle. At the same time, it is important to balance the scope of the study with the patients' risks due to relative invasiveness of the procedure. In the current study we have not controlled the participants for the blood groups. It has been previously shown, that blood group may affect the blood levels of FVIII and vWF. ABO glycoproteins are intimately intertwined with vWF, which leads to the fact, that people with 0 blood group have lower levels of vWF than those from non-0 group.

At the same time, belonging to a particular blood group can hardly explain all observed differences in other hemostatic variables. Lack of patients with type 3 vWD might be considered as a drawback. This is due to relative rarity of the patients and, as a result, difficulties in recruiting them. However, the majority of vWD patients have type 1, which makes the results of the current study applicable to the real situation.

The main strength of the current study is stringent inclusion criteria. Normally, women with vWD are often prescribed with hormonal treatment in order to control bleeding episodes during menstruation. In our study, none of the participants received hormones in any form. This was done in order to avoid bias, resulting from the influence of hormones on hemostasis.

To conclude, women with vWD are different from healthy controls with regard to the levels of hemostatic parameters. Interestingly, these differences were largely constant across the menstrual cycle. Based on that, it sounds reasonable to speculate that coagulation tests and especially global hemostatic assays, such as thrombin generation, regardless of the timepoint may still provide valuable information.

5.4 STUDY IV

In women, periodic fluctuations of the sex steroids during the menstrual cycle influence many body functions, including immune system. Summarizing the previous findings one can deduce the following pattern. Immune system is more active during the follicular phase, reflecting the rise in estrogen, followed by decrement during the luteal phase, which is at least partly dependent on the rise of the anti-inflammatory progesterone (263-265). Activity of the immune system peaks at the time of menstruation, the latter is considered to be an acute inflammatory event (266, 267). At the same time, inflammatory system and coagulation intertwine intimately, sharing the common pathways and influencing each other (268, 269). Therefore, the available data indicates the existence of a flexible cooperation between female reproductive system, inflammatory system and coagulation. In the current study we analyzed changes in the blood levels of the inflammatory and endothelial markers during a regular menstrual cycle in women with vWD.

Endostatin levels were higher during the follicular phase, although the difference was not significant ($p=0.062$). Endostatin expresses anti-angiogenic properties, but also modulates coagulation cascade, through upregulating plasmin production and downregulating FVII, FX, FXI, FXII, tissue factor and, interestingly, vWF (270, 271). Early follicular phase (cd 2-5) corresponds to the menstrual phase of the uterine cycle. During this period active angiogenesis occurs within the basal layer of the endometrium. It seems possible to hypothesize, that through synergism of the anti-angiogenic and anti-coagulant properties, endostatin contributes to the development of heavy menstrual bleeding - the common complication in vWD affected females.

Other variables did not differ between the phases, which may be due to a small number of participants in the study group.

When comparing vWD females and healthy controls, we found lower sP-selectin levels in the former group. P-selectin shares the storage site with vWF, *i.e.* Weibel-Palade bodies in endothelial cells and α -granules in platelets. One may speculate, that decrease in p-selectin levels among patients with vWD might reflect the alterations in the transportation or storage routes, common for vWF and p-selectin. Furthermore, upon stimulation with triggers, *e.g.* thrombin, P-selectin becomes externalized on the cell surface, facilitating platelet adhesion to endothelium. Therefore, lower p-selectin levels may potentially contribute to increased bleeding tendency in women with vWD.

We also observed differences in several markers, that influence angiogenesis. sVCAM-1 was lower in women with vWD, compared to controls during both phases of the menstrual cycle. VCAM-1 was previously reported to take part in *de novo* vessel formation, including cytokines-induced neoangiogenesis (272, 273). In contrast, sICAM-1 levels were higher in women with vWD, than in control group. Among other functions, sICAM-1 facilitates transendothelial migration of leukocytes, which antecedes neoangiogenesis with the inflammatory *loci* (274). sICAM-1 is triggered with a variety of stimuli, *i.a.* IL-6, the latter was also higher in vWD group. IL-6 has myriad of functions, both pro- and anti-inflammatory, including its influence on angiogenesis. Gopinathan *et al.* demonstrated that IL-6 promotes formation of defective vessels (275).

In addition, cathepsin S was lower in vWF group, than in healthy controls. This protease participates in extracellular matrix degradation, which is crucial for neoangiogenesis. Deficiency in cathepsin S was demonstrated to cause suppression of angiogenesis.

To summarize, we found that women with vWD differ from healthy controls with the levels of mediators, that play role in angiogenesis. Such dysregulation of pro- and anti-angiogenic substances may potentially contribute to the development of the specific complication of vWD - angiodysplasia. As it was written before, this term means the formation of the ectatic vessels with abnormal structure in different body regions, predominantly gastrointestinal tract (200, 276). It is assumed that the formation of angiodysplasia is directly related to the lack of vWF, which negatively modulates angiogenesis. Our results suggest, that other potent markers may also play role. It is important to note, that our study was not initially designed to study angiodysplasia. Therefore, the findings require further verification. Decreased levels of cathepsin S and p-selectin among patients with vWD, compared to controls, might contribute to the development of the bleeding complications, *i.a.* heavy menstrual bleeding.

6 GENERAL CONCLUSIONS

- The majority of women with von Willebrand disease receive versatile pharmacological treatment as a prophylaxy of the bleeding episodes. However, current therapeutic approaches are insufficient to prevent excessive blood loss during menstruation, since more than half of the women meet the clinical criteria for HMB.
- Excessive menstrual blood loss significantly influences women's lives, causing alterations in everyday life activities and diminishing the overall health-related quality of life.
- Despite the reported efficacy of hormone-releasing intrauterine devices in preventing heavy menstrual bleedings, their use among women with vWD remains low. An appropriate counseling on the advantages and actual side effects of the intrauterine devices could potentially encourage women to opt for LNG-IUS.
- Current clinical management of vWD-affected women should be optimized in order to reduce frequency of the HMB episodes and their impact on overall life activities. This could potentially be done through intensive collaboration between hematologists and gynecologists.
- Despite wide treatment coverage, women with vWD are still at higher risk of PPH, with certain subgroups being at even greater risk of experiencing major bleeding after delivery. Among different types of vWD, women with type 3 are more likely to suffer from bleeding complications following delivery.
- Instrumental assisted delivery represents an independent bleeding risk factor with almost half of vWD-affected women experiencing blood loss more than 1000mL. This reinforces the recommendations to avoid using forceps and/or vacuum in this cohort.
- Women with vWD undiagnosed at the time of the delivery have a higher risk of developing PPH. In order to overcome this challenge, a thorough identification of vWD patients is required. Validated blood scores provided in the antenatal settings are potentially beneficial.

- Levels of FVIII measured in the late-pregnancy could serve as the predictor of PPH in women with vWD.
- In women with vWD, procoagulant factors FVIII and FX are lower during the luteal phase, which may predispose them to bleed excessively during menstruation. Furthermore, higher antithrombin levels in vWD-affected females compared to healthy controls, may also contribute to the development of the bleeding complications.
- vWD-affected females require longer time to reach peak thrombin concentration, with the latter still being lower, than in healthy women. This implies, that dynamics of thrombin generation, *i.e.* rapid reaching the maximum thrombin concentration is essential to ensure effective hemostasis.
- Endostatin, which exhibits antiangiogenic and anticoagulant properties, is higher during early follicular phase, than in luteal phase in women with vWD. This may potentially contribute to the development of heavy menstrual bleeding, since reliable hemostasis and the formation of new vessels within the endometrium are both essential for the menstrual bleeding cessation.
- P-selectin levels are lower in women with vWD, compared to healthy controls, which may increase the bleeding tendency, since p-selectin promotes platelet aggregation.
- Levels of several proteins, regulating angiogenesis (sICAM-1, sVCAM-1, cathepsin S and IL-6) differ in patients with vWD, compared to healthy controls. This hypothetically contributes to the formation of angiodysplasia - common complication of vWD.

7 FUTURE PERSPECTIVES

While working on our project, as well as studying the results of other researchers, I realized that currently there are a lot of yet unresolved and controversial issues related to vWD, both in fundamental science and routine clinical practice. In part, this situation is due to the fact that vWF is a multifunctional agent that takes part in the development of many diseases.

In our project, participants were included in the study group based on stringent inclusion criteria. Therefore, they represent a valuable cohort, eligible to participate in the further studies on vWD. Following are some reflections on vWD, that, in my opinion, are of a notable interest for future researches.

Growing evidence suggests vWF role in the progression of cardiovascular diseases. Higher vWF levels in patients with atrial fibrillation present an independent risk-factor with major adverse events and all-cause mortality (277). In accordance with recent population-based study, rise in vWF and decrease in ADAMTS13 activity are associated with dementia (278). Therefore, it may be fruitful to collaborate with cardiologists on assessing the prevalence of cardiovascular diseases in our vWD group.

vWF is now also considered to play a substantial role in oncogenesis. Although this role is yet to be scrutinized, the most recent studies suggest the bidirectional effect, both in tumor progression and cancer cells apoptosis (279). Furthermore, elevated levels of vWF were reported for many malignant diseases and at least for some of them, vWF levels correlate with disease progression (280-283). The exact causality still remains to be described. A possible aim for a future research may be to study our vWD cohort in regard to the development of the malignancies among them. Due to the relative young age of participants and in order to enrich the study group, it may be reasonable to retrieve medical information on their relatives.

Emerging role of vWF in angiogenesis is another exciting field with many unsolved issues (136). In our study #4 we have also demonstrated the altered balance of pro- and anti-angiogenic mediators in patients with vWD, compared to controls. Since the abovementioned study was not initially designed to study angiodyplasia, it would be of a great interest to design such study. This could potentially bring new insights on common vWD complication - formation of angiodyplastic lesions. Furthermore, this may also provide a valuable information on other disorders, related to mesenchymal dysplasia - Ehlers-Danlos syndrome, Dieulafoy's vessels, multiple skeletal abnormalities, *etc.*, since vWD often co-occurs with them.

The angiogenic properties of vWF present a great clinical importance, as angiogenesis is currently considered to play substantial role in the progression of the various diseases, including malignancies. Studying vWF-angiogenesis relations may potentially pave the road to the new therapeutic options for cancer patients.

Undoubtedly, the development of the new scientific technologies will bring new insights and provide valuable information on vWD. To name few, using blood outgrowth endothelial cells (BOECs) to study basic mechanisms of vWD development or employing untargeted approach (omic-technologies) (284).

The development of the new drugs for vWD treatment is crucial for improving quality of life. A candidate option is interleukin-11, which was shown to increase plasma vWF and reduce bleeding symptoms, *i.a.* among patients refractory to DDAVP (285, 286). Another promising agents are aptamers - the new set of drugs, oligonucleotides, that may specifically block various proteins. For instance, an aptamer ARC1779 connects to the A1 domain of vWF, blocking its capacity to bind platelets. This may potentially be used in treating 2B vWD patients (287, 288).

Simultaneously, our project has demonstrated that the most effective current treatment option for excessive menstrual bleeding - LNG-IUS, is not widely used among patients with vWD, probably due to the prejudices towards hormonal treatment. It is of a great clinical importance to provide a reliable and most up-to-date information on the current treatment options of vWD-related gynecological complications. This might be done through designing popular science articles or a brochure to be distributed in the respective groups.

vWF has many functions, that extend far beyond the hemostasis itself. Therefore, careful study of the basic mechanisms of vWF functioning will help not only patients with vWD, but hopefully also those who suffer from a number of other diseases, in which vWF also plays role - autoimmune diseases, malignancies and atherosclerosis, *etc.* Furthermore, this will help us to better understand the mechanisms of inflammation and angiogenesis.

8 ACKNOWLEDGEMENTS

First of all, I want to thank a person without whom this project would not even have begun, my principal supervisor - **Miriam Mints**. Thank you not only for your wise and attentive mentorship, but also for the kind maternal attitude towards me all this time. You were always ready to share with me your experience and knowledge and thus I learned a lot from you. You certainly know how much I appreciate your kindness and responsiveness and I hope that we will have many opportunities to work together in the future.

I want to thank my co-supervisor, **Roza Chaireti**. Thank you for your assistance throughout the project. You always accepted my requests to discuss tricky issues and even being on maternal leave you always found time to be present during the discussion. Important, I want to appreciate your generosity in sharing valuable contacts. Your contribution to the project is difficult to overestimate.

Many thanks to my co-supervisor, **Katarina Bremme**, for your friendliness and goodwill. Your outstanding experience in clinics and science often helped me to look at the project from a different point of view.

Thanks to my co-supervisor, Head of the Coagulation Unit, **Margareta Holmström**, for sharing the priceless and most up-to-date information on coagulation. Thank you for always finding the time to carefully read the manuscripts and suggest ways to improve them. I appreciate you paving me the road through this complicated field of hemostasis.

Dear professor **Sonia Andresson**, thank you, that you always treated me and my wife kindly. You were always there to give a kind advice and helped us many times. You are no doubt always a welcome guest in our family.

I want to thank professor **Kristina Gemzell Danielsson**, Chair of Obstetrics and Gynecology, for the opportunity to be a part of the team. I was very impressed by your lectures during the course on Sexual and Reproductive Health. Your attentive attitude towards women's health is an excellent example for me to follow.

Catharina Karlsson, thank you for helping me with the administrative matters. You were always a right person to contact, irrespectively on whether that was your field or not.

I would like to express my special gratitude to **Anna Sandberg**. Thank you for constantly being there to help me managing the difficulties. You were always a lifeline for me!

Throughout the history, doctors needed nurses. I want to show my appreciation to the nurses **Doris Näslin** and **Ingela Larsson** for your help with the patients recruitment.

I want to thank professor **Dariko Niauri** for your support and wise advice, as a senior colleague.

In addition, I consider it necessary to thank **Eduard Komlichenko** and **Tatyana Pervunina** for your help in recruiting patients.

I want to thank **all the patients** who kindly agreed to participate in our project. Together, we answered some very important questions and I am sure that we will see the new amazing discoveries on von Willebrand disease in the future.

I want to say thank you to all the **researchers and doctors** whom I was fortunate enough to get acquainted with, in the laboratory and at the courses. You all helped me to become a better doctor. And we also had fun together!

A friend in need is a friend indeed. I want to thank **Zoia Razumova** for all your efforts and infinite willingness to help. I know that I can always rely on you. In your turn, you must have no doubts that I'm always at your service. Thank you for your jokes and nachos, we ate together. Without you, that would not be that fun. A special thank goes to your occasional desperate look, which made me realize that it was possibly not that bad with me. ;) You are a great young doctor and I wholeheartedly wish you success!

I also want to thank my dear colleague, **Alexander Kuznetsov**, whom we arrived together with to Karolinska Institute back in 2012. Thank you for keeping me up. I promise to pay you back during your own dissertation. In addition, thanks for the unforgettable time we had in Strix. Dear friend, we have a lot ahead of us!

I want to thank my beloved mother, **Olga Govorova**, without whom I would have never been able to enter Karolinska. It is impossible to mention all the things that you helped me with in my life. Thank you for your love, support and care. And more of that, thank you for your wisdom and patience. I was not a good boy all the time, but you were always there to maintain me. I love you. How do you like the plasticine today?

Thanks to **my entire family** for your understanding and support. Forgive me for being in a hurry, drowsy and nervous state sometimes. I promise, I will spend some time now to sleep well.

I would like to thank my colleagues and friends from Saint-Petersburg - **Olga Koz'mova, Vladimir Vorob'ev, Alexander Ivanin, Natalya Gur'eva and Andrey Denisov** for your support and help. We have had an amazing time together. It genuinely helped to reset my mind and replenish my energy.

It is impossible not to note the contributions of my hounds – **Sebastian and Mojo**. Thank you guys for restoring my mood and internal forces.

Oleg Tvorogov – a famous Russian philologist said: “A man becomes a good scientist for two reasons: the first is a good wife and the second is a bad wife”. And thus, the last but the most important, I would like to thank my darling wife, **Ellina Govorova**, who has supported me all this time and manfully endured long periods of my absence. My dear, you are my comrade in arms and I highly appreciate your sacrifice, which always helps me to move forward. Thank you for always being by my side.

9 REFERENCES

1. von Willebrand EA. Hereditar pseudoheemofili. *Fin Lakaresallsk Handl.* 1926(67):7-112.
2. Minot G, Lee R. Miscellaneous hemorrhagic conditions. *Nelson Loose-Leaf Medicine* New York, NY, Nelson & Sons. 1920.
3. Bowie E, Didisheim P, Thompson JJ, Owen CJ. von Willebrand's disease: A critical review. *Hematol Rev.* 1968;1(1).
4. Nilsson IM. In memory of Erik Jorpes. von Willebrand's disease from 1926-1983. *Scand J Haematol Suppl.* 1984;40:21-43.
5. Quick AJ. Telangiectasia: its relationship to the Minot-von Willebrand syndrome. *Am J Med Sci.* 1967;254(5):585-601.
6. Rendu M. Épistaxis répétées chez un sujet porteur de petits angiomes cutanés et muqueux. *Bull Mem Soc Med Hop Paris.* 1896;13:731-3.
7. Glanzmann E. Hereditäre haemorrhagische Thrombasthenie. Ein Beitrag zur Pathologie der Blutplättchen, *Jahrbuch fuer Kinderheilkunde.* 1918:1-42.
8. Alexander BG, R. Dual hemostatic defect in pseudoheemophilia. *J Clin Invest.* 1953;32(6):551.
9. Buchanan JC, Leavell BS. Pseudoheemophilia: report of 13 new cases and statistical review of previously reported cases. *Ann Intern Med.* 1956;44(2):241-56.
10. Singer K, Ramot B. Pseudoheemophilia type B; hereditary hemorrhagic diathesis characterized by prolonged bleeding time and decrease in antihemophilic factor. *AMA Arch Intern Med.* 1956;97(6):715-25.
11. Erlandson M, Fort E, Lee RE, Schulman I, Smith CH. Vascular hemophilia; a familial hemorrhagic disease in males and females characterized by combined antihemophilic globulin deficiency and vascular abnormality. *Pediatrics.* 1956;18(3):347-61.
12. Finch CA, Matter M, Melly A, Newcomb TF. Vascular hemophilia: the association of a vascular defect with a deficiency of antihemophilic globulin. *Am J Med Sci.* 1956;232(4):421-33.
13. Schulman I, Smith CH, Erlandson M, Fort E, Lee RE. VASCULAR HEMOPHILIA: A Familial Hemorrhagic Disease in Males and Females Characterized by Combined Antihemophilic Globulin Deficiency and Vascular Abnormality. *Pediatrics.* 1956;18(3):347-61.
14. Spurling CL, Sacks MS. Inherited hemorrhagic disorder with antihemophilic globulin deficiency and prolonged bleeding time (vascular hemophilia). *N Engl J Med.* 1959;261(7):311-9.
15. Achenbach W, Klesper R. [Angio-hemophilia A and B: nonhemophilic hemorrhagic diathesis with congenital deficiency of antihemophilic globulin-(factor IX)]. *Folia Haematol (Frankf).* 1957;1(3):251-67.
16. Karaca M, Stefanini M. Angiohemophilia: studies in vivo. *Acta Haematol.* 1963;29:102-8.
17. Larrieu MJ, Soulier JP. [Deficiency of antihemophilic factor A in a girl associated with bleeding disorder]. *Rev Hematol.* 1953;8(3):361-70.

18. Soulier JP, Alagille D. [Prolongation of clotting time combined with a deficiency of anti-hemolytic factor A]. *Rev Fr Etud Clin Biol*. 1956;1(2):187-99.
19. Lutze G, Canzler E, Franke D, Topfer G. [Pre- and postpartum hemostasis in type II Willebrand-Jurgens syndrome]. *Z Gesamte Inn Med*. 1989;44(4):117-20.
20. Lehmann W. [New studies on thrombopathy (v. Willebrand-Jurgens) at Aland Islands, Finland]. *Acta Genet Med Gemellol (Roma)*. 1959;8:38-42.
21. Quick AJ, Hussey CV. Hemophilic condition in a girl. *AMA Am J Dis Child*. 1953;85(6):698-705.
22. Creveld S, Jordan FLJ, Punt K. Deficiency of Anti-Hemophilic Factor in a Woman, Combined with a Disturbance in Vascular Function.1. *Acta Med Scand*. 2009;151(5):381-9.
23. Macfarlane R. Critical review: the mechanism of haemostasis. *QJM*. 1941;10(1):1-29.
24. Blombäck B, Blombäck M, Paul K-G, Theorell H, Thorell B. Purification of Bovine and Human Fibrinogen. *Acta Chem Scand*. 1956;10(1):147-.
25. Nilsson IM, Blombäck M, Jorpes E, Blombäck B, Johansson SA. Von Willebrand's disease and its correction with human plasma fraction 1-0. *Acta Med Scand*. 1957;159(3):179-88.
26. Ginsburg D, Handin RI, Bonthron DT, Donlon TA, Bruns GA, Latt SA, et al. Human von Willebrand factor (vWF): isolation of complementary DNA (cDNA) clones and chromosomal localization. *Science*. 1985;228(4706):1401-6.
27. Sadler JE, Shelton-Inloes BB, Sorace JM, Harlan JM, Titani K, Davie EW. Cloning and characterization of two cDNAs coding for human von Willebrand factor. *Proc Natl Acad Sci U S A*. 1985;82(19):6394-8.
28. Titani K, Kumar S, Takio K, Ericsson LH, Wade RD, Ashida K, et al. Amino acid sequence of human von Willebrand factor. *Biochemistry*. 1986;25(11):3171-84.
29. Verweij CL, de Vries CJ, Distel B, van Zonneveld AJ, van Kessel AG, van Mourik JA, et al. Construction of cDNA coding for human von Willebrand factor using antibody probes for colony-screening and mapping of the chromosomal gene. *Nucleic Acids Res*. 1985;13(13):4699-717.
30. Lynch DC, Zimmerman TS, Collins CJ, Brown M, Morin MJ, Ling EH, et al. Molecular cloning of cDNA for human von Willebrand factor: authentication by a new method. *Cell*. 1985;41(1):49-56.
31. Wagner DD, Lawrence SO, Ohlsson-Wilhelm BM, Fay PJ, Marder VJ. Topology and order of formation of interchain disulfide bonds in von Willebrand factor. *Blood*. 1987;69(1):27-32.
32. Mannucci P, Pareti F, Ruggeri Z, Capitanio A. 1-deamino-8-D-arginine vasopressin: a new pharmacological approach to the management of haemophilia and von Willebrand's disease. *The Lancet*. 1977;309(8017):869-72.
33. Rickles FR, Hoyer LW, Rick ME, Ahr DJ. The effects of epinephrine infusion in patients with von Willebrand's disease. *J Clin Invest*. 1976;57(6):1618-25.
34. Harrison RL, McKee PA. Estrogen stimulates von Willebrand factor production by cultured endothelial cells. *Blood*. 1984;63(3):657-64.

35. Michaux G, Abbitt KB, Collinson LM, Haberichter SL, Norman KE, Cutler DF. The physiological function of von Willebrand's factor depends on its tubular storage in endothelial Weibel-Palade bodies. *Dev Cell*. 2006;10(2):223-32.
36. Nightingale TD, White IJ, Doyle EL, Turmaine M, Harrison-Lavoie KJ, Webb KF, et al. Actomyosin II contractility expels von Willebrand factor from Weibel-Palade bodies during exocytosis. *J Cell Biol*. 2011;194(4):613-29.
37. Valentijn KM, van Driel LF, Mourik MJ, Hendriks GJ, Arends TJ, Koster AJ, et al. Multigranular exocytosis of Weibel-Palade bodies in vascular endothelial cells. *Blood*. 2010;116(10):1807-16.
38. Verhenne S, Libbrecht S, Vandenbulcke A, Deckmyn H, Vanhoorelbeke K, De Meyer S. The role of platelet von Willebrand factor in mice. *J Thromb Haemost*. 2013;11:231.
39. Kanaji S, Fahs SA, Shi Q, Haberichter SL, Montgomery RR. Contribution of platelet vs. endothelial VWF to platelet adhesion and hemostasis. *J Thromb Haemost*. 2012;10(8):1646-52.
40. Foster PA, Fulcher CA, Marti T, Titani K, Zimmerman T. A major factor VIII binding domain resides within the amino-terminal 272 amino acid residues of von Willebrand factor. *J Biol Chem*. 1987;262(18):8443-6.
41. Miura S, Li CQ, Cao Z, Wang H, Wardell MR, Sadler JE. Interaction of von Willebrand factor domain A1 with platelet glycoprotein Iba1(1-289). Slow intrinsic binding kinetics mediate rapid platelet adhesion. *J Biol Chem*. 2000;275(11):7539-46.
42. Sugimoto M, Mohri H, McClintock RA, Ruggeri ZM. Identification of discontinuous von Willebrand factor sequences involved in complex formation with botrocetin. A model for the regulation of von Willebrand factor binding to platelet glycoprotein Ib. *J Biol Chem*. 1991;266(27):18172-8.
43. Jenkins PV, Pasi KJ, Perkins SJ. Molecular Modeling of Ligand and Mutation Sites of the Type A Domains of Human von Willebrand Factor and Their Relevance to von Willebrand's Disease. *Blood*. 1998;91(6):2032-44.
44. Furlan M, Robles R, Lammler B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. *Blood*. 1996;87(10):4223-34.
45. Zhou YF, Eng ET, Zhu J, Lu C, Walz T, Springer TA. Sequence and structure relationships within von Willebrand factor. *Blood*. 2012;120(2):449-58.
46. Zhang C, Kelkar A, Nasirikenari M, Lau JT, Sveinsson M, Sharma UC, et al. The physical spacing between the von Willebrand factor D'D3 and A1 domains regulates platelet adhesion in vitro and in vivo. *J Thromb Haemost*. 2018;16(3):571-82.
47. Fischer BE, Kramer G, Mitterer A, Grillberger L, Reiter M, Mundt W, et al. Effect of multimerization of human and recombinant von Willebrand factor on platelet aggregation, binding to collagen and binding of coagulation factor VIII. *Thromb Res*. 1996;84(1):55-66.
48. De Meyer SF, Deckmyn H, Vanhoorelbeke K. von Willebrand factor to the rescue. *Blood*. 2009;113(21):5049-57.
49. Brinkhous K, Sandberg H, Garris J, Mattsson C, Palm M, Griggs T, et al. Purified human factor VIII procoagulant protein: comparative hemostatic response after infusions into

- hemophilic and von Willebrand disease dogs. *Proceedings of the National Academy of Sciences*. 1985;82(24):8752-6.
50. Gandhi C, Motto DG, Jensen M, Lentz SR, Chauhan AK. ADAMTS13 deficiency exacerbates VWF-dependent acute myocardial ischemia/reperfusion injury in mice. *Blood*. 2012;120(26):5224-30.
51. De Meyer SF, Savchenko AS, Haas MS, Schatzberg D, Carroll MC, Schiviz A, et al. Protective anti-inflammatory effect of ADAMTS13 on myocardial ischemia/reperfusion injury in mice. *Blood*. 2012;120(26):5217-23.
52. Smith NL, Rice KM, Bovill EG, Cushman M, Bis JC, McKnight B, et al. Genetic variation associated with plasma von Willebrand factor levels and the risk of incident venous thrombosis. *Blood*. 2011;117(22):6007-11.
53. Tsai HM. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. *J Mol Med (Berl)*. 2002;80(10):639-47.
54. De Meyer SF, Stoll G, Wagner DD, Kleinschnitz C. von Willebrand factor: an emerging target in stroke therapy. *Stroke*. 2012;43(2):599-606.
55. Wellings PJ, Ku DN. Mechanisms of platelet capture under very high shear. *Cardiovasc Eng Technol*. 2012;3(2):161-70.
56. Crawley JT, de Groot R, Xiang Y, Luken BM, Lane DA. Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor. *Blood*. 2011;118(12):3212-21.
57. Wu JJ, Fujikawa K, McMullen BA, Chung DW. Characterization of a core binding site for ADAMTS-13 in the A2 domain of von Willebrand factor. *Proc Natl Acad Sci U S A*. 2006;103(49):18470-4.
58. Dong J-f, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;100(12):4033-9.
59. van Schooten CJ, Shahbazi S, Groot E, Oortwijn BD, van den Berg HM, Denis CV, et al. Macrophages contribute to the cellular uptake of von Willebrand factor and factor VIII in vivo. *Blood*. 2008;112(5):1704-12.
60. Garcia VV, Coppola R, Mannucci PM. The role of the spleen in regulating the plasma levels of factor VIII--von Willebrand's factor after DDAVP. *Blood*. 1982;60(6):1402-6.
61. Tosetto A, Rodeghiero F, Castaman G, Goodeve A, Federici AB, Batlle J, et al. A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). *J Thromb Haemost*. 2006;4(4):766-73.
62. Goodeve A, Eikenboom J, Castaman G, Rodeghiero F, Federici AB, Batlle J, et al. Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease (MCMDM-1VWD). *Blood*. 2007;109(1):112-21.
63. Riddell AF, Gomez K, Millar CM, Mellars G, Gill S, Brown SA, et al. Characterization of W1745C and S1783A: 2 novel mutations causing defective collagen binding in the A3 domain of von Willebrand factor. *Blood*. 2009;114(16):3489-96.

64. Eikenboom JC, Matsushita T, Reitsma PH, Tuley EA, Castaman G, Briet E, et al. Dominant type 1 von Willebrand disease caused by mutated cysteine residues in the D3 domain of von Willebrand factor. *Blood*. 1996;88(7):2433-41.
65. O'Brien LA, James PD, Othman M, Berber E, Cameron C, Notley CR, et al. Founder von Willebrand factor haplotype associated with type 1 von Willebrand disease. *Blood*. 2003;102(2):549-57.
66. Bowen DJ, Collins PW. An amino acid polymorphism in von Willebrand factor correlates with increased susceptibility to proteolysis by ADAMTS13. *Blood*. 2004;103(3):941-7.
67. Castaman G, Tosetto A, Rodeghiero F. Reduced von Willebrand factor survival in von Willebrand disease: pathophysiologic and clinical relevance. *J Thromb Haemost*. 2009;7 Suppl 1:71-4.
68. Casonato A, Pontara E, Sartorello F, Cattini MG, Sartori MT, Padrini R, et al. Reduced von Willebrand factor survival in type Vicenza von Willebrand disease. *Blood*. 2002;99(1):180-4.
69. Lenting PJ, Westein E, Terraube V, Ribba AS, Huizinga EG, Meyer D, et al. An experimental model to study the in vivo survival of von Willebrand factor. Basic aspects and application to the R1205H mutation. *J Biol Chem*. 2004;279(13):12102-9.
70. Cumming A, Grundy P, Keeney S, Lester W, Enayat S, Guilliat A, et al. An investigation of the von Willebrand factor genotype in UK patients diagnosed to have type 1 von Willebrand disease. *Thromb Haemost*. 2006;96(5):630-41.
71. Castaman G. New development in von Willebrand disease. *Curr Opin Hematol*. 2013;20(5):424-9.
72. Lyons SE, Bruck ME, Bowie EJ, Ginsburg D. Impaired intracellular transport produced by a subset of type IIA von Willebrand disease mutations. *J Biol Chem*. 1992;267(7):4424-30.
73. Kunicki TJ, Montgomery RR, Schullek J. Cleavage of human von Willebrand factor by platelet calcium-activated protease. *Blood*. 1985;65(2):352-6.
74. Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM. Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor. *Proc Natl Acad Sci U S A*. 1990;87(16):6306-10.
75. Gralnick HR, Williams SB, McKeown LP, Maisonneuve P, Jenneau C, Sultan Y, et al. In vitro correction of the abnormal multimeric structure of von Willebrand factor in type IIA von Willebrand's disease. *Proc Natl Acad Sci U S A*. 1985;82(17):5968-72.
76. Ruggeri ZM, Mannucci PM, Lombardi R, Federici AB, Zimmerman TS. Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand's disease subtypes. *Blood*. 1982;59(6):1272-8.
77. Federici AB, Mazurier C, Berntorp E, Lee CA, Scharrer I, Goudemand J, et al. Biologic response to desmopressin in patients with severe type 1 and type 2 von Willebrand disease: results of a multicenter European study. *Blood*. 2004;103(6):2032-8.
78. Ware J, Dent JA, Azuma H, Sugimoto M, Kyrle PA, Yoshioka A, et al. Identification of a point mutation in type IIB von Willebrand disease illustrating the regulation

- of von Willebrand factor affinity for the platelet membrane glycoprotein Ib-IX receptor. *Proc Natl Acad Sci U S A*. 1991;88(7):2946-50.
79. Clemetson KJ, Clemetson JM, editors. *Platelet GPIb-V-IX Complex*. *Semin Thromb Hemost*; 1995: Copyright© 1995 by Thieme Medical Publishers, Inc.
80. Holmberg L, Nilsson IM, Borge L, Gunnarsson M, Sjörin E. Platelet aggregation induced by I-Desamino-8-D-arginine vasopressin (DDAVP) in type IIB von Willebrand's disease. *N Engl J Med*. 1983;309(14):816-21.
81. Hultin MB, Sussman, II. Postoperative thrombocytopenia in type IIB von Willebrand disease. *Am J Hematol*. 1990;33(1):64-8.
82. Pareti FI, Federici AB, Cattaneo M, Mannucci PM. Spontaneous platelet aggregation during pregnancy in a patient with von Willebrand disease type IIB can be blocked by monoclonal antibodies to both platelet glycoproteins Ib and IIb/IIIa. *Br J Haematol*. 1990;75(1):86-91.
83. Rick ME, Williams SB, Sacher RA, McKeown LP. Thrombocytopenia associated with pregnancy in a patient with type IIB von Willebrand's disease. *Blood*. 1987;69(3):786-9.
84. Federici AB, Mannucci PM, Castaman G, Baronciani L, Bucciarelli P, Canciani MT, et al. Clinical and molecular predictors of thrombocytopenia and risk of bleeding in patients with von Willebrand disease type 2B: a cohort study of 67 patients. *Blood*. 2009;113(3):526-34.
85. Miller JL, Cunningham D, Lyle VA, Finch CN. Mutation in the gene encoding the alpha chain of platelet glycoprotein Ib in platelet-type von Willebrand disease. *Proc Natl Acad Sci U S A*. 1991;88(11):4761-5.
86. Mazurier C, Gaucher C, Jorieux S, Goudemand M. Biological effect of desmopressin in eight patients with type 2N ('Normandy') von Willebrand disease. Collaborative Group. *Br J Haematol*. 1994;88(4):849-54.
87. Weiss H, Ball A, Mannucci P. Incidence of severe von Willebrand's disease. *The New England journal of medicine*. 1982;307(2):127-.
88. Berliner SA, Seligsohn U, Zivelin A, Zwang E, Sofferan G. A relatively high frequency of severe (type III) von Willebrand's disease in Israel. *Br J Haematol*. 1986;62(3):535-43.
89. Mannucci PM, Bloom AL, Larrieu MJ, Nilsson IM, West RR. Atherosclerosis and von Willebrand factor. I. Prevalence of severe von Willebrand's disease in western Europe and Israel. *Br J Haematol*. 1984;57(1):163-9.
90. Schub T, Richards S, Kornusky J, Pravikoff D. *von Willebrand Disease*. 2015.
91. Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. *Br J Haematol*. 2000;111(4):1236-9.
92. Benlaldj D, Moueden MA, Seghier F. [Von Willebrand disease type 3 falsely diagnosed as hemophilia A: a case report]. *Rev Med Brux*. 2017;38(1):36-8.
93. Simone JV, Cornet JA, Abildgaard CF. Acquired von Willebrand's syndrome in systemic lupus erythematosus. *Blood*. 1968;31(6):806-12.

94. Lavin M, Brophy T, Rawley O, O'sullivan J, Hayden P, Browne P, et al. Lenalidomide as a novel treatment for refractory acquired von Willebrand syndrome associated with monoclonal gammopathy. *J Thromb Haemost.* 2016;14(6):1200-5.
95. Federici AB, Rand JH, Bucciarelli P, Budde U, van Genderen PJ, Mohri H, et al. Acquired von Willebrand syndrome: data from an international registry. *Thromb Haemost.* 2000;84(2):345-9.
96. Smith SR, Auger MJ, Maccallum PK, Rodgers M, Taberner DA, Thornton JG, et al. Hypothyroidism and Von Willebrand's Disease. *The Lancet.* 1987;329(8545):1314-5.
97. Dalton R, Savidge G, Matthews K, Dewar M, Kernoff P, Greaves M, et al. HYPOTHYROIDISM AS A CAUSE OF ACQUIRED VON WDLLEBRAND'S DISEASE. *The lancet.* 1987;329(8540):1007-9.
98. Tjanheijnen VCG, Harthoornlasthuizen EJ, Kurstjens RMA, Koolen MI. A PATIENT WITH POSTPARTUM PRIMARY HYPOTHYROIDISM AND ACQUIRED VON-WILLEBRANDS DISEASE. *Neth J Med.* 1994;44(3):91-4.
99. Castaman G, Rodeghiero F. Acquired transitory von Willebrand syndrome with ciprofloxacin. *Lancet.* 1994;343(8895):492.
100. Kreuz W, Linde R, Funk M, Meyer-Schrod R, Foll E, Nowak-Gottl U, et al. Induction of von Willebrand disease type I by valproic acid. *Lancet.* 1990;335(8701):1350-1.
101. Conrad ME, Latour LF. Acquired von Willebrand's disease, IgE polyclonal gammopathy and griseofulvin therapy. *Am J Hematol.* 1992;41(2):143.
102. Gill JC, Wilson AD, Endres-Brooks J, Montgomery RR. Loss of the largest von Willebrand factor multimers from the plasma of patients with congenital cardiac defects. *Blood.* 1986;67(3):758-61.
103. Avila M, Lee KJ, Bouskill V, Rand M, James P, Carcao M. Acquired von Willebrand syndrome in paediatric patients with congenital heart disease: challenges in the diagnosis and management of this rare condition. *Haemophilia.* 2015;21(1).
104. Klovaite J, Gustafsson F, Mortensen SA, Sander K, Nielsen LB. Severely impaired von Willebrand factor-dependent platelet aggregation in patients with a continuous-flow left ventricular assist device (HeartMate II). *J Am Coll Cardiol.* 2009;53(23):2162-7.
105. Tauber H, Ott H, Streif W, Weigel G, Loacker L, Fritz J, et al. Extracorporeal membrane oxygenation induces short-term loss of high-molecular-weight von Willebrand factor multimers. *Anesth Analg.* 2015;120(4):730-6.
106. van Genderen PJ, Vink T, Michiels JJ, van 't Veer MB, Sixma JJ, van Vliet HH. Acquired von Willebrand disease caused by an autoantibody selectively inhibiting the binding of von Willebrand factor to collagen. *Blood.* 1994;84(10):3378-84.
107. Handin RI, Martin V, Moloney WC. Antibody-induced von Willebrand9s disease: a newly defined inhibitor syndrome. *Blood.* 1976;48(3):393-405.
108. Mohri H, Motomura S, Kanamori H, Matsuzaki M, Watanabe S, Maruta A, et al. Clinical significance of inhibitors in acquired von Willebrand syndrome. *Blood.* 1998;91(10):3623-9.
109. Nageswara Rao AA, Rodriguez V, Long ME, Winters JL, Nichols WL, Pruthi RK. Transient neonatal acquired von willebrand syndrome due to transplacental transfer of maternal monoclonal antibodies. *Pediatr Blood Cancer.* 2009;53(4):655-7.

110. Tefferi A, Hanson CA, Kurtin PJ, Katzmann JA, Dalton RJ, Nichols WL. Acquired von Willebrand's disease due to aberrant expression of platelet glycoprotein Ib by marginal zone lymphoma cells. *Br J Haematol.* 1997;96(4):850-3.
111. Michiels JJ, Budde U, van der Planken M, van Vliet HH, Schroyens W, Berneman Z. Acquired von Willebrand syndromes: clinical features, aetiology, pathophysiology, classification and management. *Best Pract Res Clin Haematol.* 2001;14(2):401-36.
112. Eikenboom JC, Van Der Meer FJ, Briët E. Acquired von Willebrand's disease due to excessive fibrinolysis. *Br J Haematol.* 1992;81(4):618-20.
113. Berntorp E, Ölundarson PT, editors. Prevalence of von Willebrand disease in the Nordic Region. *Hematology Meeting Reports (formerly Haematologica Reports)*; 2009.
114. Holm E, Carlsson KS, Lovdahl S, Lail AE, Abshire TC, Berntorp E. Bleeding-related hospitalization in patients with von Willebrand disease and the impact of prophylaxis: Results from national registers in Sweden compared with normal controls and participants in the von Willebrand Disease Prophylaxis Network. *Haemophilia.* 2018;24(4):628-33.
115. Sramek A, Eikenboom JC, Briet E, Vandenbroucke JP, Rosendaal FR. Usefulness of patient interview in bleeding disorders. *Arch Intern Med.* 1995;155(13):1409-15.
116. Miller CH, Haff E, Platt SJ, Rawlins P, Drews CD, Dilley AB, et al. Measurement of von Willebrand factor activity: relative effects of ABO blood type and race. *J Thromb Haemost.* 2003;1(10):2191-7.
117. Conlan MG, Folsom AR, Finch A, Davis CE, Sorlie P, Marcucci G, et al. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. *Thromb Haemost.* 1993;70(3):380-5.
118. Gallinaro L, Cattini MG, Sztukowska M, Padrini R, Sartorello F, Pontara E, et al. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. *Blood.* 2008;111(7):3540-5.
119. Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ, Jr., Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood.* 1987;69(6):1691-5.
120. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood.* 1987;69(2):454-9.
121. James PD, Goodeve AC. von Willebrand disease. *Genet Med.* 2011;13(5):365-76.
122. Bowie E, Owen C, Giles A. Animal models for the study of factor VIII and von Willebrand factor. *Coagulation and Bleeding Disorders: The Role of Factor VIII and von Willebrand Factor* New York: Marcel Dekker. 1989;305.
123. Fass DN, Bowie EJ, Owen CA, Jr., Zollman PE. Inheritance of porcine von Willbrand's disease: study of a kindred of over 700 pigs. *Blood.* 1979;53(4):712-9.
124. BRINKHOUS KM, REDDICK RL, READ MS, Nichols TC, Bellinger DA, GRIGGS TR, editors. Von Willebrand factor and animal models: contributions to gene therapy, thrombotic thrombocytopenic purpura, and coronary artery thrombosis. *Mayo Clin Proc*; 1991: Elsevier.

125. Sweeney J, Novak E, Reddington M, Takeuchi K, Swank R. The RIIS/J inbred mouse strain as a model for von Willebrand disease. *Blood*. 1990;76(11):2258-65.
126. Brooks M, Leith GS, Allen AK, Woods PR, Benson RE, Dodds WJ. Bleeding disorder (von Willebrand disease) in a quarter horse. *J Am Vet Med Assoc*. 1991;198(1):114-6.
127. Johnson GS, Turrentine MA, Kraus KH. Canine von Willebrand's disease. A heterogeneous group of bleeding disorders. *Vet Clin North Am Small Anim Pract*. 1988;18(1):195-229.
128. Bahou WF, Bowie EJ, Fass DN, Ginsburg D. Molecular genetic analysis of porcine von Willebrand disease: tight linkage to the von Willebrand factor locus. *Blood*. 1988;72(1):308-13.
129. Brooks M, Dodds WJ, Raymond SL. Epidemiologic features of von Willebrand's disease in Doberman pinschers, Scottish terriers, and Shetland sheepdogs: 260 cases (1984-1988). *J Am Vet Med Assoc*. 1992;200(8):1123-7.
130. Fuster V, Fass DN, Kaye MP, Josa M, Zinsmeister AR, Bowie EJ. Arteriosclerosis in normal and von Willebrand pigs: long-term prospective study and aortic transplantation study. *Circ Res*. 1982;51(5):587-93.
131. JOHNSON C, STEWART M, ZOECKLEIN L, BOWIE E, editors. PIGS WITH VONWILLEBRANDS DISEASE ARE RESISTANT TO THE DEVELOPMENT OF EXPERIMENTAL INFECTIVE ENDOCARDITIS. *Thromb Haemost*; 1989: FK SCHATTAUER VERLAG GMBH PO BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY.
132. Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Frequency of inherited bleeding disorders in women with menorrhagia. *Lancet*. 1998;351(9101):485-9.
133. Argyris PP, Anim SO, Koutlas IG. Maxillary pseudotumor as initial manifestation of von Willebrand disease, type 2: report of a rare case and literature review. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;121(2):e27-31.
134. Ozhan S, Arac M, Isik S, Oznur, II, Atilla S, Kemaloglu Y. Pseudotumor of the maxillary sinus in a patient with von Willebrand's disease. *AJR Am J Roentgenol*. 1996;166(4):950-1.
135. Gritti G, Cortelezzi A, Bucciarelli P, Rezzonico F, Lonati S, La Marca S, et al. Circulating and progenitor endothelial cells are abnormal in patients with different types of von Willebrand disease and correlate with markers of angiogenesis. *Am J Hematol*. 2011;86(8):650-6.
136. Starke RD, Ferraro F, Paschalaki KE, Dryden NH, McKinnon TA, Sutton RE, et al. Endothelial von Willebrand factor regulates angiogenesis. *Blood*. 2011;117(3):1071-80.
137. GIFFIN HZ. UNUSUAL TYPES OF HEMORRHAGIC DISEASE. *The American Journal of the Medical Sciences*. 1928;175(1):44-8.
138. Cardoso MF, Lourenço LC, Antunes M, e Branco JC, Santos L, Martins A, et al. Recurrent Gastrointestinal Bleeding from Dieulafoy's Lesions in a Patient with Type 1 von Willebrand Disease: A Rare Association. *GE-Portuguese Journal of Gastroenterology*. 1-5.
139. Shimizu M, Kondoh E, Ueda M, Kakui K, Tatsumi K, Konishi I. Secondary postpartum hemorrhage due to uterine artery pseudoaneurysm rupture in von Willebrand disease. *J Obstet Gynaecol Res*. 2011;37(12):1887-90.

140. Choung KC, Wei JP. Traumatic pseudoaneurysm of the supra-orbital artery associated with von Willebrand's disease: case report. *J Trauma*. 1993;35(6):966-7.
141. Garaci FG, Gandini R, Romagnoli A, Fasoli F, Varruciu V, Simonetti G. Hepatic artery pseudoaneurysm in von Willebrand's disease. *Eur Radiol*. 2003;13(8):1913-5.
142. Ricciardo BJ, Mwipatayi BP, Abbas M, Sieunarine K, Eikelboom JW. Von Willebrand disease associated with superficial temporal artery pseudoaneurysm. *Eur J Vasc Endovasc Surg*. 2005;30(3):285-7.
143. De Foer B, Stockx L, Wilms G, Vermylen J, Baert AL. Selective embolization of a superior gluteal artery pseudoaneurysm associated with von Willebrand's disease. *J Belge Radiol*. 1996;79(3):135-6.
144. Osenbach RK, Loftus CM, Menezes AH, Biller J, Tannous R, Yuh WT. Management of intraventricular haemorrhage secondary to ruptured arteriovenous malformation in a child with von Willebrand's disease. *J Neurol Neurosurg Psychiatry*. 1989;52(12):1452-4.
145. Fressinaud E, Meyer D. International survey of patients with von Willebrand disease and angiodysplasia. *Thromb Haemost*. 1993;70(3):546.
146. Wood GM, Losowsky MS. 'Angiodysplasia' of the tongue with acquired von Willebrand's disease. *Postgrad Med J*. 1982;58(675):37-8.
147. Conlon CL, Weinger RS, Cimo PL, Moake JL, Olson JD. Telangiectasia and von Willebrand's disease in two families. *Ann Intern Med*. 1978;89(6):921-4.
148. Park SO, Wankhede M, Lee YJ, Choi EJ, Fliess N, Choe SW, et al. Real-time imaging of de novo arteriovenous malformation in a mouse model of hereditary hemorrhagic telangiectasia. *J Clin Invest*. 2009;119(11):3487-96.
149. Duray PH, Marcal JM, Jr., LiVolsi VA, Fisher R, Scholhamer C, Brand MH. Gastrointestinal angiodysplasia: a possible component of von Willebrand's disease. *Hum Pathol*. 1984;15(6):539-44.
150. Thomas W, Warner E, Cameron E, Symington E. Successful treatment of acquired von Willebrand disease with lenalidomide leading to dramatic resolution of intractable gastrointestinal bleeding: A case report. *Haemophilia*. 2018;24(3):e140-e2.
151. Pickering NJ, Brody JI, Barrett MJ. von Willebrand syndromes and mitral-valve prolapse; linked mesenchymal dysplasias. *N Engl J Med*. 1981;305(3):131-4.
152. Clough V, MacFarlane IA, O'Connor J, Wood JK. Acquired von Willebrand's syndrome and Ehlers-Danlos syndrome presenting with gastro-intestinal bleeding. *Scand J Haematol*. 1979;22(4):305-10.
153. Short RV. The evolution of human reproduction. *Proc R Soc Lond B Biol Sci*. 1976;195(1118):3-24.
154. Women's NCCf, Health Cs. Heavy menstrual bleeding: RCOG Press; 2007.
155. Shankar M, Lee CA, Sabin CA, Economides DL, Kadir RA. von Willebrand disease in women with menorrhagia: a systematic review. *BJOG*. 2004;111(7):734-40.
156. Kadir RA, Economides DL, Sabin CA, Pollard D, Lee CA. Assessment of menstrual blood loss and gynaecological problems in patients with inherited bleeding disorders. *Haemophilia*. 1999;5(1):40-8.

157. Hallberg L, Hogdahl AM, Nilsson L, Rybo G. Menstrual blood loss--a population study. Variation at different ages and attempts to define normality. *Acta Obstet Gynecol Scand.* 1966;45(3):320-51.
158. Kirtava A, Crudder S, Dilley A, Lally C, Evatt B. Trends in clinical management of women with von Willebrand disease: a survey of 75 women enrolled in haemophilia treatment centres in the United States. *Haemophilia.* 2004;10(2):158-61.
159. Kouides P, Phatak P, Burkart P, Braggins C, Cox C, Bernstein Z, et al. Gynaecological and obstetrical morbidity in women with type I von Willebrand disease: results of a patient survey. *Haemophilia.* 2000;6(6):643-8.
160. Edlund M, Magnusson C, Von Schoultz B. Quality of life—a Swedish survey of 2200 women. *Dysfunctional uterine bleeding* Royal Society of Medicine Press, London. 1994.
161. Taylor JJ. Von Willebrand's disease in obstetrics and gynaecology. *J Obstet Gynaecol Br Commonw.* 1968;75(4):453-8.
162. Byams VR, Anderson BL, Grant AM, Atrash H, Schulkin J. Evaluation of bleeding disorders in women with menorrhagia: a survey of obstetrician-gynecologists. *Am J Obstet Gynecol.* 2012;207(4):269 e1-5.
163. Dilley A, Drews C, Lally C, Austin H, Barnhart E, Evatt B. A survey of gynecologists concerning menorrhagia: perceptions of bleeding disorders as a possible cause. *J Womens Health Gend Based Med.* 2002;11(1):39-44.
164. Jacobson AE, Vesely SK, Koch T, Campbell J, O'brien SH. Patterns of von Willebrand disease screening in girls and adolescents with heavy menstrual bleeding. *Obstet Gynecol.* 2018;131(6):1121-9.
165. Shankar M, Chi C, Kadir RA. Review of quality of life: menorrhagia in women with or without inherited bleeding disorders. *Haemophilia.* 2008;14(1):15-20.
166. Barr RD, Sek J, Horsman J, Furlong W, Saleh M, Pai M, et al. Health status and health-related quality of life associated with von Willebrand disease. *Am J Hematol.* 2003;73(2):108-14.
167. Miller S, Lester F, Hensleigh P. CEU: prevention and treatment of postpartum hemorrhage: new advances for low-resource settings. *Journal of Midwifery & Women's Health.* 2004;49(4):283-92.
168. Anderson FW. Maternal mortality: an enduring epidemic. *Clin Obstet Gynecol.* 2009;52(2):214-23.
169. Lutomski JE, Byrne BM, Devane D, Greene RA. Increasing trends in atonic postpartum haemorrhage in Ireland: an 11-year population-based cohort study. *BJOG.* 2012;119(3):306-14.
170. Rath WH. Postpartum hemorrhage—update on problems of definitions and diagnosis. *Acta Obstet Gynecol Scand.* 2011;90(5):421-8.
171. Oyelese Y, Ananth CV. Postpartum hemorrhage: epidemiology, risk factors, and causes. *Clin Obstet Gynecol.* 2010;53(1):147-56.
172. Szecsi PB, Jorgensen M, Klajnbard A, Andersen MR, Colov NP, Stender S. Haemostatic reference intervals in pregnancy. *Thromb Haemost.* 2010;103(4):718-27.
173. Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost.* 2003;29(2):125-30.

174. Fondevila CG, Meschengieser S, Bermejo E, Lazzari MA. Persistence of low platelet adhesiveness in von Willebrand disease during pregnancy. *Platelets*. 1997;8(2-3):155-7.
175. James AH. Von Willebrand disease. *Obstet Gynecol Surv*. 2006;61(2):136-45.
176. Sidonio RF, Haley KM, Fallaize D. Impact of diagnosis of von Willebrand disease on patient outcomes: Analysis of medical insurance claims data. *Haemophilia*. 2017;23(5):743-9.
177. Alamin A, Satti M. Evaluation of Diagnostic Criteria for Von Willebrand Disease in Sudan: LAP LAMBERT Academic Publishing; 2015.
178. Casey LJ, Tuttle A, Grabell J, Hopman W, Moorehead PC, Blanchette VS, et al. Generation and optimization of the self-administered pediatric bleeding questionnaire and its validation as a screening tool for von Willebrand disease. *Pediatr Blood Cancer*. 2017;64(10):e26588.
179. Rimmer EK, Houston DS. Bleeding by the numbers: The utility and the limitations of bleeding scores, bleeding prediction tools, and bleeding case definitions. *Transfus Apher Sci*. 2018.
180. Fressinaud E, Veyradier A, Truchaud F, Martin I, Boyer-Neumann C, Trossaert M, et al. Screening for von Willebrand disease with a new analyzer using high shear stress: a study of 60 cases. *Blood*. 1998;91(4):1325-31.
181. Favaloro EJ, Oliver S, Mohammed S, Ahuja M, Grzechnik E, Azimulla S, et al. Potential misdiagnosis of von Willebrand disease and haemophilia caused by ineffective mixing of thawed plasma. *Haemophilia*. 2017;23(5):e436-e43.
182. Collaborators WT. Effect of early tranexamic acid administration on mortality, hysterectomy, and other morbidities in women with post-partum haemorrhage (WOMAN): an international, randomised, double-blind, placebo-controlled trial. *Lancet*. 2017;389(10084):2105-16.
183. Mannucci PM. Use of desmopressin (DDAVP) during early pregnancy in factor VIII-deficient women. *Blood*. 2005;105(8):3382.
184. Trigg DE, Stergiotou I, Peitsidis P, Kadir RA. A systematic review: The use of desmopressin for treatment and prophylaxis of bleeding disorders in pregnancy. *Haemophilia*. 2012;18(1):25-33.
185. Mannucci PM. Treatment of von Willebrand's Disease. *N Engl J Med*. 2004;351(7):683-94.
186. Moake JL, Turner NA, Stathopoulos NA, Nolasco LH, Hellums JD. Involvement of large plasma von Willebrand factor (vWF) multimers and unusually large vWF forms derived from endothelial cells in shear stress-induced platelet aggregation. *J Clin Invest*. 1986;78(6):1456-61.
187. Bond L, Bevan D. Myocardial infarction in a patient with hemophilia treated with DDAVP. *N Engl J Med*. 1988;318(2):121.
188. Byrnes JJ, Larcada A, Moake JL. Thrombosis following desmopressin for uremic bleeding. *Am J Hematol*. 1988;28(1):63-5.
189. Rodeghiero F, Castaman G, Di Bona E, Ruggeri M. Consistency of responses to repeated DDAVP infusions in patients with von Willebrand's disease and hemophilia A. *Blood*. 1989;74(6):1997-2000.

190. Mannucci PM, Canciani MT, Rota L, Donovan BS. Response of factor VIII/von Willebrand factor to DDAVP in healthy subjects and patients with haemophilia A and von Willebrand's disease. *Br J Haematol*. 1981;47(2):283-93.
191. Soucie JM, De Staercke C, Monahan PE, Recht M, Chitlur MB, Gruppo R, et al. Evidence for the transmission of parvovirus B19 in patients with bleeding disorders treated with plasma-derived factor concentrates in the era of nucleic acid test screening. *Transfusion (Paris)*. 2013;53(6):1217-25.
192. Di Minno G, Canaro M, Ironside JW, Navarro D, Perno CF, Tiede A, et al. Pathogen safety of long-term treatments for bleeding disorders: still relevant to current practice. *Haematologica*; 2013.
193. Ofosu FA, Freedman J, Semple JW. Plasma-derived biological medicines used to promote haemostasis. *Thromb Haemost*. 2008;99(5):851-62.
194. Budde U, Metzner HJ, Müller H-G, editors. Comparative analysis and classification of von Willebrand factor/factor VIII concentrates: impact on treatment of patients with von Willebrand disease. *Semin Thromb Hemost*; 2006: Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.
195. Mannuccio PM, Lattuada A, Ruggeri ZM. Proteolysis of von Willebrand factor in therapeutic plasma concentrates. *Blood*. 1994;83(10):3018-27.
196. Mannucci PM, Tenconi PM, Castaman G, Rodeghiero F. Comparison of four virus-inactivated plasma concentrates for treatment of severe von Willebrand disease: a cross-over randomized trial. *Blood*. 1992;79(12):3130-7.
197. Lethagen S, Carlson M, Hillarp A. A comparative in vitro evaluation of six von Willebrand factor concentrates. *Haemophilia*. 2004;10(3):243-9.
198. Coppola A, Franchini M, Makris M, Santagostino E, Di Minno G, Mannucci PM. Thrombotic adverse events to coagulation factor concentrates for treatment of patients with haemophilia and von Willebrand disease: a systematic review of prospective studies. *Haemophilia*. 2012;18(3):e173-87.
199. Nichols WL, Hultin MB, James AH, Manco-Johnson MJ, Montgomery RR, Ortel TL, et al. von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). *Haemophilia*. 2008;14(2):171-232.
200. Franchini M, Mannucci PM. Von Willebrand disease-associated angiodysplasia: a few answers, still many questions. *Br J Haematol*. 2013;161(2):177-82.
201. Bergamaschini L, Mannucci PM, Federici AB, Coppola R, Guzzoni S, Agostoni A. Posttransfusion anaphylactic reactions in a patient with severe von Willebrand disease: role of complement and alloantibodies to von Willebrand factor. *J Lab Clin Med*. 1995;125(3):348-55.
202. Mannucci PM, Tamaro G, Narchi G, Candotti G, Federici A, Altieri D, et al. Life-threatening reaction to factor VIII concentrate in a patient with severe von Willebrand disease and alloantibodies to von Willebrand factor. *Eur J Haematol*. 1987;39(5):467-70.
203. Brown R. Recombinant von Willebrand factor for severe gastrointestinal bleeding unresponsive to other treatments in a patient with type 2A von Willebrand disease: a case report. *Blood Coagul Fibrinolysis*. 2017;28(7):570-5.

204. Gill JC, Castaman G, Windyga J, Kouides P, Ragni M, Leebeek FW, et al. Hemostatic efficacy, safety, and pharmacokinetics of a recombinant von Willebrand factor in severe von Willebrand disease. *Blood*. 2015;126(17):2038-46.
205. Alperin JB. Estrogens and surgery in women with von Willebrand's disease. *Am J Med*. 1982;73(3):367-71.
206. Foster PA. The reproductive health of women with von Willebrand Disease unresponsive to DDAVP: results of an international survey. On behalf of the Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost*. 1995;74(2):784-90.
207. Kouides PA, Phatak PD, Burkart P, Braggins C, Cox C, Bernstein Z, et al. Gynaecological and obstetrical morbidity in women with type I von Willebrand disease: results of a patient survey. *Haemophilia*. 2000;6(6):643-8.
208. Kingman C, Kadir R, Lee C, Economides D. The use of levonorgestrel-releasing intrauterine system for treatment of menorrhagia in women with inherited bleeding disorders. *BJOG*. 2004;111(12):1425-8.
209. Wortman M. Minimally invasive surgery for menorrhagia and intractable uterine bleeding: time to set standards. *J Am Assoc Gynecol Laparosc*. 1999;6(4):369-73.
210. Hagstad A. Gynecology and sexuality in middle-aged women. *Women Health*. 1988;13(3-4):57-80.
211. Ruta D, Garratt A, Chadha Y, Flett G, Hall M, Russell I. Assessment of patients with menorrhagia: how valid is a structured clinical history as a measure of health status? *Qual Life Res*. 1995;4(1):33-40.
212. Coulter A, Peto V, Jenkinson C. Quality of life and patient satisfaction following treatment for menorrhagia. *Fam Pract*. 1994;11(4):394-401.
213. Higham JM, O'Brien P, Shaw R. Assessment of menstrual blood loss using a pictorial chart. *BJOG*. 1990;97(8):734-9.
214. Sullivan M, Karlsson J, Ware JE, Jr. The Swedish SF-36 Health Survey--I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. *Soc Sci Med*. 1995;41(10):1349-58.
215. Sullivan M, Karlsson J, Taft C, Ware J. SF-36 Health Survey. Swedish manual and interpretation guide. Sahlgrenska University Hospital, Gothenburg. 2002.
216. Macfarlane DE, Stibbe J, Kirby EP, Zucker MB, Grant RA, McPherson J. Letter: A method for assaying von Willebrand factor (ristocetin cofactor). *Thromb Diath Haemorrh*. 1975;34(1):306-8.
217. Veyradier A, Fressinaud E, Meyer D. Laboratory diagnosis of von Willebrand disease. *Int J Clin Lab Res*. 1998;28(4):201-10.
218. Rosen S. Assay of factor VIII:C with a chromogenic substrate. *Scand J Haematol Suppl*. 1984;40(S40):139-45.
219. Chaireti R, Gustafsson KM, Bystrom B, Bremme K, Lindahl TL. Endogenous thrombin potential is higher during the luteal phase than during the follicular phase of a normal menstrual cycle. *Hum Reprod*. 2013;28(7):1846-52.
220. Chaireti R, Lindahl TL, Bystrom B, Bremme K, Larsson A. Inflammatory and endothelial markers during the menstrual cycle. *Scand J Clin Lab Invest*. 2016;76(3):190-4.

221. Rugeri L, Beguin S, Hemker C, Bordet JC, Fleury R, Chatard B, et al. Thrombin-generating capacity in patients with von Willebrand's disease. *Haematologica*. 2007;92(12):1639-46.
222. James AH, Kouides PA, Abdul-Kadir R, Edlund M, Federici AB, Halimeh S, et al. Von Willebrand disease and other bleeding disorders in women: consensus on diagnosis and management from an international expert panel. *Am J Obstet Gynecol*. 2009;201(1):8.
223. Pacheco LD, Costantine MM, Saade GR, Mucowski S, Hankins GD, Sciscione AC. von Willebrand disease and pregnancy: a practical approach for the diagnosis and treatment. *Am J Obstet Gynecol*. 2010;203(3):194-200.
224. Kirtava A, Drews C, Lally C, Dilley A, Evatt B. Medical, reproductive and psychosocial experiences of women diagnosed with von Willebrand's disease receiving care in haemophilia treatment centres: a case-control study. *Haemophilia*. 2003;9(3):292-7.
225. Rae C, Furlong W, Horsman J, Pullenayegum E, Demers C, St-Louis J, et al. Bleeding disorders, menorrhagia and iron deficiency: impacts on health-related quality of life. *Haemophilia*. 2013;19(3):385-91.
226. Byams VR, Kouides PA, Kulkarni R, Baker JR, Brown DL, Gill JC, et al. Surveillance of female patients with inherited bleeding disorders in United States Haemophilia Treatment Centres. *Haemophilia*. 2011;17 Suppl 1(s1):6-13.
227. Leminen H, Hurskainen R. Tranexamic acid for the treatment of heavy menstrual bleeding: efficacy and safety. *International journal of women's health*. 2012;4:413-21.
228. Chi C, Huq FY, Kadir RA. Levonorgestrel-releasing intrauterine system for the management of heavy menstrual bleeding in women with inherited bleeding disorders: long-term follow-up. *Contraception*. 2011;83(3):242-7.
229. Barr RD, Sek J, Horsman J, Furlong W, Saleh M, Pai M, et al. Health status and health-related quality of life associated with von Willebrand disease. *Am J Hematol*. 2003;73(2):108-14.
230. Solovieva S. Clinical severity of disease, functional disability and health-related quality of life. Three-year follow-up study of 150 Finnish patients with coagulation disorders. *Haemophilia*. 2001;7(1):53-63.
231. de Wee EM, MAUSER-BUNSCHOTEN EP, van der Bom JG, DEGENAAR-DUJARDIN ME, Eikenboom H, Fijnvandraat K, et al. Health-related quality of life among adult patients with moderate and severe von Willebrand disease. *J Thromb Haemost*. 2010;8(7):1492-9.
232. Mehrabadi A, Hutcheon JA, Lee L, Liston RM, Joseph KS. Trends in postpartum hemorrhage from 2000 to 2009: a population-based study. *BMC Pregnancy Childbirth*. 2012;12(1):108.
233. Jangsten E, Mattsson LA, Lyckestam I, Hellstrom AL, Berg M. A comparison of active management and expectant management of the third stage of labour: a Swedish randomised controlled trial. *BJOG*. 2011;118(3):362-9.
234. Siboni SM, Spreafico M, Calo L, Maino A, Santagostino E, Federici AB, et al. Gynaecological and obstetrical problems in women with different bleeding disorders. *Haemophilia*. 2009;15(6):1291-9.
235. Ramsahoye BH, Davies SV, Dasani H, Pearson JF. Pregnancy in von Willebrand's disease. *J Clin Pathol*. 1994;47(6):569-70.

236. Kadir RA, Lee CA, Sabin CA, Pollard D, Economides DL. Pregnancy in women with von Willebrand's disease or factor XI deficiency. *Br J Obstet Gynaecol.* 1998;105(3):314-21.
237. Stoof SC, van Steenberg HW, Zwagemaker A, Sanders YV, Cannegieter SC, Duvekot JJ, et al. Primary postpartum haemorrhage in women with von Willebrand disease or carriership of haemophilia despite specialised care: a retrospective survey. *Haemophilia.* 2015;21(4):505-12.
238. Holmgren P. Postpartumblödningar. I Hagberg. *H m fl(Red) Obstetrik upplaga.* 2008;1(2):553-63.
239. Huq FY, Kadir RA. Management of pregnancy, labour and delivery in women with inherited bleeding disorders. *Haemophilia.* 2011;17 Suppl 1:20-30.
240. Chee YL, Townend J, Crowther M, Smith N, Watson HG. Assessment of von Willebrand disease as a risk factor for primary postpartum haemorrhage. *Haemophilia.* 2012;18(4):593-7.
241. Lassila R, Holme PA, Landorph A, Petrini P, Onundarson PT, Hillarp A. Nordic Haemophilia Council's practical guidelines on diagnosis and management of von Willebrand disease. *Semin Thromb Hemost.* 2011;37(5):495-502.
242. James AH. Diagnosis and management of women with bleeding disorders--international guidelines and consensus from an international expert panel. *Haemophilia.* 2011;17 Suppl 1(SUPPL. 1):3-5.
243. Chi C, Kadir RA. Inherited bleeding disorders in pregnancy. *Best Pract Res Clin Obstet Gynaecol.* 2012;26(1):103-17.
244. Larsson C, Saltvedt S, Wiklund I, Pahlen S, Andolf E. Estimation of blood loss after cesarean section and vaginal delivery has low validity with a tendency to exaggeration. *Acta Obstet Gynecol Scand.* 2006;85(12):1448-52.
245. Biggs R, Matthews JM. The treatment of haemorrhage in von Willebrand's disease and the blood level of factor VIII (AHG). *Br J Haematol.* 1963;9(2):203-14.
246. Mannucci PM, Ruggeri ZM, Ciavarella N, Kazatchkine MD, Mowbray JF. Precipitating antibodies to factor VIII/von Willebrand factor in von Willebrand's disease: effects on replacement therapy. *Blood.* 1981;57(1):25-31.
247. Lebech AM, Kjaer A. Lipid metabolism and coagulation during the normal menstrual cycle. *Horm Metab Res.* 1989;21(8):445-8.
248. Koh SC, Prasad R, Fong Y. Hemostatic status and fibrinolytic response potential at different phases of the menstrual cycle. *Clinical and applied thrombosis/hemostasis.* 2005;11(3):295-301.
249. Dapper DV, Didia BC. Haemorheological changes during the menstrual cycle. *East Afr Med J.* 2002;79(4):181-3.
250. Cederblad G, Hahn L, Korsan-Bengtson K, Pehrsson N, Rybo G. Variations in blood coagulation, fibrinolysis, platelet function and various plasma proteins during the menstrual cycle. *Pathophysiol Haemost Thromb.* 1977;6(5):294-302.
251. Knol HM, Kemperman RF, Kluin-Nelemans HC, Mulder AB, Meijer K. Haemostatic variables during normal menstrual cycle. A systematic review. *Thromb Haemost.* 2012;107(1):22-9.

252. Blomback M, Eneroth P, Landgren BM, Lagerstrom M, Anderson O. On the Intraindividual and Gender Variability of Hemostatic Components. *Thromb Haemost.* 1992;67(1):70-5.
253. Blomback M, Landgren BM, Stiernholm Y, Andersson O. The effect of progesterone on the haemostatic mechanism. *Thromb Haemost.* 1997;77(1):105-8.
254. Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptive. *Thromb Haemost.* 1999;82(5):1456-61.
255. Koh SC, Prasad RN, Fong YF. Hemostatic status and fibrinolytic response potential at different phases of the menstrual cycle. *Clin Appl Thromb Hemost.* 2005;11(3):295-301.
256. Önundarson PT, Gudmundsdottir BR, Arnfinnsdottir AV, Kjeld M, Ólafsson Ö. Von Willebrand factor does not vary during normal menstrual cycle. *Thromb Haemost.* 2001;85(01):183-4.
257. Repina MA, Korzo TM, Zinina TA. Effect of hormone replacement therapy with femoston on hemostasis in peri- and postmenopausal women. *Med Sci Monit.* 2002;8(9):PI78-84.
258. Siegbahn A, Odland V, Hedner U, Venge P. Coagulation and fibrinolysis during the normal menstrual cycle. *Ups J Med Sci.* 1989;94(2):137-52.
259. Mandalaki T, Louizou C, Dimitriadou C, Symeonidis P. Variations in factor VIII during the menstrual cycle in normal women. *N Engl J Med.* 1980;302(19):1093-4.
260. Miller CH, Dilley AB, Drews C, Richardson L, Evatt B. Changes in von Willebrand factor and factor VIII levels during the menstrual cycle. *Thromb Haemost.* 2002;87(6):1082-3.
261. Govorov I, Ekelund L, Chaireti R, Elfvinge P, Holmstrom M, Bremme K, et al. Heavy menstrual bleeding and health-associated quality of life in women with von Willebrand's disease. *Exp Ther Med.* 2016;11(5):1923-9.
262. Giardina EG, Chen HJ, Sciacca RR, Rabbani LE. Dynamic variability of hemostatic and fibrinolytic factors in young women. *J Clin Endocrinol Metab.* 2004;89(12):6179-84.
263. Alvergne A, Tabor VH. Is Female Health Cyclical? Evolutionary Perspectives on Menstruation. *Trends Ecol Evol.* 2018;33(6):399-414.
264. Oertelt-Prigione S. Immunology and the menstrual cycle. *Autoimmun Rev.* 2012;11(6-7):A486-92.
265. Lorenz TK, Heiman JR, Demas GE. Interactions Among Sexual Activity, Menstrual Cycle Phase, and Immune Function in Healthy Women. *The Journal of Sex Research.* 2017:1-9.
266. Critchley HO, Kelly RW, Brenner RM, Baird DT. The endocrinology of menstruation—a role for the immune system. *Clin Endocrinol (Oxf).* 2001;55(6):701-10.
267. Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. *Rev Endocr Metab Disord.* 2012;13(4):277-88.
268. Levi M, van der Poll T, Büller HR. Bidirectional relation between inflammation and coagulation. *Circulation.* 2004;109(22):2698-704.

269. Lipinski S, Bremer L, Lammers T, Thieme F, Schreiber S, Rosenstiel P. Coagulation and inflammation. Molecular insights and diagnostic implications. *Hamostaseologie*. 2011;31(2):94-102, 4.
270. Abdollahi A, Hahnfeldt P, Maercker C, Grone HJ, Debus J, Ansorge W, et al. Endostatin's antiangiogenic signaling network. *Mol Cell*. 2004;13(5):649-63.
271. Reijerkerk A, Voest E, Gebbink M. No grip, no growth: the conceptual basis of excessive proteolysis in the treatment of cancer. *Eur J Cancer*. 2000;36(13):1695-705.
272. Koch AE, Halloran MM, Haskell CJ, Shah MR, Polverini PJ. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature*. 1995;376(6540):517-9.
273. Vanderslice P, Munsch CL, Rachal E, Erichsen D, Sughrue KM, Truong AN, et al. Angiogenesis induced by tumor necrosis factor- α ; is mediated by α 4 integrins. *Angiogenesis*. 1998;2(3):265-75.
274. Gho YS, Kleinman HK, Sosne G. Angiogenic activity of human soluble intercellular adhesion molecule-1. *Cancer Res*. 1999;59(20):5128-32.
275. Gopinathan G, Milagre C, Pearce OM, Reynolds LE, Hodivala-Dilke K, Leinster DA, et al. Interleukin-6 Stimulates Defective Angiogenesis. *Cancer Res*. 2015;75(15):3098-107.
276. Makris M. Gastrointestinal bleeding in von Willebrand disease. *Thromb Res*. 2006;118 Suppl 1:S13-7.
277. Zhong C, Xin M, He L, Sun G, Shen F. Prognostic value of von Willebrand factor in patients with atrial fibrillation: A meta-analysis. *Medicine*. 2018;97(27):e11269.
278. Wolters FJ, Boender J, de Vries PS, Sonneveld MA, Koudstaal PJ, de Maat MP, et al. Von Willebrand factor and ADAMTS13 activity in relation to risk of dementia: a population-based study. *Sci Rep*. 2018;8(1):5474.
279. O'sullivan JM, Preston RJ, Robson T, O'donnell JS, editors. Emerging roles for von Willebrand factor in cancer cell biology. *Semin Thromb Hemost*; 2017: Thieme Medical Publishers.
280. Mohren M, Jentsch-Ullrich K, Koenigsmann M, Kropf S, Schalk E, Lutze G. High coagulation factor VIII and von Willebrand factor in patients with lymphoma and leukemia. *Int J Hematol*. 2016;103(2):189-95.
281. Rohsig LM, Damin DC, Stefani SD, Castro CG, Jr., Roisenberg I, Schwartzmann G. von Willebrand factor antigen levels in plasma of patients with malignant breast disease. *Braz J Med Biol Res*. 2001;34(9):1125-9.
282. Wang W-S, Lin J-K, Lin T-C, Chiou T-J, Liu J-H, Yen C-C, et al. Plasma von Willebrand factor level as a prognostic indicator of patients with metastatic colorectal carcinoma. *World journal of gastroenterology: WJG*. 2005;11(14):2166.
283. Zietek Z, Iwan-Zietek I, Paczulski R, Kotschy M, Wolski Z. von Willebrand factor antigen in blood plasma of patients with urinary bladder carcinoma. *Thromb Res*. 1996;83(5):399-402.
284. Starke RD, Paschalaki KE, Dyer CE, Harrison-Lavoie KJ, Cutler JA, McKinnon TA, et al. Cellular and molecular basis of von Willebrand disease: studies on blood outgrowth endothelial cells. *Blood*. 2013;121(14):2773-84.

285. Ragni MV, Jankowitz RC, Jaworski K, Merricks EP, Kloos MT, Nichols TC. Phase II prospective open-label trial of recombinant interleukin-11 in women with mild von Willebrand disease and refractory menorrhagia. *Thromb Haemost.* 2011;106(4):641-5.
286. Ragni MV, Novelli EM, Murshed A, Merricks EP, Kloos MT, Nichols TC. Phase II Prospective Open-Label Trial of Recombinant Interleukin-11 in DDAVP-Unresponsive Von Willebrand Disease and Mild or Moderate Hemophilia A. *Thromb Haemost.* 2013;109(2):248.
287. Jilma B, Paulinska P, Jilma-Stohlawetz P, Gilbert JC, Hutabarat R, Knobl P. A randomised pilot trial of the anti-von Willebrand factor aptamer ARC1779 in patients with type 2b von Willebrand disease. *Thromb Haemost.* 2010;104(3):563-70.
288. Sundaram P, Kurniawan H, Byrne ME, Wower J. Therapeutic RNA aptamers in clinical trials. *Eur J Pharm Sci.* 2013;48(1-2):259-71.