Von Willebrand disease

– aspects of diagnosis, prophylaxis and outcome

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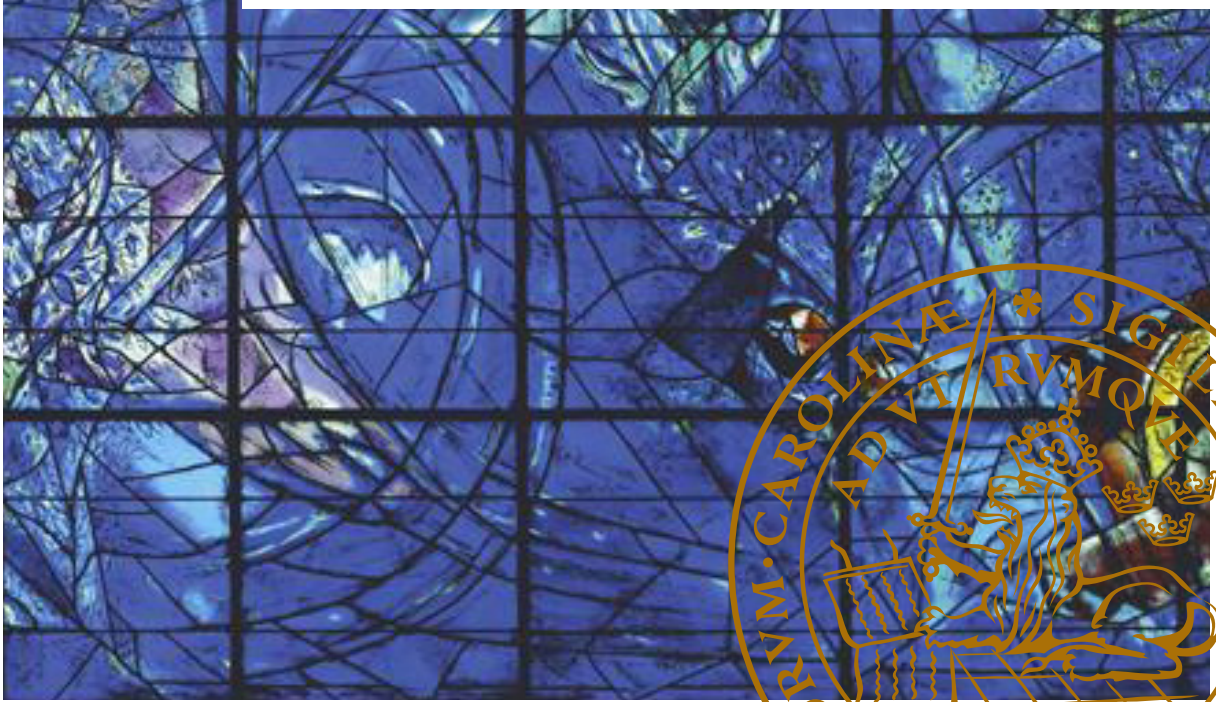

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Abstract

Von Willebrand disease (VWD) is an autosomally inherited bleeding disorder, affecting men and women to the same degree. Typical symptoms include bleeding from mucous membranes such as the mouth and nose, gastrointestinal (GI) bleeding, and heavy menstrual bleeding which can affect the patient's state of health and lead to hospitalisation. Joint bleeding is also common in the more severe forms of the disease. From a clinical point of view, VWD is somewhat overlooked. It is estimated that 1-2% of the population could be carriers of VWD but only a small proportion are symptomatic and around thousand patients are registered at coagulation centres in Sweden. The disease exists in three main forms, type 1 VWD (70% of all patients), characterized by a deficiency of von Willebrand factor (VWF) in the blood, type 2 VWD, with a qualitative deficiency of VWF with deficient function and type 3, inherited autosomal recessive, with lack of VWF in blood and platelets. Type 3 is quite rare with a prevalence of 5/1,000,000 in Sweden and higher in countries in which intermarriage is common. This type can be associated with very serious bleeding – both mucosal (primary haemostasis defect) and joint (defect in plasma coagulation). Sweden is a forerunner for prophylactic treatment for both haemophilia and VWD. Prophylaxis has been shown to be effective in an international cooperative study (von Willebrand Disease Prophylaxis Network). We showed in paper I that prophylactic treatment has a dramatic effect in patients with joint bleeding and epistaxis, however, the effect is less pronounced in those with GI bleeding. In paper II we studied 185 patients referred to the coagulation clinic and found four women with previously undiagnosed mild VWD, illustrating the importance of screening for this disease. In paper III we studied the hospitalization rates among patients with VWD compared to matched controls using register data as well as the impact of prophylaxis. Paper IV describes cardiovascular morbidity and mortality among all patients with VWD in Sweden compared to age- and gender-matched controls over a very long period of observation.

Key words: von Willebrand disease, prophylaxis, registry, cardiovascular disease.

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Nothing replaces work:
Not titles, not nerve, not luck
- Gabrielle Chanel

To Hjördis and all families with von Willebrand disease.

Contents

Abbreviations

VWD	von Willebrand Disease
VWD PN	von Willebrand Disease Prophylaxis Network
VIP	VWD International Prophylaxis Study
FVIII	Coagulation factor VIII
CVD	Cardiovascular disease
GI	Gastrointestinal
WHO	World Health Organization
ICD	International Classification of Diseases
TGA	Thrombin generation assay
BS	Bleeding Score
CBDS	Congenital Bleeding Disorders in Sweden
NPR	National Patient Register

Original publications

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text:

I. Changes in bleeding patterns in von Willebrand disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network. Holm E, Abshire TC, Bowen J, Alvarez MT, Bolton-Maggs P, Carcao M, Federici A, Cox Gill J, Halimeh S, Kempton C, Key N, Kouides P, Lail A, Landorph A, Leebeek F, Makris M, Mannucci P, Mauser-Bunschoten E, Nugent D, Valentino L, Winikoff R, Berntorp E. Blood Coagulation & Fibrinolysis: An International Journal in Haemostasis and Thrombosis. 2015 Jun; 26(4):383-8.

II. Patients referred for bleeding symptoms of unknown cause: Does evaluation of thrombin generation contribute to diagnosis? Holm E, Zetterberg E, Lovdahl S, Berntorp E. Mediterranian Journal of Hematology and Infectious Diseases. 2016;8(1):1-6.

III. Bleeding-related hospitalization in patients with von Willebrand disease and the impact of prophylaxis: Results from national registries in Sweden compared with normal controls and participants in the von Willebrand Disease Prophylaxis Network. Holm E, Steen Carlsson K, Lövdahl S, Lail AE, Abshire TC, Berntorp E. Haemophilia. 2018:1-6.

IV. Cardiovascular disease-related hospitalization and mortality among persons with von Willebrand disease: A nation-wide register study in Sweden. Holm E, Osooli M, Steen Carlsson K, Berntorp E. Manuscript.

Introduction

History

Clinical Coagulation, a collective term for thrombosis and bleeding disorders, is a young discipline in our country and internationally. Haemophilia was described in the Babylonian Talmud in the 3rd century by the rabbi Judah ha-Nasi [1], venous blood clotting in the 19th century by Rudolf Virchow[2] and the first functional coagulation schedule by Moravitz in 1905[3]. Modern coagulation research first gained momentum in the 1930's. Pioneering discoveries about blood coagulation were made in Scandinavia by Paul Owren (known for factor V and coagulation analysis) in Norway[4], by Henrik Dam in Denmark (vitamin K)[5] and by Erik Jorpes[6] in Sweden (heparin). Heparin began to be used as a medicine for treatment of blood clots. In the early 1950s, Inga Marie Nilsson's and Margareta and Birger Blombäck's research was established at the Karolinska Institute in Stockholm. Margareta and Birger Blombäck determined the chemical structure of fibrinogen in 1956 [7]. Birger Blombäck and Inga Marie Nilsson isolated coagulation factor VIII (anti haemophilia globulin, AHF) from blood plasma. Further, between 1956 to 1957 they made the first concentrate for the treatment of haemophilia A [8]. Inga Marie Nilsson moved to Malmö in the mid-1950s and established the coagulation centre there under Professor Jan Waldenström. Arguably, her most important discovery occurred on May 11th, 1956, when she gave AHF to a girl with severe bleeding due to von Willebrand disease. Nilsson showed that the condition was caused by a lack of plasma protein that could be supplied with AHF, which was distinct from the haemophilia factor. Nilsson engaged very early in the treatment of bleeding disorders and was the first, globally, to introduce prophylactic treatment for patients with severe haemophilia. Results from twenty-five years of research were compiled and it was shown in a 1992 publication[9] that it is, to a great extent, possible to prevent haemorrhage and hence avoidance of the joint injuries that cause pronounced motor disabilities as early as the teenage years. The Swedish approach of prophylactic treatment is currently used in a number of countries as a model to prevent severe haemophilia-related bleeding.

The highly specialised coagulation activities in Sweden are organised in three centres located at the university hospitals in Stockholm, Gothenburg and Malmö. These are responsible for the investigation and treatment of both hereditary and acquired bleeding and thrombotic diseases. Exceptional for these coagulation centres is that they are responsible for the investigation and treatment of patients with haemophilia, von Willebrand disease and other rare hereditary bleeding disorders in Sweden. The units have a clinical component, associated with haematology, and a laboratory component, organised in clinical chemistry. All centres have both adult and paediatric services and access to routine and specialist coagulation analyses through the expert capacity available in clinical chemistry. The patients' data are organised in a national registry and regular follow-ups of treatment results are made. All state-of-the-art coagulation analyses are available as well as molecular genetic diagnostics for a series of hereditary diseases. Furthermore, 24-hour on call services are available. The research is well-organised with strong international affiliations. Thanks to this cooperation, I was able to prepare several of my works.

Von Willebrand disease

Von Willebrand disease is the most common hereditary bleeding disorder. The disease is autosomally inherited and consequently, unlike haemophilia, is present in equal proportions among men and women. The first case was described by the Finnish doctor Erik von Willebrand in 1926.

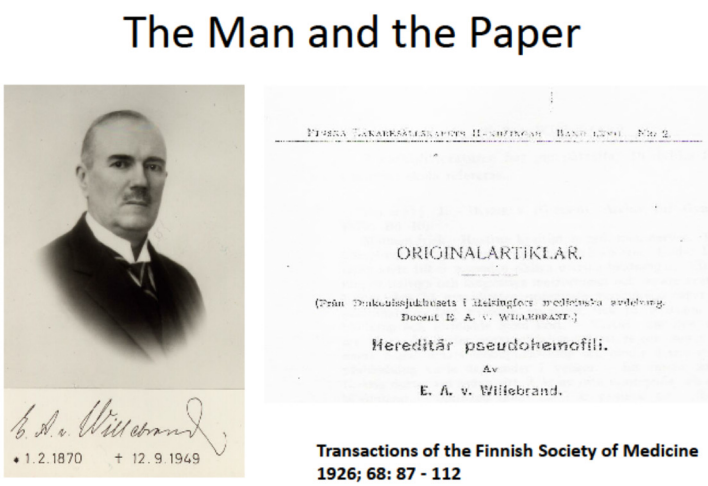

Figure 1.
The first publication about VWD.

Erik von Willebrand reported the clinical symptoms and heredity in a family from the Åland islands[10]. The index case was Hjördis, a 5-year old girl who had an increased bleeding tendency. She was the ninth of eleven siblings. Six of her ten siblings had bleeding symptoms.

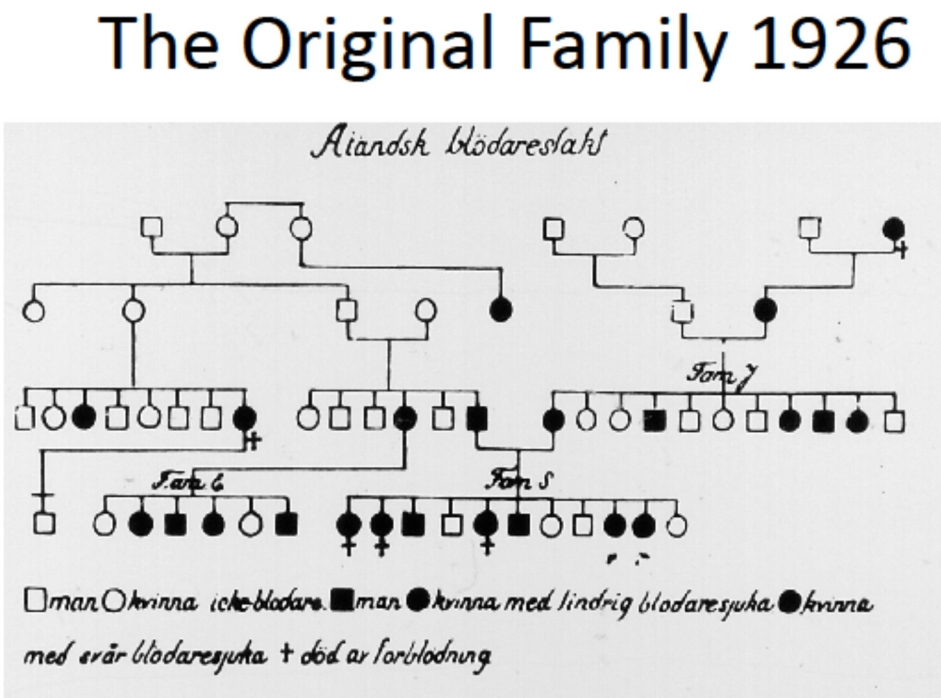

Figure 2.
The family tree from Åland. Hjördis is located as number 9 in family S.

Hjördis had life-threatening bleeding during her fourth menstrual period and died at the age of 13. This story was the first clue in the discovery of von Willebrand disease, a disorder that Erik von Willebrand named *hereditary pseudohemophilia.*

For a long time, the disease was thought to be due to lack or deficiency of FVIII. A platelet disorder could be identified as well and blurred the picture of the disease[11] However, in the 1950's, von Willebrand factor (VWF) was identified as having two important functions: 1. it stimulates platelet adhesion and aggregation (primary haemostasis); and 2. it protects FVIII from degradation, which promotes a longer half-life in circulation[12,13].

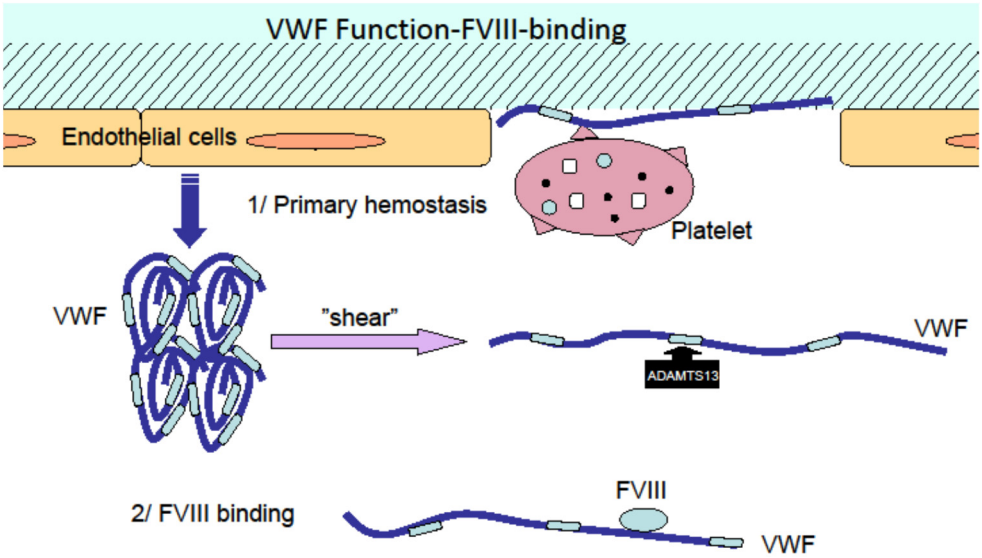

Figure 3.
Functions of VWF. (Courtesy of Andreas Hillarp).

The von Willebrand factor is manufactured in endothelial cells and megakaryocytes. Biosynthesis involves continuous release to the circulation but the von Willebrand factor is also stored in Weibel-palade bodies in endothelial cells and in platelet alpha-granules.

There are different types of von Willebrand disease. Types 1 and 3 are the result of quantitative defects in von Willebrand factor.

Type 1: Partial quantitative deficiency of VWF, type 3: virtually complete deficiency of VWF

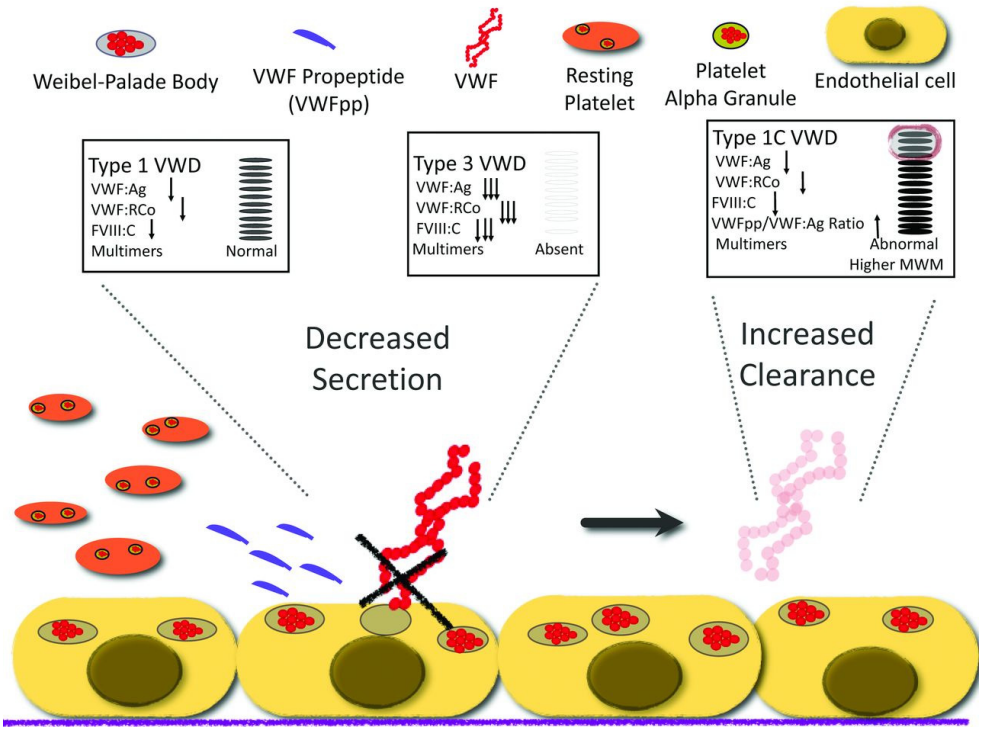

Figure 4.
Pathogenesis at VWD type 1 and type 3. (Branchford and Di Paola[14]).

Type 2: depends on qualitative defects of von Willebrand factor.

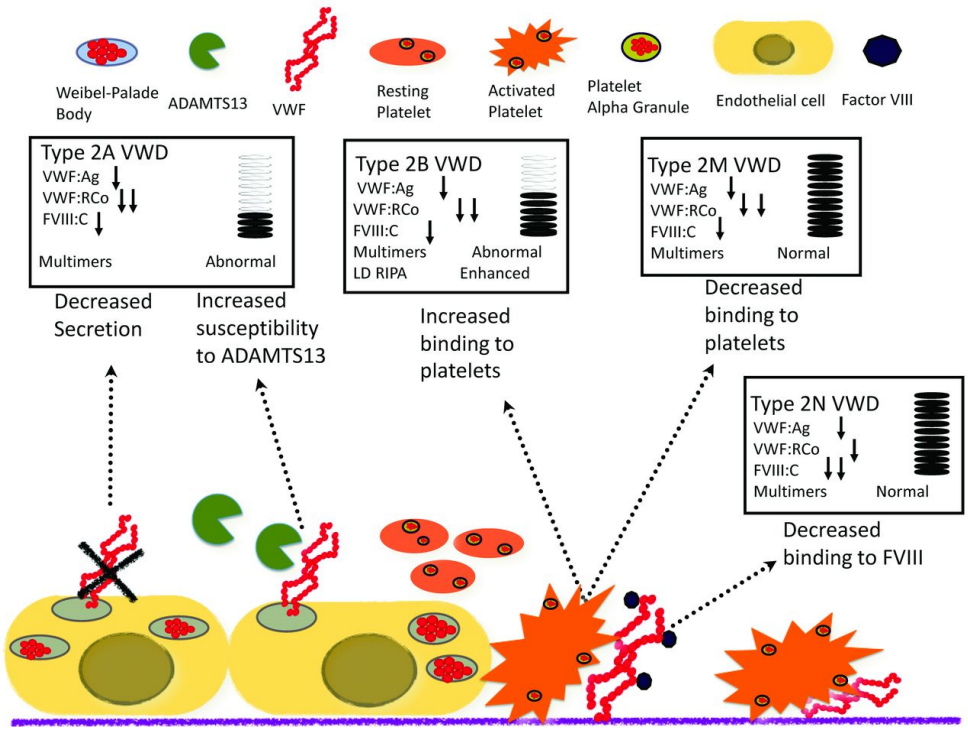

Figure 5.
Pathogenesis of VWD type 2. (Branchford and Di Paola[14]).

Type 2A: lack of high-molecular weight multimers and reduced VWF dependent adhesion.

Type 2B: lack of high-molecular weight multimers and increased affinity for platelet receptor GPIB.

Type 2M: decreased VWF dependent platelet adhesion without a selective deficiency of high molecular weight multimers.

Type 2N: markedly decreased binding affinity for factor VIII.

As a thumb role, seventy percent of all patients with von Willebrand disease have type 1, 25% have type 2 and 5% suffer from the most severe, type 3. However, exact figures are difficult to estimate and e.g. in countries where consanguinity is common the prevalence/incidence of type 3 is higher than in other countries[12] . The clinical symptoms include mouth and nasal bleeding, bowel bleeding, joint bleeding (in severe cases), and menorrhagia. Treatment includes acute care of bleeding episodes and preventive treatment of severe recurrent bleeding that may significantly affect

quality of life. As many cases have a dual reduction of von Willebrand factor and FVIII, symptoms may reflect deficiency in the primary haemostasis (VWF) as well as in plasma coagulation (FVIII).

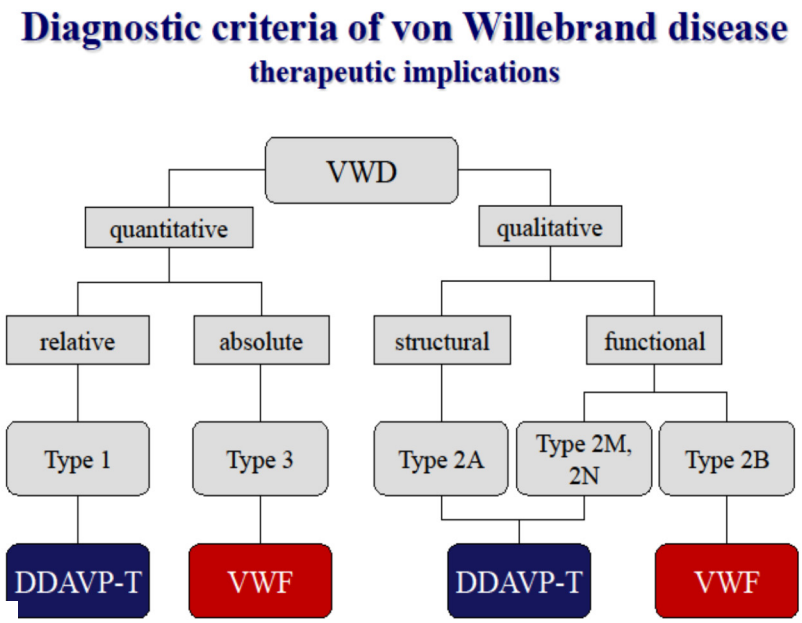

Figure 6.
Treatment approaches for the different types of VWD (Nordic guidelines for VWD[13]).

Desmopressin, which is a synthetic variant of antidiuretic hormone, stimulates endogenous VWF secretion into plasma and increases VWF levels temporarily. FVIII levels are also increased after treatment with desmopressin which contributes to enhanced haemostasis. Desmopressin administered as nasal spray[15-17] can be successfully used for the treatment of the majority of patients with type 1 and type 2 disease, with the exception of type 2B due to increased risk of pronounced thrombocytopenia. Desmopressin has no effect on type 3 VWD where VWF is missing completely and there is nothing to release.

Patients with VWD type 3 and type 2B are eligible for treatment with plasma-derived factor concentrates. Furthermore, patients with type 1 and type 2 VWD who are insufficiently responsive to desmopressin, or patients with contraindications for desmopressin may be treated with factor concentrates. A pasteurized product has been on the market since early 1980s , is licensed both in Europe and United States,

and has a good safety and efficacy record[18]. This concentrate contained both FVIII and VWF and the main indication was haemophilia. Concentrates with primary indication VWD has since been developed. The concentrates differ in their content of VWF and FVIII and in the VWF multimeric pattern[19]. Recently a recombinant VWF concentrate[20] is entering the market, several decades after the firs recombinant FVIII products aimed for haemophilia treatment[21,22].

Prophylaxis in VWD is not as well established as in haemophilia and few publications are available. With the advent of AHF, as described above, prophylaxis was also started at a small scale. A long-term follow up of the 35 Swedish patients on prophylaxis (Type 3, n=28; type 2A, n=3; type 2B, n=4. Females, n=18;males, n=17) was published in 2005[23] showing the benefit of prophylaxis with substantial reduction in bleeding frequency after institution of prophylaxis. The primary indication in small children was mouth and nose bleeds whereas menorrhagia and joint bleeds were more prevalent as indication in adolescents and adults. In the elderly population gastrointestinal bleeds was a dominant cause of prophylaxis. Interestingly children who started prophylaxis before the age of 5 years never developed arthropathy which indicated a preventive effect on joint bleeds. More recently other studies have corroborated these findings but usually in small, and not well controlled chohorts[24-26]. Based on the earlier studies the health technology assessment in Sweden in 2011 concluded that evidence for prophylaxis in VWD was weak and that more studies are needed[27].

The von Willebrand Disease Prophylaxis Network (VWD PN)

The von Willebrand disease prophylaxis network was formed in 2004 as an international study group with the goal of evaluating prophylactic regimens for people with severe VWD and extensive bleeding problems[28]. The VWD International Prophylaxis (VIP) study was initiated by the VWD PN and several retrospective and prospective studies were designed. The first report from the retrospective study, including 59 patients from 20 centres in 10 countries, showed significant reductions in bleeding after initiation of prophylaxis[29]. The only prospective study on prophylaxis in VWD was recently published by the VWD PN group[30]. Although small (10 patients fulfilled the study) prophylaxis was proven effective and the use of a dose-escalation regimen model was proposed. In another retrospective study the network compiled data from 48 patients with GI bleeds[31] of whom 38% had verified angiodysplasia. Prophylaxis was the most efficient therapy but a number of treatments were used in addition.

Registers in Sweden

The personal identity number was introduced in Sweden in 1947 and provided the opportunity to create a national register with records containing data of high-quality. Registers are kept by the National Board of Health and Welfare Statistics Sweden (SCB) and supported by the government[32]. There is a unique opportunity to link records from different registers through the personal identity numbers. The World Health Organization (WHO) has overseen the creation and updating of the ICD-codes (International Classification of Diseases) since 1948.

National registers

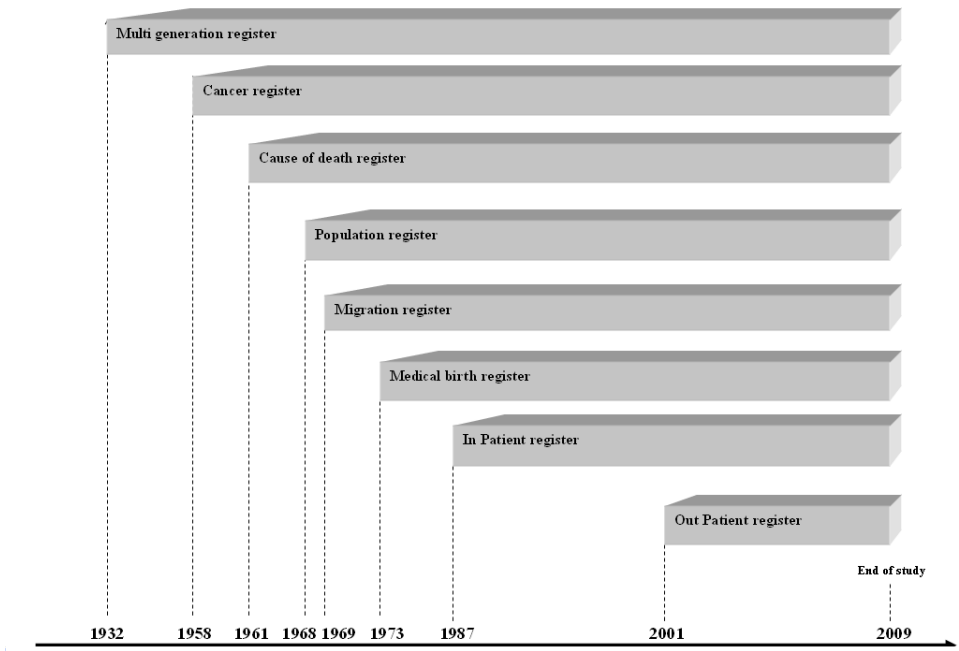

Figure 7.
National registers in Sweden. (Lövdahl et al, with permission[32]).

The ability to study patients with rare diseases using data from a variety of registers provides the opportunity to answer a multitude of questions.

Population register

This register has been available since 1968. It contains demographic information including name, gender, age, native country, and residence among other variables. The matched control cohort in papers III and IV was randomly selected from the population registry.

Patient register

Records of admissions for inpatient and outpatient care can be found in the patient register. The inpatient register, available from 1987 onwards, contains complete records of hospital admissions. The outpatient register has been available since 2001. Persons with VWD were identified in this register using ICD codes.

Cause of death register

This register includes the date and the cause of death, classified by ICD codes, from 1961. This registry was used in paper IV.

Cancer register

The cancer register is the oldest resource. Individual records have been kept since 1958 with an estimated 4% missing data. The register was used in paper IV.

Thrombin generation assay (TGA) and bleeding scores (BS)

Traditional coagulation tests such as INR and APTT have clot formation as an endpoint and measure the time at which clotting occurs. This happens when only approximately 5% of all relevant physiological thrombin has been formed. Therefore, the results do not always reflect the patient's clinical symptoms in terms of bleeding or thrombosis. The in vitro capacity to generate thrombin over time is called endogenous thrombin potential[33-36]. It determines the full capacity of thrombin formation i.e., the combined effects of promotors and inhibitors of thrombin formation when a plasma sample is activated in vitro. Activation occurs with tissue factor in the presence of phospholipid and calcium.

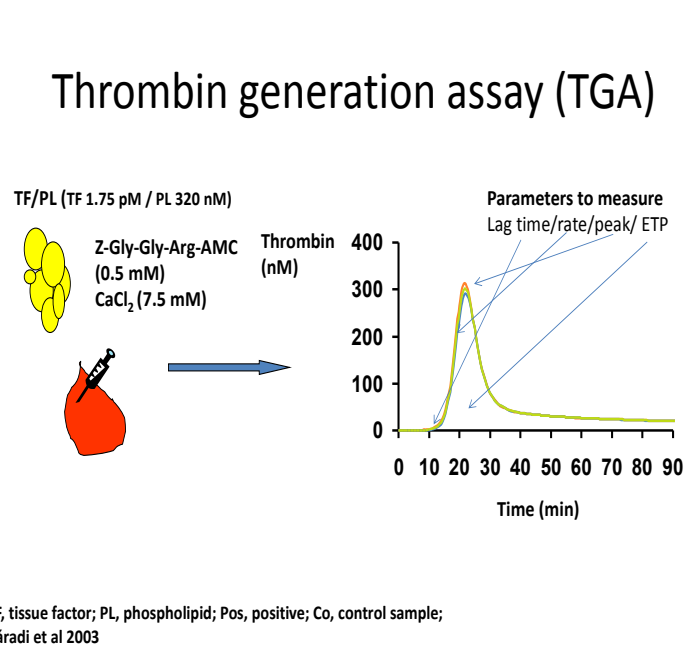

Figure 8.
Thrombin generation assay. (Varadi et al[36] with permission).

The TGA test can be used to monitor the effect of treatment in haemophilia or to assess the risk of thrombosis although the full clinical implications of the test have yet to be further evaluated[37,38].

The Bleeding Assessment Tool (BAT) is used in the clinic setting to estimate a patient's bleeding patterns. Bleeding scores (BS) for individuals without a bleeding tendency are less than 4.

Table 1.
Questionnaire administered to patients with bleeding symptoms (translated to Swedish and modified after Tosetto et al[39] and Bowman et al[40]).

BLÖDNINGS-SCORE

Patient:	Datum:	Dr:

Symptom	-1	0	1	2	3	4	POÄNG
Näsblödning	---	<5 ggr/år el. endast ena näsborren	>5 ggr/år eller >10 min.	Endast konsultation	Tamponering, etsning, Tx* och/el. desmopressin	Blodtransfusion	
Blåmärken	---	Obetydliga (<1 cm)	Rec. på bålen utan trauma >3 cm	Endast konsultation	>5 cm i diameter med resistens		
Blödning vid mindre sår	---	Obetydlig (<5)	>5 ggr/år el. >5 min. ”Byta plåster”	Endast konsultation	Kirurgisk hemostas, Tx* och/el. desmopressin	Blodtransfusion	
Munhåle-blödning	---	Nej	Sällan	Endast konsultation	Kirurgisk hemostas, Tx* och/el. desmopressin	Blodtransfusion	
GI blödning	---	Nej	Pga ulcus, varicer, hemorrojder el. angiodysplasi	Spontan	Kirurgisk hemostas, blodtransfusion, Tx* och/el. desmopressin	Blodtransfusion > 5 ggr	
Vid tandextraktion	Ingen blödning vid minst två extraktioner	Ingen ex. eller ingen blödning vid en ex.	Ökad blödning efter en extrakt. Sökt tandläkare/sjukvård	Ökad blödning efter ≥2 ex. Sökt tandläkare/sjukvård	(Re)sutur, tamponering, Tx* och/el. desmopressin	Blodtransfusion	
Vid kirurgi	Ingen blödning vid minst två tillfällen	Ingen op. eller ingen blödning vid en op.	Ökad blödning efter en op. Sökt sjukvård	Ökad blödning efter ≥2 op. Sökt sjukvård	Kirurgisk hemostas, Tx* och/el. desmopressin	Blodtransfusion	
Muskelhematom	---	Aldrig	Posttraumatisk, ingen behandling	Spontan, ingen behandling	Spontan el. traumatisk, hemostatisk behandling	Spontan el. traumatisk, behov av kirurgiskt ingrepp el. blodtransfusion	
Hemartros	---	Aldrig	Posttraumatisk, ingen behandling	Spontan, ingen behandling	Spontan el. traumatisk, hemostatisk behandling	Behov av kirurgiskt ingrepp el. blodtransfusion	
Menorragi från menarche >7 dgr, frekv. byten	---	Nej	Endast konsultation	Tx* och/el. desmopressin, järnmedicin, p-piller, hormonspiral	Blodtransfusion, ablation	Hysterektomi	
Post partum-blödning	Ingen blödning vid minst två förlossningar	Ingen förlossning eller ingen blödning vid en förlossning	Endast konsultation	Skrapning, järnmedicin, Tx* och/el. desmopressin	Blodtransfusion	Hysterektomi	
CNS-blödning		Nej			Subdural Intervention med. el. kir.	Intracerebral Intervention med. el. kir.	
Förstagrads-heredited		Nej	Ja				

*Tx=antifibrinolytisk behandling

SUMMA:

Poäng för personer utan blödningsbenägenhet enligt Tosetto et al (2006) och Bowman et al (2008): <4

Aim of the thesis

The goal of this work was to evaluate the impact of prophylactic treatment in patients with VWD and a severe bleeding phenotype with respect to bleeding site and bleeding-related hospitalizations using data from the VWD International Prophylaxis (VIP) Study. Using national registers in Sweden, we were able to present data about need for hospitalization among patients with VWD, and cardiovascular disease outcomes for all VWD cohorts in Sweden compared with age- and gender-matched controls. The study of BS and TGA demonstrates the importance of use of coagulation analyses beyond those traditionally used in patients with bleeding symptoms in order to identify people with bleeding disorders including VWD to enhance future management and treatment.

Specific aims

Paper I: To study the impact of prophylactic treatment in a large cohort of patients with VWD and describe the efficacy of the treatment by bleeding site.

Paper II: To describe patients referred to our department of coagulation in Malmö with bleeding symptoms, but without diagnosis, and examine the correlation between TGA and BS.

Paper III: To study the role and impact of prophylactic treatment on hospitalization in patients with VWD using national registers and data from the VIP study.

Paper IV: To investigate cardiovascular morbidity and mortality in patients with VWD in Sweden.

Materials and methods

Papers I and III report on subjects from 20 centers in 10 countries enrolled in the prospective or retrospective VIP studies.

Inclusion criteria for the prospective study of bleeding patterns were:

- Joint bleeding: two episodes from the same joint, or three from different joints, during six months.
- Gastrointestinal bleeding: two or more bleeding episodes which required blood transfusion and clotting factor concentrate treatment.
- Menorrhagia: Pictorial Blood Assess Chart (PBAC) score > 185
- Epistaxis: three or more bleeding events with need of blood transfusion and/or concentrate treatment.

Inclusion criteria in the retrospective study were:

- Ongoing prophylaxis for at least six months.
- Previous prophylaxis for at least six months.
- Access to all documentation.

Data from the same group of patients were included in the second part of paper III to evaluate the hospitalization rate prior to and after the start of prophylactic treatment in cases of severe VWD.

Paper II examined eighty-five persons referred to the coagulation department in Malmö due to bleeding symptoms without known disease and with normal results from traditional laboratory tests.

The Bleeding Score described by Tosetto[39] and Bowman[40] was used to assess the rate of bleeding symptoms.

A careful review of notes in the patients' records was used to describe the bleeding type in these patients.

Papers III and IV included data for all registered Swedish persons with VWD, regardless of the type and severity, during an observation period between 1987 through 2008. For each person with VWD, up to five age- and sex-matched persons

without congenital bleeding disorders were included as controls. The National Patient Register (NPR) was the main source of data for hospitalization rate, cardiovascular disease (CVD) and bleeding outcomes. The NPR includes inpatient care visits from 1987 and outpatient hospital visits from 2004. ICD codes (ICD-9 for 1987-1996, and ICD-10 for 1997-onwards) were used for registration of primary and other diagnoses for each admission.

Statistical analyses

Paper I: Annualized bleeding rates were calculated for the periods prior to start of prophylaxis and during prophylactic treatment for each of the primary indication sites. To describe bleeding rates medians and interquartile ranges (IQR) were used. A paired Wilcoxon signed-rank test of the differences in the medians was used to compare the bleeding rate by primary indication.

Paper II: Spearman's correlation was used to calculate correlations between BS and thrombin generation assay results. A p-value <0.05 was considered to indicate statistical significance.

For comparison of the scores, all participants were divided into four age groups (1-20; 21-40; 41-60 and >60 years old). The Kruskal Wallis test was used and a p-value <0.008 was considered to indicate statistical significance.

Paper III: Results are presented for all patients in population-based registers in Sweden and by decade of birth. The mean number of visits per year per person and the 95% confidence intervals were reported for outpatient care. The primary diagnosis at registration and the first surgical procedure code were used for surgical events. To estimate the incidence rate ratio of hospital admission, number of inpatient days and hospital visits we used population-average negative binomial regression.

For data from the VWD PN studies we used the Wilcoxon signed-rank test and a p-value <0.05 was considered statistically significant.

Paper IV: Cox regression models were used to estimate the hazard rate ratios (HR) of hospitalization for CVD and for both all-cause and CVD mortality.

The VIP study was approved by the human subjects committees of collaborating institutions in a compliance with guidelines of the Declaration of Helsinki. The VIP study is registered at www.ClinicalTrials.gov (NCT 00557908).

The register study was approved by the regional ethics committee of Lund, Sweden on 7 August 2008, registration number 241/2008.

Results

Paper I

The study was conducted between 2008 to 2013 and included 105 patients from 10 countries. The mean age of participants was 26 years (range 1-81) at the start of prophylaxis. The proportions of adults and children were similar.

The majority of patients were white and had type 3 VWD. In the overall group, there were as many men as women. It may be noted that this study of 105 persons to our knowledge is the largest investigation of prophylaxis in VWD conducted thus far.

Table 2.
Gender, race and VWD type by age group. Figures denote number (%).

	Adult	Child	Overall
Gender			
Female	34 (32.4%)	18 (17.1%)	52 (49.5%)
Male	22 (20.9%)	31 (29.5%)	53 (50.5%)
Race			
Asian	3 (2.9%)	3 (2.9%)	6 (5.7%)
Black	0	4 (3.8%)	4 (3.8%)
Hispanic	1 (0.95%)	12 (11.4%)	13 (12.4%)
White	52 (49.5%)	27 (25.7%)	79 (75.2%)
Other	0	3 (2.9%)	3 (2.9%)
VWD Type			
Type 1	8 (7.6%)	5 (4.8%)	13 (12.4%)
Type 2A	16 (15.2%)	9 (8.6%)	25 (23.8%)
Type 2B	7 (6.7%)	2 (1.9%)	9 (8.6%)
Type 2M	1 (0.95%)	2 (1.9%)	3 (2.9%)
Type 2, Unknown	1 (0.95%)	0	1 (0.95%)
Type 3	23 (21.9%)	31 (29.5%)	54 (51.4%)

In total, 1116 bleeding episodes were analysed in the study and their distribution is presented in the table below.

Table 3.
Distribution of bleeding events prior to and during prophylaxis by location.

Location of Bleeding	Frequency	Percentage
Epistaxis	365	32.7
Gastrointestinal	259	23.2
Intracranial	4	0.4
Joint	257	23.0
Menorrhagia	46	4.1
Muscle	23	2.1
Oral	67	6.0
Soft tissue	67	6.0
Other	28	2.5
Total	1116	100.0

Gastrointestinal bleeding was the most common reason for the initiation of prophylaxis in adults and these patients had generally poorer response rates, requiring higher doses of concentrate and higher treatment frequency (Tables 4 and 5).

Table 4.
Annualized rate of bleeding episodes prior to and during prophylaxis and median change in annualized rate and percentage change by primary bleeding indication.

Indication	N	Prior to Prophylaxis Median (IQR)	During Prophylaxis Median (IQR)	Median Rate Change (IQR)	Median Percent Change (IQR)
Epistaxis	28	11.1 (6.0, 48.0)	3.8 (0.21, 16.8)	-6.1 (-42.0, -1.5)	-86.7 (-95.5, -49.8)
GI bleeding	18	9.3 (6.0, 21.6)	6.0 (3.6, 7.1)	-3.0 (-6.0, 0.0)	-44.3 (-72.2, 0)
Joint bleeding	25	11.9 (6.0, 18.0)	0.8 (0.0, 3.2)	-8.5 (-12.0, -4.2)	-86.9 (-100.0, -52.5)
Menorrhagia	9	9.6 (8.4, 12.0)	0.0 (0.0, 0.4)	-9 (-9.3, -6.0)	-100.0 (-100.0, -95.8)

Table 5.
Dose (VWF:RCo, U/kg) by bleeding type prior to and during prophylaxis and average dose by regimen (N=80).

		During prophylaxis	
Bleeding type	Prior to prophylaxis	<3 times per week/cycle	≥3 times per week/cycle
Epistaxis			
N	25	9	12
Mean	44.9	38.8	48.8
STD	16.8	15.5	18.3
Gastrointestinal			
N	13	2	10
Mean	48.2	45.0	49.3
STD	15.0	7.1	11.7
Joint			
N	19	9	4
Mean	45.8	50.8	73.3
STD	16.2	20.8	51.5
Menorrhagia			
N	5	1	2
Mean	51.9	50	39.0
STD	20.1	-	1.4

The total study group showed significant reductions in the number of bleeding events after initiation of prophylaxis.

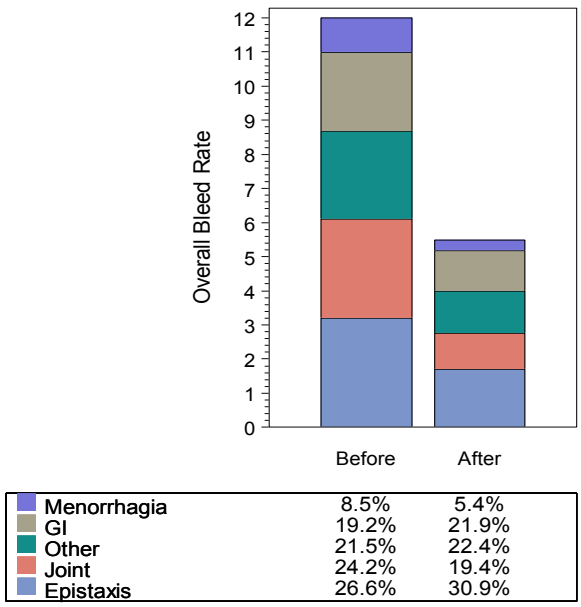

Figure 9.
Annualized rates of bleeding for the total study group.

Patients with the most severe form of von Willebrand disease, type 3, achieved obvious benefits after initiation of prophylaxis with significant reduction in the number of bleeding events, see figure 10.

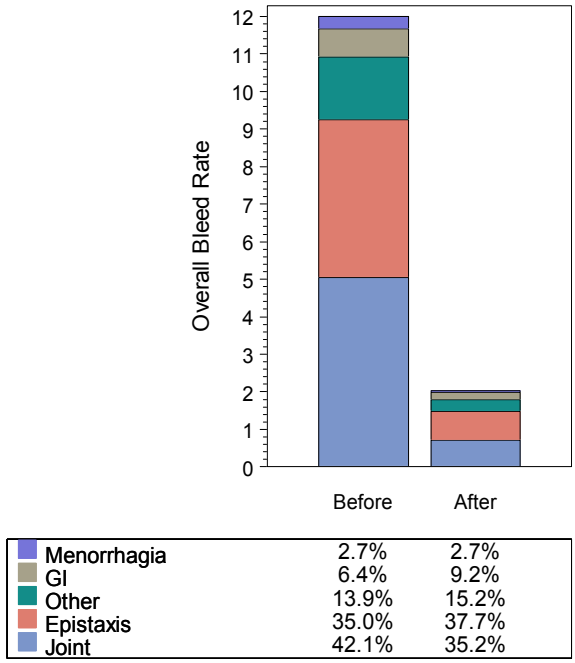

Figure 10.
Annualized rates of bleeding for patients with VWD type 3.

Paper II

The purpose of the study was to investigate whether there was an association between BS and thrombin generation in patients referred to the coagulation department by a variety of specialists due to bleeding symptoms but with no known diagnosis of a bleeding disorder. A secondary objective was to determine whether there were patients with undiagnosed VWD among these patients.

We identified one man with mild haemophilia A and five unrelated women with type 1 VWD. The ages of the women were 16, 21, 41, 44 and 55 years. They had BS, from the youngest to the oldest, of 4, -1, 7, 15 and 2. One woman, aged 21, referred because of bruising, was pregnant and had high levels of peak TG. The other four women had low levels of peak TG. These patients received relevant

information about their disease, adequate treatment for their bleeding symptoms and appropriate interventions and surgery. They were excluded from further analyses.

Table 6.
Description of primary bleeding type in each patient as cause for referral.

Bleeding site	N (%)
Epistaxis	34 (18.9)
Menorrhagia	49 (27.4)
Extreme bleeding after surgery	36 (20.)
Bruises	28 (15.6)
Postpartum bleeding	6 (3.4)
Extreme bleeding after tooth extraction	5 (2.8)
Haematuria	3 (1.7)
Gastrointestinal bleeding	3 (1.7)
Joint and muscle bleeding	2 (1.1)
Other	13 (7.3)
Total	179 (100.0)

The most common reason for referral was menorrhagia .

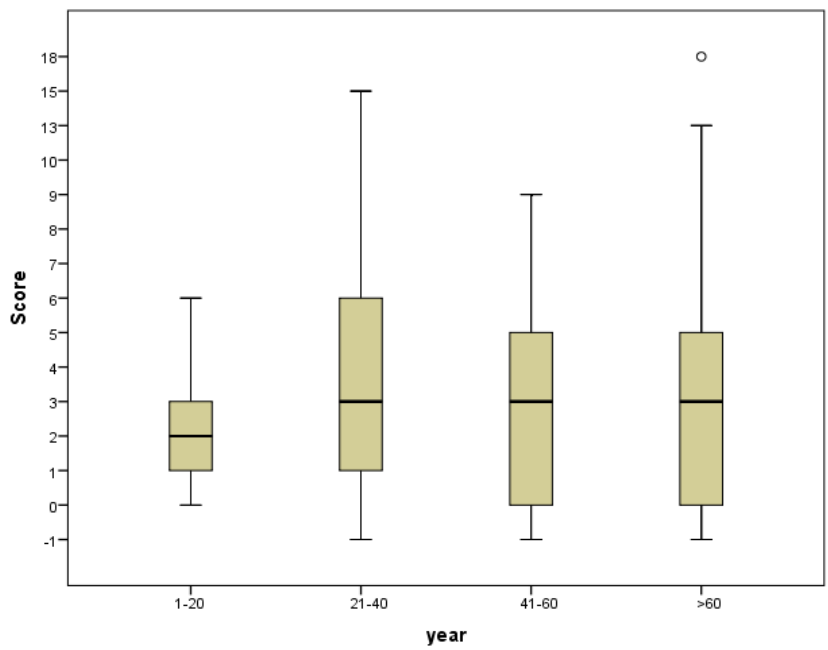

Figure 11.
Bleeding Scores in the different age cohorts.

In general, study participants had low BS. In addition to other samples, blood was taken for TGA assays. We found a slightly significant correlation between BS and TGA in women, but the results did not support a recommendation for use of this method in everyday clinical practice.

Paper III

Data from national registers in Sweden showed considerably higher numbers of hospitalizations over the period 1987-2009, and inpatient days during the years 2004-2009 for people with VWD compared to age- and gender- matched controls as seen in figure 12 and figure 13.

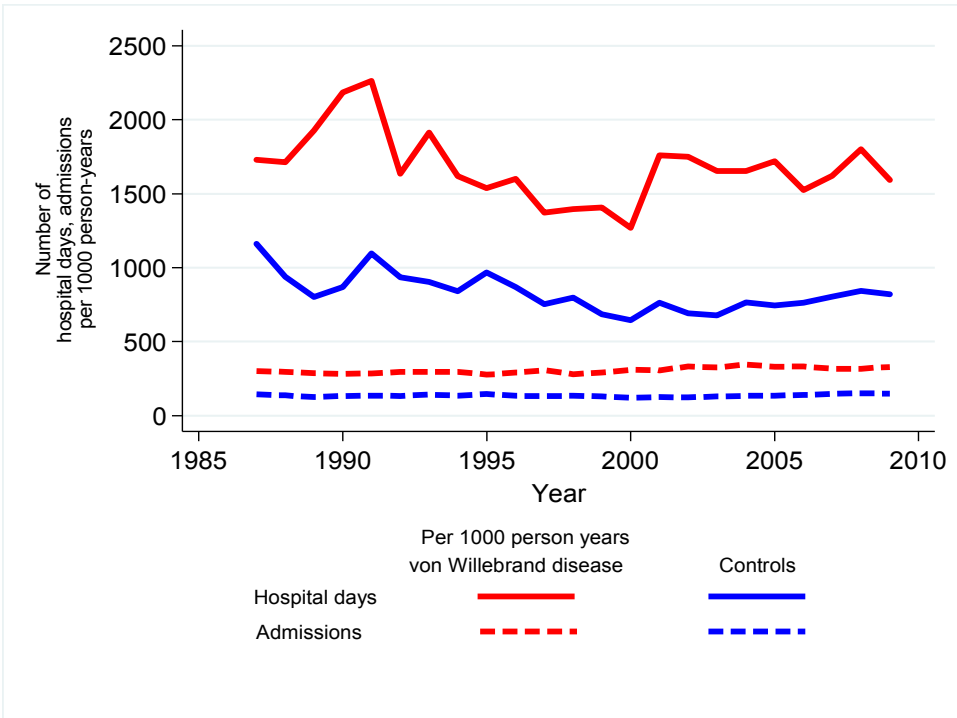

Figure 12.
Data from national registries. Annual number of hospital days and admissions per 1,000 person years from 1987-2009 for people with von Willebrand disease and matched controls from the general population.

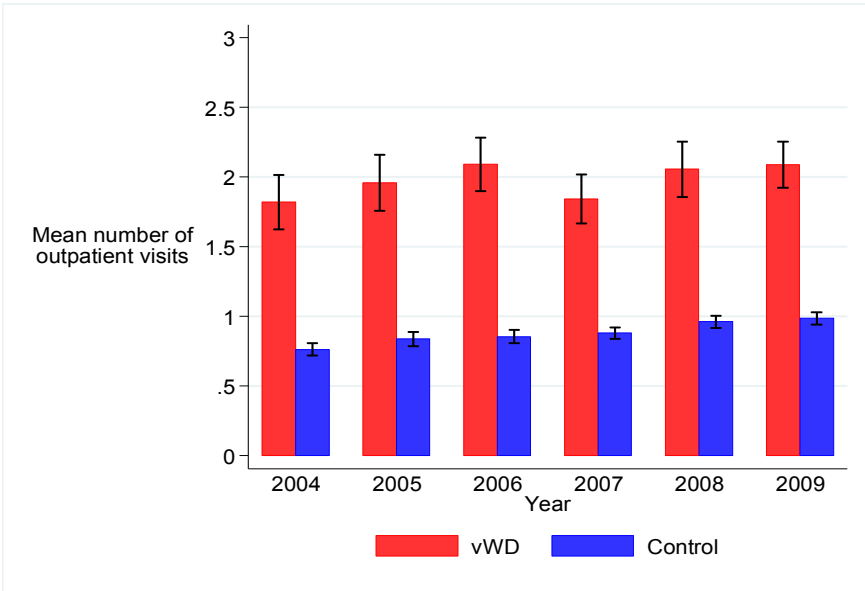

Figure 13.
Data from national registries. Annual number of outpatient hospital visits (mean, 95% confidence interval) for people with von Willebrand disease and controls from 2004-2009.

Gastrointestinal bleeding followed by menorrhagia and epistaxis were the most common causes of hospitalization.

In the second part of this study, using data from the VWD PN, we found that significantly fewer hospitalizations occurred after initiation of prophylactic treatment in patients with severe VWD (75 prior to and 45 after, p=0.006).

Gastrointestinal bleeding remained the most common reason for hospitalization after initiating prophylaxis.

Paper IV

The sample contained 2,790 people with von Willebrand disease in Sweden with at least one registration in the National Patient Register at the National Board of Health and Welfare. There were 1,902 women (68%) and 888 men (32%). The median year of birth was 1961 (interquartile range 1942 - 1978). At the time of data retrieval, 2,445 subjects were alive and registered as living in Sweden, while 302 were deceased and 43 had emigrated. The sample contained one subject with HIV and 151 subjects with hepatitis. Figure 14 shows the distribution of the number of people with VWD by decade of birth.

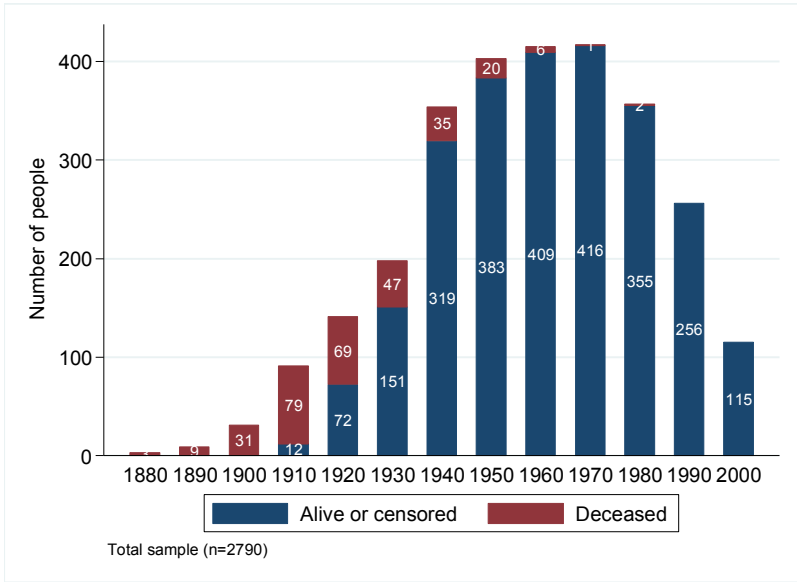

Figure 14.
People with von Willebrand disease registered in the National Patient Register at the National Board of Health and Welfare by decade of birth and status: alive/censored and deceased, respectively.

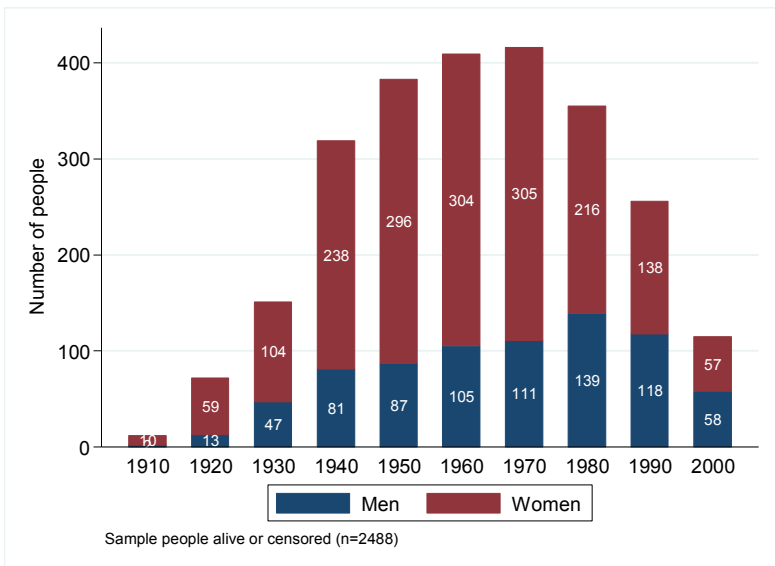

Figure 15.
Number of men and women with VWD by decade of birth.

A comparison group included 13,938 participants individually matched for birthdate and sex to those with VWD.

Overall, the hazard of CVD-related hospitalization was 30% higher (95% CI: 1, 1, 1.5) among participants with VWD after adjusting for sex, birthdate, diabetes and cancer. However, this group had a 60% (95% CI: 0.3, 0.6) lower hazard of CVD-related mortality than the general population sample.

Limitations

None of the studies conducted included randomized samples. Paper I, which primarily contained retrospective data, confirmed that we need to include patients with severe VWD in prospective studies. We did not study differences in outcome between brands of VWD concentrates as these may require different dosing schedules. Virtually all patients used the same type of concentrate (Humate-P/Haemate, CSL Behring, Marburg, Germany).

In paper II we did not have controls for comparison. Secondly, the ISTH BAT was not available at the time of study start. We used, instead, an older variant of BS assessment with poorer quality without the intention of improving the sensitivity of bleeding assessment across all age groups.

TGA parameters were analysed at one time, only, and we could not evaluate whether these were consistent over time.

Paper III and IV: We could not rule out possible misclassification of diseases in the registers. Further, we did not have details of the subtypes of VWD. In addition, we could not adjust for other risk factors for CVD such as smoking, obesity and family history as these parameters were not available.

Conclusions and Future Perspectives

Knowledge, understanding and treatment of von Willebrand disease have improved considerably since the disease was first described in 1926.

The main conclusions of this thesis are the following:

- Prophylactic treatment has a dramatic effect on joint bleeding and epistaxis in all age groups.
- Patients with GI bleeding need higher factor concentrate doses and more frequent treatment.
- Future prospective studies may form the basis for more adapted treatment regimens for patients with VWD where indication of prophylaxis is present.
- VWD screening is important for future treatment of bleeding and, possibly, prior to surgery.
- Individuals with VWD have significantly higher healthcare consumption compared to controls and the introduction of prophylaxis may reduce the need for hospitalization due to bleeding.
- Hospital care for CVD was about twice as common among those with VWD. On the other hand, however, CVD mortality was 60% lower. Total mortality did not differ from controls.

Svensk populärvetenskaplig sammanfattning

Von Willebrand sjukdom (VWD) är den vanligaste ärftliga blödningsrubbningen. Det finns sannolikt ett stort mörkertal av oupptäckta fall som har mer eller mindre uttalade blödningsbesvär. Man räknar med att 1-2% av befolkningen bär på anlaget för VWD. Sjukdomen nedärvs autosomalt och drabbar alltså män och kvinnor i samma utsträckning. Symtom kan vara näsblödningar, rikliga menstruationer, blödningar från magtarmslemhinna, ledblödningar som kan påverka patientens hälsotillstånd och livskvalité och i vissa fall leda till sjukhusvistelse.

VWD indelas i tre kategorier – typ 1, typ 2 och typ 3. Typ 2 fördelas i sin tur på flera undergrupper (subtyper). När det inte finns tillräckligt med von Willebrand faktor (VWF) i blodet, eller om VWF inte fungerar normalt, tar det längre tid för blödningar att upphöra. Typ 1 är den vanligaste formen av VWD och utgör cirka 70 % av alla patienten. Vid typ 1 fungerar VWF normalt, men det finns för lite i blodet. Många personer med typ 1 har inga blödningsbesvär alls förrän de råkar ut för en skada eller operation. Då kan de få en allvarlig blödning.

Vid typ 2 kan mängden VWF i blodet vara normalt. Däremot fungerar VWF inte som den ska, vilket ger upphov till ökad blödningsbenägenhet.

VWD typ 3 är mycket ovanlig och finns endast hos ungefär 5/1 000 000 individer i befolkningen. Personer med typ 3 har ingen eller nästa ingen VWF. Dessa patienter kan blöda ofta och blödningarna kan bli svåra om de inte behandlas. Spontana ledblödningar, svåra magtarmblödningar, kraftiga menstruationsblödningar förekommer.

Sverige är ett föregångsland avseende profylaxbehandling (förebyggande behandling) vid såväl hemofili som VWD. Genom ett internationellt samarbete (Von Willebrand Disease Prophylaxis Network) har man visat att profylaxbehandling är effektiv. Med hjälp av en stor databas från ett tiotal länder har vi studerat effekten av profylaxbehandling på olika typer av blödningar. Förebyggande behandling har en dramatisk effekt på ledblödningar samt blödningar från nässlemhinna. Däremot är effekten sämre vid blödningar från magtarmslemhinna och dessa patienten har högre doser och mer frekvent behandling. Behandlingen hade effekt i alla åldersgrupper.

I ett annat arbete har vi studerat hur stor sjukvårdsbörda patienter med VWD genererar jämfört med matchade kontroller. För detta har använts svenska populations- och diagnosregister. Registren innehåller 2790 personer med von Willebrand sjukdom som har fått diagnosen under åren 1987-2009 samt 13 920 köns-och åldersmatchade kontroller. Individer med VWD hade dubbelt så många sjukhusbesök och öppenvårdsbesök jämfört med kontrollerna. Vanligaste blödningsorsaker för sjukhusvård var magtarmblödningar, rikliga menstruationer och näsblödningar. Vi studerade även behovet av sjukhusvård hos 105 patienter med VWD ingående i en internationell studie före och efter insättande av profylaxbehandling. Resultatet visar att antalet vårdepisoder nästan halverades efter insättning av profylax. Sammanfattningsvis har individer med VWD betydligt högre sjukvårdskonsumtion jämfört med kontroller och insättande av profylax kan minska behovet av sjukhusvård pga blödning.

I ett av arbeten studerades ett betydande antal patienter i olika åldrar som har remitterats till blödningsmottagning i Malmö för bedömning pga olika blödningar men utan känd diagnos. Av 185 patienter fick fyra kvinnor diagnos mild Von Willebrand sjukdom och en man diagnostiserades med mild hemofili (blödarsjuka). Screening för dessa sjukdomar är viktig. 179 patienter med majoritet av kvinnor analyserades avseende relationen mellan trombingenerering (en laboratoriemetod som inte ingår i standardanalyser) och blödningsscore (ett formulär som poängsätter blödningssymtom). Vi fann en svag men signifikant korrelation hos kvinnor. Studien visade att VWD är förhållandevis förekommande i en grupp som är undersökt vid lokalt sjukhus utan att man kunnat ställa diagnos.

I vårt sista arbete studeras sjuklighet och överlevnad i hjärtkärlsjukdomar hos personer med VWD med hjälp av svenska nationella register inom ramen för ett stort projekt (Congenital Bleeding Disorders in Sweden, CBDS). 2 735 individer med von Willebrand sjukdom med 5 kön-och åldermatchade kontroller (n = 12 948) ingår i analysen. Median för födelseår var 1961, 1874 (68 %) var kvinnor och 861(32 %) var män. Sjukhusvård för hjärtkärlsjukdom var ungefär dubbelt så vanlig bland VWD men dödligheten pga hjärtkärlsjukdom var 60 % lägre hos personer med VWD.

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References

1. Ingram GI. The history of haemophilia. *Haemophilia : the official journal of the World Federation of Hemophilia* 1997; **3, (Suppl. 1)**: 5-15.
2. Titford M. Rudolf Virchow: Cellular Pathologist. *Laboratory Medicine* 2010; **41**(5): 311-2.
3. Douglas S. Historical review. *British journal of haematology* 1999; **107**: 22-32.
4. Stormorken H. The discovery of factor V: a tricky clotting factor. *Journal of thrombosis and haemostasis : JTH* 2003; **1**(2): 206-13.
5. Dam H. The antihaemorrhagic vitamin of the chick. *Biochem J* 1935; **29**(6): 1273-85.
6. Jorpes JE. Heparin, its chemistry, pharmacology and clinical use. *Am J Med* 1962; **33**: 692-702.
7. Blombäck B. A journey with bleeding time factor. *Compr Biochem* 2007; **45**: 209-55.
8. Nilsson IM, Blomback M, Von Francken I. On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time. *Acta medica Scandinavica* 1957; **159**(1): 35-57.
9. Nilsson IM, Berntorp E, Lofqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *Journal of internal medicine* 1992; **232**(1): 25-32.
10. von Willebrand EA. Hereditär pseudohemofili. *Fin Läkaresällsk Handl* 1926; (68): 87-112.
11. Jurgens R, Forsius H. [On Willebrand-Jurgens constitutional thrombopathy in the Aland Islands]. *Schweiz Med Wochenschr* 1951; **81**(50): 1248-53.
12. Sadler JE, Mannucci PM, Berntorp E, et al. Impact, diagnosis and treatment of von Willebrand disease. *Thrombosis and haemostasis* 2000; **84**(2): 160-74.
13. 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. *Seminars in thrombosis and hemostasis* 2011; **37**(5): 495-502.
14. Branchford BDP, J. Making a diagnosis of von Willebrand disease. *Hematology Am Soc Hematol Educ Program* 2012; (2012): 161-7.

15. Federici AB, Mazurier C, Berntorp E, 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.
16. Lethagen S. Desmopressin--a haemostatic drug: state-of-the-art review. *Eur J Anaesthesiol Suppl* 1997; **14**: 1-9.
17. Lethagen S, Harris AS, Sjorin E, Nilsson IM. Intranasal and intravenous administration of desmopressin: effect on F VIII/vWF, pharmacokinetics and reproducibility. *Thrombosis and haemostasis* 1987; **58**(4): 1033-6.
18. Berntorp E. Haemate P/Humate-P: a systematic review. *Thrombosis research* 2009; **124 Suppl 1**: S11-4.
19. Castaman G, Goodeve A, Eikenboom J, European Group on von Willebrand D. Principles of care for the diagnosis and treatment of von Willebrand disease. *Haematologica* 2013; **98**(5): 667-74.
20. Mannucci PM, Kempton C, Millar C, et al. Pharmacokinetics and safety of a novel recombinant human von Willebrand factor manufactured with a plasma-free method: a prospective clinical trial. *Blood* 2013; **122**(5): 648-57.
21. Bray GL, Gomperts ED, Courter S, et al. A multicenter study of recombinant factor VIII (recombinate): safety, efficacy, and inhibitor risk in previously untreated patients with hemophilia A. The Recombinate Study Group. *Blood* 1994; **83**(9): 2428-35.
22. Lusher JM, Arkin S, Abildgaard CF, Schwartz RS. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A. Safety, efficacy, and development of inhibitors. Kogenate Previously Untreated Patient Study Group. *The New England journal of medicine* 1993; **328**(7): 453-9.
23. Berntorp E, Petrini P. Long-term prophylaxis in von Willebrand disease. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis* 2005; **16 Suppl 1**: S23-6.
24. Borel-Derlon A, Federici AB, Roussel-Robert V, et al. Treatment of severe von Willebrand disease with a high-purity von Willebrand factor concentrate (Wilfactin): a prospective study of 50 patients. *Journal of thrombosis and haemostasis : JTH* 2007; **5**(6): 1115-24.
25. Berntorp E, Windyga J, European Wilate Study G. Treatment and prevention of acute bleedings in von Willebrand disease--efficacy and safety of Wilate, a new generation von Willebrand factor/factor VIII concentrate. *Haemophilia : the official journal of the World Federation of Hemophilia* 2009; **15**(1): 122-30.
26. Halimeh S, Krumpel A, Rott H, et al. Long-term secondary prophylaxis in children, adolescents and young adults with von Willebrand disease. Results of a cohort study. *Thrombosis and haemostasis* 2011; **105**(4): 597-604.
27. Berntorp E, Astermark J, Baghaei F, et al. Treatment of haemophilia A and B and von Willebrand's disease: summary and conclusions of a systematic review as part of a Swedish health-technology assessment. *Haemophilia : the official journal of the World Federation of Hemophilia* 2012; **18**(2): 158-65.

28. Berntorp E, Abshire T, von Willebrand Disease Prophylaxis Network Steering C. The von Willebrand disease prophylaxis network: exploring a treatment concept. *Journal of thrombosis and haemostasis : JTH* 2006; **4**(11): 2511-2.
29. Abshire TC, Federici AB, Alvarez MT, et al. Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN). *Haemophilia : the official journal of the World Federation of Hemophilia* 2013; **19**(1): 76-81.
30. Abshire T, Cox-Gill J, Kempton CL, et al. Prophylaxis escalation in severe von Willebrand disease: a prospective study from the von Willebrand Disease Prophylaxis Network. *Journal of thrombosis and haemostasis : JTH* 2015; **13**(9): 1585-9.
31. Makris M, Federici AB, Mannucci PM, et al. The natural history of occult or angiodysplastic gastrointestinal bleeding in von Willebrand disease. *Haemophilia : the official journal of the World Federation of Hemophilia* 2014.
32. Lovdahl S, Henriksson KM, Baghaei F, et al. Incidence, mortality rates and causes of deaths in haemophilia patients in Sweden. *Haemophilia : the official journal of the World Federation of Hemophilia* 2013; **19**(3): 362-9.
33. Hemker HC, Willems GM, Beguin S. A computer assisted method to obtain the prothrombin activation velocity in whole plasma independent of thrombin decay processes. *Thrombosis and haemostasis* 1986; **56**(1): 9-17.
34. Hemker HC, Wielders S, Kessels H, Beguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thrombosis and haemostasis* 1993; **70**(4): 617-24.
35. Hemker HC, Beguin S. The generation of thrombin in whole plasma. Biochemical possibilities and physiological realities. *Verh K Acad Geneeskd Belg* 1985; **47**(5): 321-39.
36. Varadi K, Negrier C, Berntorp E, et al. Monitoring the bioavailability of FEIBA with a thrombin generation assay. *Journal of thrombosis and haemostasis : JTH* 2003; **1**(11): 2374-80.
37. Salvagno GL, Astermark J, Lippi G, et al. Thrombin generation assay: a useful routine check-up tool in the management of patients with haemophilia? *Haemophilia : the official journal of the World Federation of Hemophilia* 2009; **15**(1): 290-6.
38. Berntorp E, Salvagno GL. Standardization and clinical utility of thrombin-generation assays. *Seminars in thrombosis and hemostasis* 2008; **34**(7): 670-82.
39. Tosetto A, Rodeghiero F, Castaman G, et al. A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). *Journal of thrombosis and haemostasis : JTH* 2006; **4**(4): 766-73.
40. Bowman M, Riddel J, Rand ML, Tosetto A, Silva M, James PD. Evaluation of the diagnostic utility for von Willebrand disease of a pediatric bleeding questionnaire. *Journal of thrombosis and haemostasis : JTH* 2009; **7**(8): 1418-21.

Paper I

Changes in bleeding patterns in von Willebrand disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network

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Clinically, the leading symptom in von Willebrand disease (VWD) is bleeding, chiefly of mucosal type, for example, epistaxis, gingival, or gastrointestinal bleeding, and menorrhagia. In severe forms of VWD with secondary deficiency of factor VIII, spontaneous joint bleeding, resembling that observed in severe haemophilia A, may also be observed. The bleeding patterns of VWD can affect quality of life, and may be life-threatening. The von Willebrand Disease Prophylaxis Network is an international study group formed with the goal of investigating the role of prophylaxis in clinically severe VWD. The objective of the present study is to investigate the response to prophylaxis focusing primarily on epistaxis, joint bleeding, gastrointestinal bleeding, and heavy bleeding associated with menses. Data from 105 subjects, 10 enrolled in a prospective study and 95 in a retrospective study between 2008 and 2013, were available for analysis. The median annualized rate reductions in bleeding were significant for epistaxis ($P<0.0001$), gastrointestinal bleeding ($P=0.0003$), joint bleeding ($P<0.0001$), and menorrhagia ($P=0.008$). Doses on a group level were approximately the same prior to and during prophylaxis, but more patients with gastrointestinal bleeding had prophylaxis three or more times per week as well as higher dosages. Our study, which primarily used retrospective data, indicates that prospective studies are needed to better delineate the doses and dose intervals that should be used for prophylactic treatment of VWD. *Blood Coagul Fibrinolysis* **26:383–388**

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Introduction

The rationale for initiating long-term prophylaxis in von Willebrand disease (VWD) is two-fold. First, joint hemorrhages with development of arthropathy can occur, particularly in type 3 VWD, and frequent mucous membrane bleeds, gastrointestinal bleeding, and menorrhagia can jeopardize quality of life or even be life-threatening. Second, it has been successfully used in severe hemophilia, showing that it is feasible to implement early in life in a home setting, and that prevention of joint disease and other bleeds is possible [1,2]. It is logical to consider application of the experience learned in hemophilia to VWD. Data to support long-term prophylaxis in VWD come from a few, less well-controlled cohort studies using retrospective designs [3,4].

The von Willebrand Disease Prophylaxis Network (VWD PN) is an international study group formed with the goal of evaluating prophylactic regimens for people with severe VWD. The VWD International Prophylaxis

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study (VIP) is an initiative of the VWD PN comprising a group of retrospective and prospective studies. In a recently published VIP retrospective study report, it was concluded that prophylactic treatment of VWD is efficacious [5]. This study was the largest so far comprising data from 59 patients enrolled in 20 centers from 10 countries. The number of patients with type 3 VWD was large ($n = 34$; 57%) compared with other reports. The results showed significant overall reduction of hemorrhages for each indication examined.

In the above-referenced reports and in other studies of replacement therapy in VWD [6], it is clear that prophylaxis is efficacious. However, the response of specific bleeding types has not been comprehensively studied. Combining data from the prospective and retrospective VIP studies provides a substantial number of bleeding events occurring prior to and during prophylaxis. The objective of the present study is to investigate the response to prophylaxis focusing primarily on epistaxis, joint bleeding, gastrointestinal bleeding, and heavy bleeding associated with menses (menorrhagia).

Methods

Subject population

Subjects enrolled in the prospective or retrospective VIP studies were included. Prophylaxis was defined as receiving factor infusions at least once per week to prevent or decrease the severity of bleeding with the intention of maintaining this regimen for 45 or more weeks per year, or receiving factor infusions on a regular basis during menstrual periods to prevent or decrease the severity of menorrhagia.

The prospective study evaluated the effect of prophylaxis in a treatment escalation study design. Subjects were required to meet criteria specific to type of VWD and demonstrate patterns of bleeding specific to one of the indications under study.

The following VWD-type criteria were used for the prospective study:

(1) Type 1: eligible for participation if
   (a) 20% or lesser von Willebrand factor (VWF):Ristocetin cofactor activity (VWF:RCo) and/or 20% or lesser factor VIII (FVIII); and
   (b) Desmopressin-nonresponsive, defined as occurrence of bleeding episodes not responding satisfactorily to desmopressin, or deemed nonresponsive *a priori* by the investigator.
(2) Type 2: eligible for participation if desmopressin-nonresponsive, defined as occurrence of bleeding episodes not responding satisfactorily to desmopressin, or deemed nonresponsive *a priori* by the investigator (type 2B).
(3) Type 3: all were eligible.

Criteria for inclusion by bleeding indication were as follows:

(1) Joint bleeding – documentation of at least two spontaneous bleeding episodes in the same joint in the 6 months prior to enrolment; or three or more apparently spontaneous bleeding episodes in different joints in the 6 months prior to enrolment.
(2) Gastrointestinal bleeding – history of two or more severe gastrointestinal bleeding episodes associated with either a drop in hemoglobin of 2 g/dl or higher or required red blood cell transfusion or treatment with VWD concentrate.
(3) Excessive bleeding during menstruation – a diagnosis of menorrhagia, prospectively completed Pictorial Blood Assessment Chart score more than 185, and normal cervical cytology.
(4) Epistaxis – three or more bleeding episodes during a 6-month period that required treatment with VWD concentrates or red blood cell transfusions.

Subjects were excluded from the prospective study if they had acquired von Willebrand syndrome, a history of inhibitors, were already on prophylaxis, or had a history of noncompliance.

Inclusion criteria for the retrospective study included:

(1) Currently on a prophylactic regimen for VWD that was initiated at least 6 months prior to enrolment
   -OR-
(2) A history of use of prophylaxis, for a period of at least 6 months, which was discontinued because it was no longer required.
(3) Availability of records to document, or reliably estimate, the type and frequency of bleeding episodes prior to, and after, the initiation of prophylaxis.

For subjects enrolled in the retrospective study, the primary indication for prophylaxis was defined as the bleeding symptom accounting for half or more of his bleeding episodes. Subjects were excluded from the retrospective if they had a history of noncompliance.

Data were collected between 2008 and 2013. The human-subjects committees of collaborating institutions approved the VIP study in a compliance with the guidelines of the Declaration of Helsinki. The VIP study is registered at www.ClinicalTrials.gov (NCT00557908).

Clinical data collection

Sources of information included center records, diaries, and bleeding logs. Variables included subject demographics, VWD type, location, and frequency of bleeding prior to, and after, the initiation of prophylaxis. Bleeding log data including site, treatment type, and dose were available for a total of 1116 bleeding episodes collected in the retrospective study.

The retrospective study period was defined as beginning at least 12 months prior to initiation of the first prophylactic regimen and ending at enrolment or at the conclusion of prophylaxis. For those enrolled in the prospective study, it was 12 months prior to initiation of the first prophylactic regimen and 1 year following the onset of prophylaxis.

Statistical methods

Annualized bleeding rates were calculated for the periods prior to prophylaxis and during prophylaxis. Rates were calculated for the primary indication by multiplying the total annual number of bleeding episodes by the proportion that occurred at the primary indication site. Medians and interquartile ranges are used to describe bleeding rates. In addition, a 'paired' approach was used to calculate the percentage change in number of bleeding episodes within individuals by subtracting the number of bleeds that occurred before prophylaxis from the number of bleeds during prophylaxis, then dividing by the number of bleeds that occurred before prophylaxis. A paired Wilcoxon signed-rank test was used to compare the bleeding rate by primary indication. A general linear model was used to compare percentage change in bleeding rate by indication while controlling for VWD diagnosis.

Results

One hundred and five subjects, 10 enrolled in the prospective study and 95 in the retrospective study, were available for analysis. The mean age at start of prophylaxis was 26 years (range 1–81). There were similar proportions of adults ($n = 56$; 53.3%, mean age 42.7, range 18.5–80.6) and children ($n = 49$; 46.7%, mean age 7.8, range 1.0–17.9) in our study. A child was defined as any patient below 18 years at onset of prophylaxis, and an adult was any patient 18 years or older.

A description of the cohort can be found in Table 1. Among adults, there were more women participating than men, and among children, there were more boys than girls. The majority of subjects were of European descent, with smaller proportions of participants of African descent, Hispanic, Asian, and other races or ethnicities. The most common VWD type was type 3 (51.4%), followed by type 2A (23.8%), and type 1 (12.4%).

Table 1 Sex, race/ethnicity, and VWD type by age group ($n = 105$)

	Adult	Child	Overall
Sex			
Female	34 (32.4%)	18 (17.1%)	52 (49.5%)
Male	22 (20.9%)	31 (29.5%)	53 (50.5%)
Race/ethnicity			
Asian	3 (2.9%)	3 (2.9%)	6 (5.7%)
Black	0	4 (3.8%)	4 (3.8%)
Hispanic	1 (0.95%)	12 (11.4%)	13 (12.4%)
White	52 (49.5%)	27 (25.7%)	79 (75.2%)
Other	0	3 (2.9%)	3 (2.9%)
VWD type			
Type 1	8 (7.6%)	5 (4.8%)	13 (12.4%)
Type 2A	16 (15.2%)	9 (8.6%)	25 (23.8%)
Type 2B	7 (6.7%)	2 (1.9%)	9 (8.6%)
Type 2M	1 (0.95%)	2 (1.9%)	3 (2.9%)
Type 2, Unknown	1 (0.95%)	0	1 (0.95%)
Type 3	23 (21.9%)	31 (29.5%)	54 (51.4%)

VWD, von Willebrand disease.

Gastrointestinal bleeding was the most common reason for initiation of prophylaxis among adults (34%), followed by joint bleeding (20.8%). For children, the most frequent sites were epistaxis (43.2%) and joint bleeding (31.8%).

Change in annualized bleeding rates by indication

Reductions in annualized bleeding rates before and during prophylaxis and percentage change for those with primary bleeding indications of epistaxis, gastrointestinal bleeding, joint bleeding, and menorrhagia ($N = 80$) were calculated (Table 2). The median reductions in bleeding, within individuals, were significant for epistaxis ($P < 0.0001$), gastrointestinal bleeding ($P = 0.0003$), joint bleeding ($P < 0.0001$), and menorrhagia ($P = 0.008$). The results from a model controlling for VWD type showed that overall, indication did not predict the percentage change in bleed rates after onset of prophylaxis ($P = 0.12$). However, the difference in percentage change between gastrointestinal bleeding and menorrhagia was significant ($P = 0.02$) as well as the difference in percentage change between gastrointestinal bleeding and joint bleeding ($P = 0.04$).

Figure 1 displays the contribution of each of the major sites to the annualized bleeding rate prior to and during prophylaxis. Although the overall bleeding rate is much lower during prophylaxis in each VWD type, the contribution of each site to the total does not vary greatly, perhaps with the exception of type 1, compared with that observed in the period before prophylaxis. The greatest decrease in overall bleeding rate occurred among those with type 3 VWD.

Dose by bleeding type prior to and during prophylaxis and average dose by regimen

All data reported in the retrospective study bleeding logs prior to and during prophylaxis were examined (Table 3). Data from the prospective study were not used in this analysis because dose and frequency were specified by the protocol.

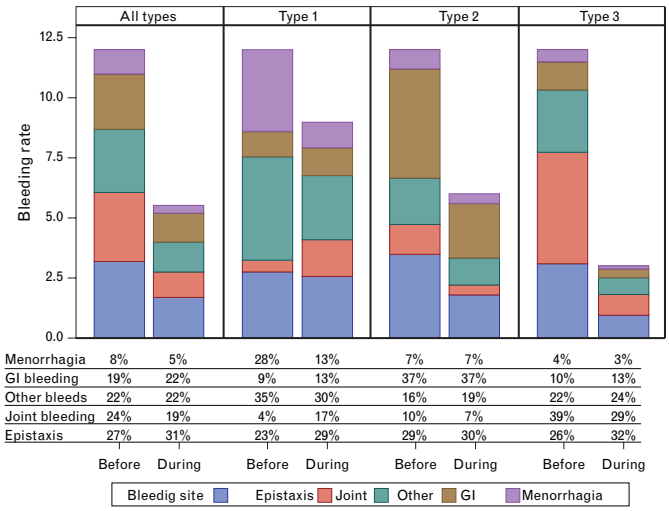
Focusing on the treatment for epistaxis, gastrointestinal bleeding, joint bleeding, and menorrhagia, dose was calculated by averaging across all the doses for the specific bleed type (Table 4). There is not a notable difference in the mean doses given prior to the onset of prophylaxis for the treatment of episodic bleeding events and those given during prophylaxis. Prophylactic regimen was divided into two categories: those receiving prophylaxis less than three times per week/per cycle and those

Table 2 Annualized rate of bleeding episodes prior to and during prophylaxis and median change in annualized rate and percentage change by primary bleeding indication (*N* = 80)

Indication	*N*	Prior to prophylaxis, median (IQR)	During prophylaxis, median (IQR)	Median rate change (IQR)	Median percentage change (IQR)
Epistaxis	28	11.1 (6.0–48.0)	3.8 (0.21–16.8)	−6.1 (−42.0 to −1.5)	−86.7 (−95.5 to −49.8)
Gastrointestinal bleeding	18	9.3 (6.0–21.6)	6.0 (3.6–7.1)	−3.0 (−6.0 to 0.0)	−44.3 (−72.2 to 0)
Joint bleeding	25	11.9 (6.0–18.0)	0.8 (0.0–3.2)	−8.5 (−12.0 to −4.2)	−86.9 (−100.0 to −52.5)
Menorrhagia	9	9.6 (8.4–12.0)	0.0 (0.0–0.4)	−9 (−9.3 to −6.0)	−100.0 (−100.0 to −95.8)

IQR, interquartile range.

Fig. 1



Annualized rates of bleeding for the total study group and by von Willebrand disease (VWD) type are shown prior to and during prophylaxis in the stacked bars. Frequency distributions of bleeding by site prior to and during prophylaxis are shown in the columns that appear under the corresponding stacked bar. The category of 'Other' contains bleeding sites that occurred with low frequency. These include, for example, intracranial haemorrhage, hematomas in soft tissue, oral, dental extraction, scraped knees, ovarian cysts or bleeding. The percentages in each column sum to 100%.

with an infusion frequency of three or more times per week/per cycle. The latter group generally had higher doses than those who received prophylaxis less frequently. This observation was strongest for joint bleeding. Data that would permit calculation of total product usage prior to and during prophylaxis were not collected as part of this study.

Table 3 Distribution of bleeding events prior to and during prophylaxis recorded in logs or diaries by location

Location of Bleeding	Frequency	Percentage
Epistaxis	365	32.7
Gastrointestinal	259	23.2
Intracranial	4	0.4
Joint	257	23.0
Menorrhagia	46	4.1
Muscle	23	2.1
Oral	67	6.0
Soft tissue	67	6.0
Other	28	2.5
Total	1116	100.0

Discussion

It has been shown that long-term prophylaxis with VWF-containing concentrates in cases of VWD is useful and capable of preventing recurrent bleeding and arthropathy, thereby improving quality of life. It is likely that health economic benefits will occur as a result of prophylaxis for VWD.

Severe VWD is a rare disease, and an international collaboration such as the VIP study makes it possible to answer a number of questions and develop guidelines for treatment and follow-up of those most severely

Table 4 Dose (VWF:RCo, U/kg) by bleeding type prior to and during prophylaxis and average dose by regimen

Bleeding type	Prior to prophylaxis for episodic bleeding*	During prophylaxis	
		<3 times per week/cycle	≥3 times per week/cycle
Epistaxis			
N	25	9	12
Mean	44.9	38.8	48.8
STD	16.8	15.5	18.3
Gastrointestinal			
N	13	2	10
Mean	48.2	45.0	49.3
STD	15.0	7.1	11.7
Joint			
N	19	9	4
Mean	45.8	50.8	73.3
STD	16.2	20.8	51.5
Menorrhagia			
N	5	1	2
Mean	51.9	50	39.0
STD	20.1	–	1.4

*Numbers for subjects prior to treatment do not equal the total treated for the indication because of missing dosage data; numbers in dosage categories during prophylaxis may not sum to the total treated for the indication prior to prophylaxis because of cessation of bleeding for that site or dosage missing data. VWF:RCo, von Willebrand factor:Ristocetin cofactor activity. STD, standard deviation.

affected by the disease. Abshire *et al.* [5] showed that prophylactic treatment of VWD is effective in reducing bleeding and the current analysis extends that work to examine more closely the outcomes of specific bleeding types.

Data collected as part of the VIP retrospective and prospective studies were used. There were similar findings among adults and children participating, showing that the need for treatment was present across the age groups. Type 3 was the most common VWD-type in both age groups – approximately half of the total – followed by type 2. The high proportion of patients with type 3 is expected, given that the inclusion criteria required need for treatment with VWF concentrates.

The figure showed that the median annualized bleeding rate for the total study group and for each type was 12 – one bleeding episode per month. This is likely a reflection of the fact that participants in this study, regardless of VWD diagnosis, exhibited a frequency of bleeding that indicated a need for prophylaxis, confirming observations from cohort and registry studies that a subset of people with VWD experiences a significant degree of bleeding-related morbidity. It may also be because of, to some degree, a form of digit preference related to the way data were collected. If a complete log of every bleeding episode for the year prior to prophylaxis was not available, investigators made an assessment of all available documentation to determine 'on average, how many episodes of bleeding occurred each month.'

The most frequent indication for starting prophylaxis for adults was gastrointestinal bleeding and the lowest response in terms of reduction of bleeding events occurred among this group. Gastrointestinal bleeding occurred primarily among those with VWD type 2. This fits well with previous studies reporting angiodysplasia as a cause of gastrointestinal bleeding together with the coagulation defect. Angiodysplasia is reported to be more frequent in VWD compared with that seen in normal controls and especially frequent in type 2 and type 3 VWD wherein the function and level of VWF are more greatly impaired than in type 1. This lends credence to the speculation by Randi *et al.* and Franchini and Mannucci [7,8] that VWF has antiangiogenic properties.

Doses on a group level were approximately the same prior to and during prophylaxis, but more patients with gastrointestinal bleeding had prophylaxis three or more times per week with a higher dosage each time. Clearly, new approaches for treating gastrointestinal bleeding are needed.

Epistaxis was the most common treatment indication for children. The proportional contribution of this indication was similar prior to and during prophylaxis. Treatment doses were also similar prior to and during prophylaxis and higher when patients were receiving three or more infusions per week. Dose escalation and more treatment occasions may be necessary to reach an optimal reduction of episodes of epistaxis.

Joint bleeding was represented equally in adults and children, mostly occurring among those with type 3 VWD. Treatment doses were higher during prophylaxis, particularly so among patients treated three or more times per week. Those with type 3 VWD and joint bleeding had the best response to prophylaxis, possibly because of the higher dosage.

Conclusion

This is by far the largest study presented in which the treatment and replacement effect of VWF-containing concentrates in VWD not responding to desmopressin are described. Institution of regular replacement therapy has a dramatic effect on bleeding frequency but does not change the proportions of type of bleeds that occur to a great extent. There is variation among patients with regard to response to therapy, both among and within subtypes of VWD. Gastrointestinal bleeding stands out, having a lower response to prophylaxis than other bleeding sites. Gastrointestinal bleeds appear to require more frequent prophylactic infusions. It was also noted that when the treatment frequency is escalated for joint bleeding, higher doses were used. Our study, mainly using retrospective data, indicates that prospective studies are needed to better delineate the doses and dose intervals that should be used for prophylactic treatment of VWD. It is likely that more tailored dosing can be used than seen in the present study in which doses were generally similar for all subtypes and indications. This is imperative from a health economic point of view.

Further health economic analyses of cost per avoided bleed are necessary and relevant. It should also be borne in mind that different brands of VWF concentrates may require different dosing schedules, as their content of FVIII and VWF vary.

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Conflicts of interest

There are no conflicts of interest.

References

1 Manco-Johnson MJ, Abshire TC, Shapiro AD, Riske B, Hacker MR, Kilcoyne R, *et al.* Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357**:535–544.
2 Fischer K, Steen Carlsson K, Petrini P, Holmstrom M, Ljung R, van den Berg HM, *et al.* Intermediate-dose versus high-dose prophylaxis for severe hemophilia: comparing outcome and costs since the 1970s. *Blood* 2013; **122**:1129–1136.
3 Halimeh S, Krümpel A, Rott H, Bogdanova N, Budde U, Manner D, *et al.* Long-term secondary prophylaxis in children, adolescents and young adults with von Willebrand disease. *Thromb Haemost* 2011; **104**:984–989.
4 Berntorp E, Astermark J, Donfield SM, Nelson GW, Oldenburg J, Shapiro AD, *et al.* Haemophilia Inhibitor Genetics Study - evaluation of a model for studies of complex diseases using linkage and association methods. *Haemophilia* 2005; **11**:427–429.
5 Abshire TC, Federici AB, Alvarez MT, Bowen J, Carcao MD, Cox Gill J, *et al.* Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN). *Haemophilia* 2013; **19**:76–81.
6 Berntorp E, Astermark J, Baghaei F, Bergqvist D, Holmström M, Ljungberg B, *et al.* Treatment of haemophilia A and B and von Willebrand's disease: summary and conclusions of a systematic review as part of a Swedish health technology assessment. *Haemophilia* 2012; **18**:158–165.
7 Randi AM, Laffan MA, Starke RD. Von Willebrand factor, angiodysplasia and angiogenesis. *Mediterr J Hematol Infect Dis* 2013; **5**:e2013060.
8 Franchini M, Mannucci PM. Von Willebrand disease-associated angiodysplasia: a few answers, still many questions. *Br J Haematol* 2013; **161**:177–182.

Paper II

Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Patients Referred for Bleeding Symptoms of Unknown Cause: Does Evaluation of Thrombin Generation Contribute to Diagnosis?

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Competing interests: The authors have declared that no competing interests exist.

Abstract. ***Introduction.*** **Patients with mild to moderate bleeding symptoms referred for coagulation investigation sometimes never receive a definitive diagnosis. Bleed assessment tools have been developed and validated to assess the severity of symptoms. Global coagulation assays, e.g., the thrombin generation test (thrombogram) have a potential to identify hemostatic defects that are not detected in specific assays.**

Material and Methods: **One hundred and eighty-five patients referred to our centre because of bleeding symptoms were evaluated using the bleeding assessment tool (BAT) described by Tosetto and colleagues in 2006. Blood samples were investigated for thrombin generation (TG) capacity (Technoclone), in platelet poor (PPP) plasma, and specific clotting factors, i.e., von Willebrand factor, factor VIII and IX, as well as INR, APTT, platelet count, and platelet adhesion.**

Results: **Of the 185 patients, five women were diagnosed with mild von Willebrand disease and one male with mild hemophilia A. The remaining 179 subjects (76% females and 24% males with average ages of 33 and 28 years, respectively) were evaluated further. In the total cohort and among women, peak TG, and lag time correlated with bleeding score (p=0.01 and p=0.04, respectively with correlation coefficients). No such correlations were found among males.**

Discussion and Conclusion: **Although our study showed some correlation between TG and bleeding score, results are generally consistent with a previous report which failed to demonstrate the value of TG measurement in a similar setting. In conclusion, the complexity of the mechanisms underlying clinical bleeding complicates the ability to use TG tests as reliable predictors of bleeding. Mild congenital bleeding disorders, especially VWD, should be specifically screened for in patients with mild/moderate symptoms.**

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Introduction. There are many patients with mild to moderate bleeding symptoms in whom no underlying coagulation defect can be found, despite thorough evaluation for clotting factor deficiencies, von Willebrand disease (VWD) and platelet function disorders. Typically, these patients are referred because of frequent bleeding episodes such as nose bleeds, heavy bleeding during menstruation, and bleeding during medical procedures. How to prevent haemorrhages during procedures, and offer targeted pharmacological treatment in the event of symptomatic bleeding in these patients, is currently left to the treating physician's clinical judgement.

To assess the severity of a bleeding disorder, bleeding assessment tools (BATs) have been developed and validated. One BAT was described by Tosetto et al in 2006[1]and was shortly thereafter introduced at our department as a routine tool in the check up of patients referred for bleeding symtoms of unknown cause. It was originally developed to discriminate VWD type 1, but has been used to evaluate other bleeding conditions as well.[2] This BAT has been further modified and was in 2010 published as the ISTH BAT

Thrombin generation (TG) is a key process that determines the extent of a haemostatic plug or a thrombotic process. The inappropriate generation of thrombin may lead to haemorrhagic or thrombotic diseases. More than twenty years ago Hemker and colleagues[3] introduced a method in which the amount of thrombin activity in plasma could be monitored continuously in platelet poor plasma (PPP) or platelet rich plasma (PRP). The method is currently referred to as thrombogram, previously thrombin generation curve, where dynamics of thrombin generation as well as the total amount of thrombin can be evaluated. Thrombin generation has been shown to be increased in patients with thrombotic disorders and decreased in patients with known clotting factor deficiencies such as haemophilia A and B.[4,5]

The aim of the present study was to assess whether thrombin generation parameters reflect the clinical phenotype in patients referred to a specialized centre because of bleeding symptoms, but where no diagnosis of clotting factor deficiency or platelet disorder could be determined.

Materials and Methods.

Subjects: One hundred and eighty-five consecutive patients referred to the Centre for Thrombosis and Haemostasis in Malmö during the years 2008 through 2011 for bleeding symptoms of unknown cause were enrolled. Exclusion criteria were platelet count $<80x10^9$/L, haemoglobin <10g/dL, known liver or kidney disease, use of medication such as aspirin, clopidogrel, non-steroidal anti-inflammatory drugs, antidepressants during the past seven days, anticoagulation therapy, and pregnancy as well as presence of a known clotting factor deficiency or platelet disorder.

Patients were included after signed informed consent and all study-related activities were performed according to the Helsinki declaration. The study was approved by the ethics committee, Lund University.

Bleeding score: All patients were evaluated by the bleeding assessment tool described by Tosetto et al.[1] and Bowman et al.[6] The bleeding score (BS) obtained has been validated by Tosetto in adult patients with von Willebrand disease type 1, and a reference group without apparent bleeding symptoms.

The BS is calculated by summing the severity of all bleeding symptoms reported by a subject, and is graded according to an arbitrary scale. The grading of bleeding symptoms ranges from 0 (absence of abnormal haemorrhage) to 4 (blood transfusion and/or surgical intervention). In the case of no bleeding during two events of tooth extraction, surgery or delivery, one score is deducted from the sum. The BS has been used and validated for bleeding symptoms that were present at diagnosis before use of any prophylaxis. The cut off value described for persons without known bleeding disorders was set to <4.[1,6]

Blood samples: Blood samples were collected by venepuncture using a 21-gauge needle into vacuum tubes (Vacutainer, Becton Dickinson, Plymouth, UK) containing 0.129 M sodium citrate without corn trypsin inhibitor (CTI), yielding a final concentration of 1:9 citrate/blood. The blood was centrifuged for 20 minutes at 1830 g, plasma transferred to a new tube and centrifuged for another 20 minutes, obtaining platelet poor plasma (PPP). The PPP was frozen to minus 80°C and thawed at 37°C just before performing the assay.

Clotting factor and other laboratory analyses: To exclude the possibility of presence of a clotting factor deficiency or platelet disorder, the following routine assays were performed at the Coagulation Laboratory, Department of Clinical Chemistry: international normalized ratio (INR), activated partial thromboplastin time (APTT), platelet count, prothrombin time (PT), von Willebrand factor ristocetin cofactor activity (VWF:RCo)[7] and von Willebrand factor Ag (VWF:Ag).[8] Factor VIII was measured using a chromogenic assay (Coatest SP4, Chromogenix, Mölndal, Sweden) and FIX with a clotting based assay (Stago PTT Reagent). Platelet adhesion was measured using a modified Adeplat S test (Semmelweis, Milan, Italy) which is based on Hellem´s method.[9] The test has a normal range of 16% - 34% with a coefficient of variation (CV) of 7.1% as tested in our laboratory. The intra-individual day-to-day variation has been tested in two healthy volunteers on six different occasions over a period of 4 years. The CV was found to be 8% and 13%, respectively.[10]

Reagents: Human thrombin was obtained from Enzyme Research Lab (Dia-Service, Gothenburg, Sweden) and frozen (freeze dried) human plasma from the local plasma pool. All other reagents and buffer components were commercially available.

Thrombin generation assay: The assay described by Varadi et al[11] was used. Thrombin generation in citrated plasma (40uL) was triggered by a low concentration of TF/PL-complex phospholipid/tissue

factor mix, (Rb containing 3,2 μM PCPS 80/20 (phosphatidyl-choline-phosphatidyl-serine) + 17,9 pM rTF. Technoclone Vienna, Austria), (10uL) in the presence of $CaCl_2$ (15mM) and fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem Ag, Bubendorf, Switzerland). Continuous fluorescence was measured on a FLx800 fluorescence luminescence reader (BioTek Instruments, Inc., Vermont, USA) and converted to fluorogenic units (RFU) by the kinetic program of the fluorometer. The rate of the increase of the RFU (RFU/min) was calculated at all-time points and converted to thrombin concentration by using a reference curve prepared from known concentrations of purified thrombin (Technothrombin TGA, Vienna, Austria.) From the resulting thrombin generation curve lag phase (time to thrombin to burst), peak thrombin and endogenous thrombin potential were calculated.

Statistical analysis: Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 20.0, SPSS; Chicago Illinois USA). A *p*-value <0.05 was considered to indicate statistical significance.

Correlations between variables were calculated with Spearman's correlation. For comparison of the scores in the four age groups, the Kruskal Wallis test was used followed by a post hoc test using the Mann-Whitney U test with Bonferroni correction. Thus, a *p*-value < 0.008 was considered to indicate statistical significance.

Results. Five unrelated women were diagnosed with mild VWD Type 1 and one male with mild haemophilia A. One of these women aged 21 was pregnant and had high levels of peak TG and bleeding score -1 (referred because of bruising). The other four women were 16, 41, 44 and 55 years old. They had bleeding score from the youngest to the oldest, 4, 7, 15 and 2. They all had low levels of peak TG. The diagnosis was set by a specialist in coagulations disorders based on clinical data and low values of VWF:RCo. The male with mild haemophilia A was 19 years old and had Factor VIII:C 0,14 kIU/L, bleeding score 3. Demographics of the 179 remaining subjects were as follows: women n=137 (77%) with a median age of 33 years (range 3-81) and males, n=42 (23%), median age 28 years (range 6-78). One hundred and twenty of the179 patients (64.5%) had a bleeding score <4 and 66 patients (33.5%) had a bleeding score ≥ 4. The main bleeding symptom for referral of each patient is given in **Table 1**. The most frequent symptoms encountered were menorrhagia, abnormal bleeding in connection with surgery, epistaxis and bruises. None of the patients was referred because of abnormal laboratory values. Four subjects were from one and the same family, without any common diagnosis after investigation. Otherwise subjects were not related.

Table 1. Description of primary bleeding type in each patient as cause for referral.

Bleeding	**N (%)**
Menorrhagia	49 (27.4)
Abnormal bleeding in connection with surgery	36 (20.1)
Epitaxis	34 (18.9)
Bruises	28 (15.6)
Postpartum bleeding	6 (3.4)
Extreme bleeding after tooth extraction	5 (2.8)
Hematuria	3 (1.7)
Gastrointestinal bleeding	3 (1.7)
Joint and muscle bleeding	2 (1.1)
Other	13 (7.3)
Total	179 (100)

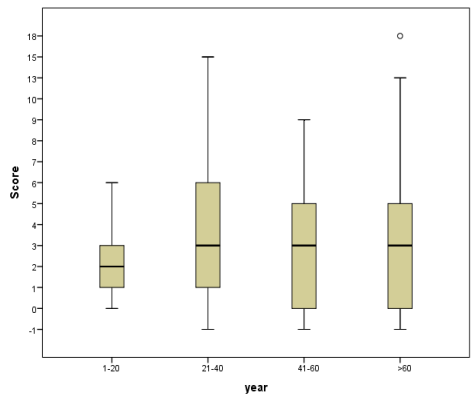

Figure 1. Bleeding score in different age cohorts

The entire cohort (n=179) was divided into four age groups (**Figure 1**). Group 0: 1-20 years (34.4%); Group 1: 21-40 years (33.3%); Group 2: 41-60 years (24.7%) and Group 3: >60 years (7.5%). The median bleeding score was lowest 2, in the youngest group (p=0.003) whereas the other groups had a score close to 3.

In the entire cohort, only peak thrombin significantly correlated with BS although the correlation coefficients were low. Among men, no such correlation was found. In contrast, peak TG and lag phase correlated significantly to bleeding score in women (**Figure 2a** and **2b**) (p=0.01 and p=0.04, r=0.22 and r=0.18, respectively). There was no significant correlation between TG results and platelet adhesion (data not shown).

The most common laboratory deviation was low platelet adhesion value (<16%), as shown in **Table 2**. The 68 patients with low platelet adhesion values did not have higher bleeding scores than patients with normal platelet adhesion (data not shown).

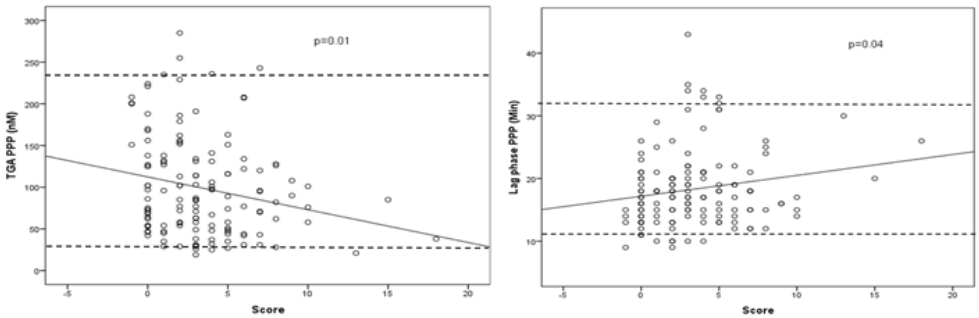

Figure 2a and 2b. Correlation between bleeding score and peak thrombin generation (2a) and lag phase (2b) in female participants (n=137). Dotted lines indicate reference ranges for 11 hemostatically normal women (31-236 nM and 12-33 nM, respectively).

Table 2. Laboratory results

Laboratory parameter	n	Median (range)	Normal range
APTT, s	179	31 (21-49)	26-33
FVIII level ,kIU/L	179	1.06 (0.39-2.14)	0.50-2.00
FIX activity, kIU/L	25	0.94 (0.58-1.64)	0.70-1-30
Platelet count, $x10^9$/L	179	251 (106-449)	165-387 (female) 145-348 (male)
INR	179	1.0 (0.9-1.8)	<1.2
PT, s	179	12.11 (10-21)	10-13
Platelet adhesion, %	179	15.46 (3-30)	16-34
VWF:RCo kIU/L	179	0.90 (0.44-1.5)	0.52-1.58
VWF:Ag, kIU/L	30	0.76 (0.4-1.92)	0.60-2.73

Discussion. Patients referred to our centre because of bleeding symptoms undergo clinical examination and a comprehensive medical history including administration of the Tosetto questionnaire which produces a bleeding score. This has been routine for many years and assures that a comprehensive history is taken, an important part in decision-making with respect to the direction of further investigations. Laboratory samples are routinely collected to exclude the most common coagulation disturbances. In many patients, abnormal values are never found, even in the presence of an obvious history of bleeding, and it is of importance to explore whether these cases have impaired haemostatic capacity as measured by thrombin generation capacity. The aim of our study, therefore, was to investigate whether there was a correlation between bleeding score and TGA parameters in patients at varying ages without known or diagnosed bleeding disorders. Correlations were seen in in the total cohort and in women, i.e., in PPP low values of peak thrombin and, also in women, long lag phase correlated with higher BS. These findings were not seen in males but this could be due to lack of statistical power, as men made up a much smaller proportion of the study cohort. The results indicate that some patients with bleeding tendencies have a lower capacity to produce thrombin upon haemostatic challenges, even when routine coagulation assays are shown to be normal. However the correlation coefficients were usually low which jeopardizes the practical value of the test in the clinical setting. Many, approximately 2/3, of our examined patients had low bleeding scores (<4) and most likely do not require further evaluation or treatment in connection with bleeding events or surgical procedures. Few patients with high BS had comparatively low peak thrombin generation. However, many patients with normal BS also had low thrombin generation capacity and the range of thrombin generation values in the BS group <4 was quite broad. A few patients (n=6) had abnormal values of APTT and/or PT but were not outliers in terms of TGA results. One of the patients had a bleeding score >4. None of the patients were considered to have a bleeding disorder. Of note is that five of the enrolled patients turned out to have VWD type 1 in mild form and one patient mild haemophilia A.

Our study has several strengths.

i) Drawing and handling of blood samples and analysis of TG were done by the same staff during the entire period.

ii) Blood sampling was done in a stable situation when there was no bleeding.
iii) The intra-assay CV in the TGA test is below 10% at our laboratory.
iv) The BAT was administered by a small number of experienced physicians in a consistent manner. In addition, the number of patients evaluated is relatively high and represents a consecutively enrolled population referred to our centre.

Our study also has several limitations.

i) The cross-sectional design where we could not evaluate weather TGA parameters was consistent with time. As our center is a referral center for large parts of the country additional sampling occasions were not feasible to perform.
ii) Mild platelet disorders cannot be ruled out using the platelet retention test which was the only routine test available during the entire study period. The experience from our laboratory is that the specificity and sensitivity of the platelet retention test for bleeding symptoms are rather low in subjects where no indication of plasma coagulation aberration can be revealed (unpublished data from a population-based prevalence study on bleeding disorders in young women).[12] This is substantiated by the finding in the present study that 68 subjects with low platelet adhesion values did not have higher bleeding scores than those with normal platelet adhesion.
iii) Controls not referred for bleeding symptoms were not included.

One previous study[2] compared TGA parameters in patients with mild to moderate bleeding complications with age and gender matched controls, and no significant differences were found. The findings in our study corroborate, to some extent, the previous report but also show a correlation between thrombin generation parameters analysed in PPP and bleeding scores in women. Given that these findings derive from a large group, it is not clear whether they are of significant value in the evaluation of an individual patient. In those with high BS, however, TGA could add value in the routine check up to discriminate between platelet function impairment and impairment of the plasma coagulation system, even if routine assays such as APTT and PT are normal.

The outcome of the study is not only dependent on the TG assay but also on the quality of the BS assessment. Several bleeding assessment tools (BATs) have been published and in 2010 ISTH/SSC proposed a standardized questionnaire developed from previously published tools[13] with the intention to improve the sensitivity of BATs across all age groups. As our study started prior to publication of the ISTH/SSC-BAT we have not been able to include this tool in the calculations.

Conclusions. the complexity of clinical bleeding – an interplay of coagulation and fibrinolysis, platelets and vessel walls, as well as life style – complicates the ability to identify thrombin generation, an apparent key parameter, as having major importance as a predictor of haemorrhagic tendency. Sensitive platelet function tests, usually not available as routine analyses, perhaps might disclose more abnormalities and explain mild bleeding symptoms. The study also show the importance of looking for mild congenital bleeding disorders such as VWD type 1 and mild hemophilia in patients referred with mild/moderate bleeding symptoms.

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References:

1. Tosetto A, Rodeghiero F, Castaman G, Goodeve A, Federici AB, Batlle J, Meyer D, Fressinaud E, Mazurier C, Goudemand J, Eikenboom J, Schneppenheim R, Budde U, Ingerslev J, Vorlova Z, Habart D, Holmberg L, Lethagen S, Pasi J, Hill F, Peake I. A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). Journal of thrombosis and haemostasis : JTH. 2006;4:766-73. http://dx.doi.org/10.1111/j.1538-7836.2006.01847.x PMid:16634745
2. Ay C, Haselbock J, Laczkovics C, Koder S, Pabinger I. Thrombin generation in patients with a bleeding tendency of unknown origin. Annals of hematology. 2011;90:1099-104. http://dx.doi.org/10.1007/s00277-011-1201-8 PMid:21359651
3. Hemker HC, Wielders S, Kessels H, Beguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. Thrombosis and haemostasis. 1993;70:617-24. PMid:7509511
4. Ten Cate H. Thrombin generation in clinical conditions. Thrombosis research. 2012;129:367-70. http://dx.doi.org/10.1016/j.thromres.2011.10.017 PMid:22079443
5. Dargaud Y, Beguin S, Lienhart A, Al Dieri R, Trzeciak C, Bordet JC, Hemker HC, Negrier C. Evaluation of thrombin generating capacity in plasma from patients with haemophilia A and B. Thrombosis and haemostasis. 2005;93:475-80. http://dx.doi.org/10.1160/th04-10-0706
6. Bowman M, Mundell G, Grabell J, Hopman WM, Rapson D, Lillicrap D, James P. Generation and validation of the Condensed MCMDM-1VWD Bleeding Questionnaire for von Willebrand disease. Journal of thrombosis and haemostasis : JTH. 2008;6:2062-6. http://dx.doi.org/10.1111/j.1538-7836.2008.03182.x PMid:18983516
7. Strandberg K, Lethagen S, Andersson K, Carlson M, Hillarp A. Evaluation of a rapid automated assay for analysis of von Willebrand ristocetin cofactor activity. Clin Appl Thromb Hemost. 2006;12:61-7. http://dx.doi.org/10.1177/107602960601200110 PMid:16444436
8. Veyradier A, Fressinaud E, Sigaud M, Wolf M, Meyer D. A new automated method for von Willebrand factor antigen measurement using latex particles. Thrombosis and haemostasis. 1999;81:320-1. PMid:10064018
9. Hellem AJ. Platelet adhesiveness in von Willebrand's disease. A study with a new modification of the glass bead filter method. Scandinavian journal of haematology. 1970;7:374-82. PMid:5312568
10. Lethagen S, Olofsson L, Frick K, Berntorp E, Bjorkman S. Effect

kinetics of desmopressin-induced platelet retention in healthy volunteers treated with aspirin or placebo. Haemophilia : the official journal of the World Federation of Hemophilia. 2000;6:15-20. http://dx.doi.org/10.1046/j.1365-2516.2000.00355.x PMid:10632736

11. Varadi K, Negrier C, Berntorp E, Astermark J, Bordet JC, Morfini M, Linari S, Schwarz HP, Turecek PL. Monitoring the bioavailability of FEIBA with a thrombin generation assay. Journal of thrombosis and haemostasis: JTH. 2003;1:2374-80. http://dx.doi.org/10.1046/j.1538-7836.2003.00450.x PMid:14629472
12. Friberg B, Orno AK, Lindgren A, Lethagen S. Bleeding disorders among young women: a population-based prevalence study. Acta Obstet Gynecol Scand. 2006;85:200-6. http://dx.doi.org/10.1080/00016340500342912 PMid:16532915
13. Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, Neunert C, Lillicrap D, VWF ISj, Perinatal/Pediatric Hemostasis Subcommittees Working G. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. Journal of thrombosis and haemostasis: JTH. 2010;8:2063-5. http://dx.doi.org/10.1111/j.1538-7836.2010.03975.x PMid:20626619

Paper III

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ORIGINAL ARTICLE

WILEY Haemophilia

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

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Introduction: Patients suffering from von Willebrand disease (VWD) have a variety of bleeding symptoms and require both outpatient care for treatment and, in more severe cases, hospitalization.

Aim: To investigate the impact of having VWD on frequency of hospitalization compared to a control group and to evaluate whether regular replacement therapy (prophylaxis) is associated with reduction in the number of hospitalizations.

Methods: Linkage of national population-based registries was used in the Congenital Bleeding Disorders study in Sweden (CBDS). Data were from the von Willebrand Disease Prophylaxis Network (VWD PN).

Results: The national registries contained 2790 subjects with a diagnosis of VWD between 1987 and 2009. A total of 13 920 age- and gender-matched controls were identified. There were 2.0 times (range 1.5-2.5) as many inpatient hospitalizations among subjects with VWD compared to controls. The most common causes of hospitalization were gastrointestinal (GI) bleeding (n = 232 as primary diagnosis), menorrhagia (n = 198) and epistaxis (n = 192). Outpatient visits per year were also twice as common among those with VWD. From the VWD PN, 105 subjects were included (VWD type 3, 52.4%; type2A, 22.9%; type 1, 12.4% and other types, 3.9%). A total of 122 hospitalizations due to bleeding episodes, dominated by GI bleeds, were analysed. Significantly fewer hospitalizations occurred after initiation of prophylaxis (75 prior to and 45 after, $P = .006$).

Conclusion: Our study indicates that subjects with VWD have a considerably higher consumption of healthcare resources compared to controls and that initiation of prophylaxis may reduce the number of hospitalizations due to bleeding.

KEYWORDS
bleeding, hospitalization, Prophylaxis, VWD

1 | INTRODUCTION

Von Willebrand disease (VWD) is the most common congenital bleeding disorder. Occurrence of bleeding events in severe VWD adversely affects short- and long-term quality of life and may be life-threatening. Strategies for the treatment of VWD vary by type and severity of disease and include desmopressin acetate, antifibrinolytics and therapy to replace the missing von Willebrand factor (VWF).[1]

Sweden has a long tradition of national registers containing high-quality data[2] including that kept by the National Board of Health and Welfare and Statistics, Sweden (SCB). National registers offer a view of the general population and enable studies with long-term outcomes that can be examined retrospectively. Both inpatient and outpatient admissions are recorded in the registers. The inpatient register contains an overview of the hospitalization including dates of admission and discharge as well as the primary diagnosis, additional diagnoses and interventions. The outpatient register contains all visits to hospital specialists.

The von Willebrand Disease Prophylaxis Network (VWD PN) is an international study group formed with the goal of evaluating prophylactic regimens for people with clinically severe VWD. Retrospective and prospective studies from the VWD PN showed that prophylactic treatment of severe forms of VWD is efficacious and a significant overall reduction in haemorrhages occurred after initiation of prophylaxis.[3-6]

Hospitalization due to bleeding in patients with VWD is demanding for both the individuals and the healthcare system—being hospitalized is stressful and affects quality of life for patients and is costly for society. Therefore, prophylactic treatment in VWD constitutes an approach which addresses both problems. The objective of this study was to describe hospitalizations in patients in Sweden having VWD without known severity, compared to matched controls, using national registry data and examine the occurrence of hospitalizations due to bleeding before and after initiation of prophylactic treatment using data from the VWD PN in which patients were treated according to specified criteria. Two different datasets were used to address the research questions.

2 | METHODS

2.1 | Registers

The first segment of this investigation is observational and based on population-based registers from the National Board of Health and Welfare (NBHW) and Statistics Sweden and is part of the study congenital bleeding disorders in Sweden (CBDS). The NBHW covers inpatient care visits from 1987 and outpatient hospital specialist care from 2004. Primary and secondary diagnoses were coded using International Classification of Disease (ICD) codes (ICD-9 for 1987-1996, and ICD-10 for 1997 and onwards). Five age- and sex-matched controls were selected for each person with VWD (for more details of these registries, see reference[2]). Matching according to diagnosis/procedure was not performed. All patients with VWD, regardless of the type and severity (the latter cannot be identified in NBHW), were included in the study (N = 2790). A total of 13 920 control subjects were identified.

The following study variables were analysed: annual overall hospital care use including the number of hospital admissions for inpatient care, number of hospital days and number of outpatient visits. Second, pooled data were used for the full study period of CBDS (inpatient care 1987-2009; outpatient care 2004-2009) to report on the same study variables by selected diagnoses related to bleeding episodes known to be most frequent in severe forms of VWD as observed in the VWD PN cohort (epistaxis, menorrhagia, gastrointestinal haemorrhage and joint haemorrhage) and to selected invasive procedures (hysterectomy, gastroscopy, colonoscopy, knee replacement and hip replacement).

The study was approved by the regional ethics committee of Lund, Sweden, on 7 August 2008, registration number 241/2008. The opt-out method was used instead of individual informed consent and advertised through mass media in Sweden. This method requires that an individual who does not want to be enrolled in the study actively contacts the investigator and requests that they be excluded from enrolment. All data were de-identified for study purposes.

2.2 | Subject population in the VWD PN studies

In the second segment of this study, subjects were enrolled in prospective or retrospective studies as detailed in.[3,4,6] The VWD PN studies are designed and supervised by an international network (see Appendix S1). The objectives were to describe demographics of the use of prophylaxis in VWD and to develop evidence-based guidelines for prophylaxis in VWD. Both retrospective and a prospective studies have been performed. Briefly, prophylaxis was defined as receiving factor infusions at least once per week to prevent or decrease the severity of bleeding with the intention of maintaining this regimen for 45 or more weeks per year, or receiving factor infusions on a regular basis during menstrual periods to prevent or decrease the severity of menorrhagia. Only 7 of the VWD PN also occurred in the CBDS cohort, that is 7 of 2790 subjects.

The prospective study evaluated the effect of prophylaxis in a treatment escalation design. Subjects were required to meet criteria specific to type of VWD and demonstrate patterns of bleeding specific to one of the indications under study.

Causes of all hospitalizations were collected prior to and after initiation of prophylactic treatment. Events were divided into two groups—bleeding associated and others.

Studies of the VWD PN are registered at www.ClinicalTrials.gov (NCT00557908). Ethical approval and informed consent were obtained at each centre.

2.3 | Statistical methods

The descriptive analysis was based on counts of hospital admissions and number of inpatient days. These are presented as number

of observed events and number of hospital days, respectively, per 1000 person-years. We used population-averaged negative binomial regression to estimate the incidence rate ratio of hospital admission, number of inpatient days and hospital visits using data from all available years controlling for gender and year of observation. Results are presented for all patients and by decade of birth. For surgical events, we used the primary diagnosis of the registration and the first registered surgical procedure code. For outpatient care, we report the mean number of visits per year per person and the 95% confidence intervals.

The Wilcoxon signed-rank test was used for data from the VWD PN studies. A *P*-value less than .05 was considered statistically significant.

3 | RESULTS

3.1 | Data from national registries

As shown in Figure 1, the number of hospital admissions and inpatient days was consistently higher for people with VWD compared to matched controls. There were, on average, 2.3 times more admissions in the group with VWD (range 1.9-2.7) over the period 1987-2009, and 2.0 (range 1.5-2.5) times as many days of hospitalization. People with VWD were also more likely to have at least one inpatient admission in a year (VWD 17%; control 9%). During the years 2004-2009, people with VWD had considerably greater outpatient visits compared with controls (Figure 2), approximately two visits per year compared to one visit per year. Using regression analyses, people with VWD had twofold increase in the incidence rate of hospital admissions and hospital days (1987-2009); and in outpatient hospital visits (2004-2009); the incidence rate ratio was 2.23 (95% CI 2.07-2.39), 1.97 (1.72-2.25) and 2.31 (1.95-2.73), respectively. The overall increase in use of hospital care was present and significant in all age groups as measured by decade of birth in the longitudinal regression analysis with the eldest age group (born <1960) having the biggest effect (results by decade of birth shown in Table S1).

The most common cause of hospitalization for persons with VWD was gastrointestinal bleeding (n = 232 as the primary diagnosis) among 56 533 person-years between 1987 and 2009. Menorrhagia (n = 198) and epistaxis (n = 192) were equally common as causes of hospitalization. Joint bleeding was rare in patients with VWD, but did occur, and was absent in healthy controls.

The most common reason for outpatient visits was menorrhagia in both patients with VWD and controls; however, the occurrence was much greater in those with VWD (22.51 per 1000 person-years compared to 5.77 per 1000 person-years). Epistaxis was a frequent reason for outpatient visits in people with VWD (20. 43 per 1000 person-years) and substantially less frequent among controls (1.33 per 1000 person-years). Gastrointestinal haemorrhages and joint bleeding were rare causes for ambulatory visits in both groups but more common in the group with VWD (Table 1).

Patients with VWD had a greater need for a variety of interventions, delivered as inpatient and outpatient hospitalizations, than the general population. Table 2 illustrates that people with VWD undergo gastroscopy, colonoscopy, hysterectomy and knee replacement consistently to a larger extent than controls except for hip replacement which was almost as common in both groups.

3.2 | Data from the VWD PN

One hundred and nine subjects were enrolled in the VWD PN study—96 in a retrospective study and 13 (two of whom withdrew after enrolment) in a prospective study. Of these, 105 subjects were included in the current analysis, ten subjects from the prospective and ninety-five from the retrospective study.

The most common type of VWD was type 3 (52%) followed by type 2A (22%), type 1 (12%), type 2B (9%) and other types (4%). The

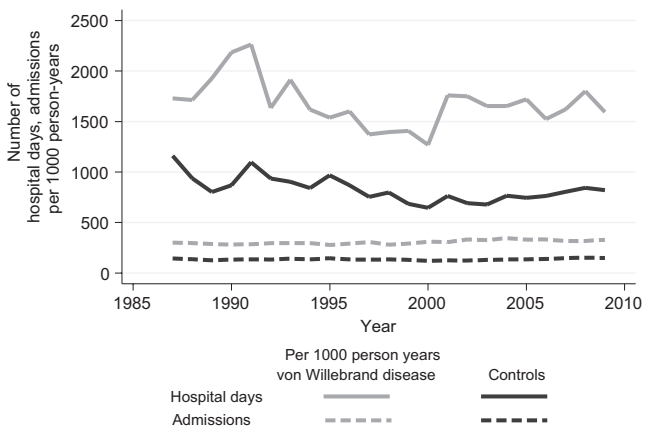

FIGURE 1 Data from national registries. Annual number of hospital days and admissions per 1000 person-years from 1987 to 2009 for people with von Willebrand disease and matched controls from the general population

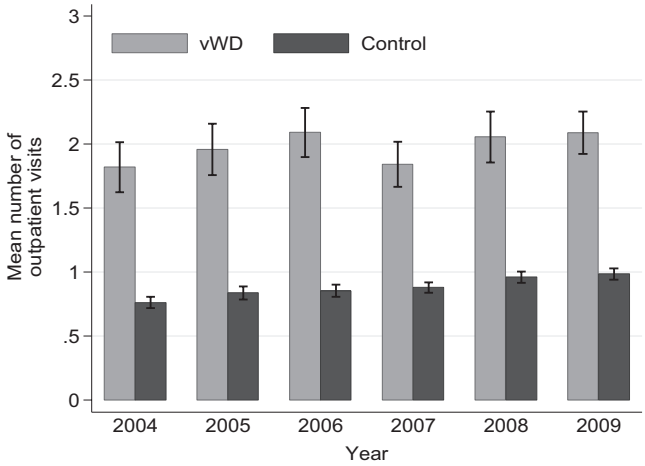

FIGURE 2 Data from national registries. Annual number of outpatient hospital visits (mean, 95% confidence interval) for people with von Willebrand disease and controls from 2004 to 2009

TABLE 1 Inpatient admissions from 1987 to 2009 and outpatient hospital visits from 2004 to 2009 due to four types of haemorrhagic events: epistaxis, menorrhagia, gastrointestinal or joint

	Inpatient admissions 1987-2009					
	von Willebrand disease (56 553 person-years)			Control (267 377 person-years)		
	Primary diagnosis	Number (per 1000 person-years)	Hospitals days (episodes with main diagnosis)	Primary diagnosis	Number (per 1000 person-years)	Hospitals days (episodes with main diagnosis)
Epistaxis[a]	192	3.40	466	33	0.12	66
Menorrhagia[b]	198	3.50	588	101	0.38	282
Gastrointestinal haemorrhages[c]	232	4.10	1034	108	0.40	451
Joint bleeding[d]	2	0.04	1	0	0.00	0
	Outpatient visits 2004-2009					
	von Willebrand disease (14 881 person-years)			**Control (70 034 person-years)**		
	Primary diagnosis	**Number (per 1000 person-years)**		**Primary diagnosis**	**Number (per 1000 person-years)**	
Epistaxis[a]	304	20.43		93	1.33	
Menorrhagia[b]	335	22.51		404	5.77	
Gastrointestinal haemorrhages[c]	78	5.24		73	1.04	
Joint bleeding[d]	6	0.40		2	0.03	

[a](ICD 9) 784H, (ICD 10) R040.
[b](ICD 9) 626 all variants, (ICD 10) N92 all variants.
[c](ICD 9) 569D, 578X, 578A, 578B; (ICD 10) K625, K920, K921, K922.
[d](ICD 9) 719B; (ICD 10) M250.
Data from the national Swedish Patient Register on 323 930 person-years (inpatient care) and 84 915 person-years (outpatient hospital care) for persons with von Willebrand disease and matched controls from the general population.
Admissions and visits were identified using criteria according to the International Classification of Diagnoses version 9 and version 10.

median age for the start of prophylactic treatment was 22 years. The youngest participant was 1 year old, and the oldest was 80 years. Gender distribution was similar.

A total of 122 hospitalizations due to bleeding episodes, 75 prior to and 47 after start of prophylaxis were analysed corresponding to 712 and 448 events per 1000 patient years. We found significantly

TABLE 2 Inpatient admissions between 1997 and 2009 and outpatient hospital visits between 2004 and 2009 due to five invasive procedures: hysterectomy, gastroscopy, colonoscopy, knee replacement and hip replacement

	von Willebrand disease (32 488 person-years)		Control (152 973 person-years)	
	Surgery code 1	Number per 1000 patient years	Surgery code 1	Number per 1000 patient years
Procedure 1997-2009				
Hysterectomy[a]	95	2.92	135	0.88
Gastroscopy[b]	167	5.14	218	1.43
Colonoscopy[c]	71	2.19	60	0.39
Knee replacement[d]	48	1.48	117	0.76
Hip replacement[e]	52	1.60	186	1.22
	von Willebrand disease (14 881 person-years)		Control (70 034 person-years)	
Outpatient visits 2004-2009	Surgery code 1	Number per 1000 patient years	Surgery code 1	Number per 1000 patient years
Hysterectomy[a]	0	0	2	0.03
Gastroscopy[b]	254	17.07	482	6.88
Colonoscopy[c]	132	8.87	364	5.20
Knee replacement[d]	1	0.07	2	0.03
Hip replacement[e)]	0	0	3	0.04

[a]LCD.
[b]UJD.
[c]UJF.
[d]NGB or NGC.
[e]NFB or NFC (ICD-codes for procedures).
Data from the national Swedish Patient Register for 185 461 person-years (inpatient care) and 84 915 person-years (outpatient hospital care) for persons with von Willebrand disease and matched controls from the general population.

fewer hospitalizations after initiation of prophylactic treatment ($P = .006$).

Gastrointestinal haemorrhages were dominated as the cause of admissions both before the start of prophylaxis (49%) and after (53%). This type of bleeding remained the most common reason for hospitalization after initiating prophylaxis. Epistaxis was the cause of 21 admissions prior to and 10 admissions following the start of prophylaxis. Two intracranial haemorrhages occurred in two subjects prior to prophylaxis and none during prophylaxis.

4 | DISCUSSION

In accordance with the management of haemophilia, prophylactic treatment of severe forms of VWD has received greater attention in clinical practice in several countries. Studies have demonstrated significant reductions in the number of bleeding episodes after initiation of prophylaxis. The purpose of our work was to examine the need for hospital admissions, outpatient visits, the bleeding sites and procedures in patients with VWD compared with matched controls using national registries in Sweden. Each person with VWD in the register had five sex- and age-matched controls. We then analysed the influence of prophylaxis on the number of hospitalizations using data from an international study covering the interval 1 year before and 1 year after the onset of prophylaxis. Reports on this topic are lacking as these issues have not been thoroughly studied. We show clear differences in health-care needs between people with VWD and controls. Gastrointestinal haemorrhages dominated the reasons for hospitalization in both segments of our study and underline the need for further research to identify more effective therapies for these patients.[7] Initiation of prophylaxis, however, made a difference in the number of admissions due to gastrointestinal bleeding. People with VWD were more likely to have a hysterectomy, gastroscopy, colonoscopy and surgery for knee replacement.

International cooperation with the purpose of evaluating the efficacy of prophylaxis in VWD has existed for a number of years. With data collected as part of the VWD PN, we were able to demonstrate that prophylaxis significantly influenced the need for hospitalization in patients with VWD. Repeated or severe bleeding, hospitalization, frequent outpatient visits and a variety of interventions affect quality of life and lead to increased costs. Our results indicate that prophylaxis in VWD substantially reduces these problems.

The major weakness of register-based studies is that it is not always possible to obtain information about confounding factors. Further, the national registries, as well as data compiled as part of the VWD PN, do not contain adequate information to exclude

sources of bias or to examine outcomes by types of VWD or type of treatment. The strengths of our study include general population data obtained from national registries over many years as well as matched controls. The VWD PN enrolled the largest cohort using prophylaxis for the management of VWD. Prophylaxis using well-defined regimens, as in this study, reduced the need for in- and outpatient visits which should translate to increased quality of life for patients and their families. As length of hospital stay was not recorded, health economic analyses could not be performed. It could, however, be anticipated that cost is substantially reduced if cost is exempted for the increased consumption of concentrate that is the consequence of regular, long-term prophylaxis similar to the situation in haemophilia.[8,9]

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DISCLOSURES

EH, KSC and AL report no conflict of interests. EB has participated in Advisory boards for CSL Behring and received funding for this research as well as from Bayer. SL is an employee of NovoNordisk. TA CSL Behring and Shire ext Advisory boards.

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REFERENCES

1. Castaman G, Goodeve A, Eikenboom J. Principles of care for the diagnosis and treatment of von Willebrand disease. *Haematologica*. 2013;98:667-674.
2. Lovdahl S, Henriksson KM, Baghaei F, et al. Incidence, mortality rates and causes of deaths in haemophilia patients in Sweden. *Haemophilia*. 2013;19:362-369.
3. Abshire T, Cox-Gill J, Kempton CL, et al. Prophylaxis escalation in severe von Willebrand disease: a prospective study from the von Willebrand Disease Prophylaxis Network. *J Thromb Haemost*. 2015;13:1585-1589.
4. Abshire TC, Federici AB, Alvarez MT, et al. Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN). *Haemophilia*. 2013;19:76-81.
5. Berntorp E, Abshire T, von Willebrand C. Disease Prophylaxis Network Steering, The von Willebrand disease prophylaxis network: exploring a treatment concept. *J Thromb Haemost*. 2006;4:2511-2512.
6. Holm E, Abshire TC, Bowen J, et al. Changes in bleeding patterns in von Willebrand disease after institution of long-term replacement therapy: results from the von Willebrand Disease Prophylaxis Network. *Blood Coagul Fibrinolysis*. 2015;26:383-388.
7. Franchini M, Makris M, Santagostino E, Coppola A, Mannucci PM. Non-thrombotic-, non-inhibitor-associated adverse reactions to coagulation factor concentrates for treatment of patients with haemophilia and von Willebrand's disease: a systematic review of prospective studies. *Haemophilia*. 2012;18:e164-e172.
8. Carlsson KS, Astermark J, Donfield S, Berntorp E. Cost and outcome: comparisons of two alternative bypassing agents for persons with haemophilia A complicated by an inhibitor. *Thromb Haemost*. 2008;99:1060-1067.
9. Steen Carlsson K, Höjgård S, Glomstein A, et al. On-demand vs. prophylactic treatment for severe haemophilia in Norway and Sweden: differences in treatment characteristics and outcome. *Haemophilia*. 2003;9:555-566.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Paper IV

Cardiovascular disease-related hospitalization and mortality among persons with von Willebrand disease: a nation-wide register study in Sweden

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Key words: VWD, cardiovascular disease, hospitalization, mortality

Abstract

Introduction: It has been hypothesized that persons with von Willebrand disease (VWD) may be protected against arterial thrombosis despite having atherosclerosis.

Aim: To calculate a nation-wide estimate of the absolute and comparative burden of cardiovascular disease (CVD) hospitalization and mortality among persons with VWD using birthdate and sex-matched comparisons from the general population in Sweden.

Methods: Persons with VWD regardless of the type and severity, diagnosed by a medical doctor, who lived in Sweden for some time during the observation period 1987 through 2008 were included. For each participant with VWD, up to five randomly-selected birthdate- and sex-matched persons from general population were selected as controls.

Results: 2,790 participants with VWD including 888 male and 1,902 female subjects and 13,938 controls were included. Overall, the hazard of CVD-related hospitalization was 1.3-fold (95% CI: 1.1, 1.5) among participants with VWD after adjusting for sex, birthdate, diabetes, and cancer. However, they had a 0.4-fold (95% CI: 0.3, 0.6) hazard of CVD-related mortality compared to general population sample.

Conclusions: In this nation-wide, long-term register study with individually matched controls we have been able to show that persons with VWD have a higher hospitalization rate due to CVD events. However, the mortality rates appear lower than in the control population. The latter finding is consistent with previous studies and indicates a protective effect of the clotting factor deficiency inherited with VWD.

Introduction

von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by a deficiency and/or defect of von Willebrand factor (VWF). The prevalence of VWD is approximately 1%, although estimates are uncertain and the phenotypic prevalence may be less [1,2]. The common symptoms are mucocutaneous bleeding, hematomas and bleeding after surgery and trauma. In more severe cases with pronounced factor VIII (FVIII) deficiency hemophilia-like joint bleeding occurs. Treatment to prevent or stop bleeding has primarily consists of desmopressin, in milder cases, and replacement therapy using von Willebrand factor (VVF) concentrates in more severe forms of VWD [1].

It has been hypothesized that patients with von Willebrand disease may be protected against arterial thrombosis [3]. Conventional risk factors for arterial thrombosis, and hence cardiovascular disease (CVD), include e.g. hypertension, hypercholesterolemia, type 2 diabetes, smoking and hypercoagulation[4] [5-7]. Additional clotting factors with pro-thrombotic effects include fibrinogen, VWF and FVIII. von Willebrand factor is a glycoprotein essential for normal hemostasis as a carrier of factor VIII (FVIII), for mediating platelet adhesion and aggregation, is involved in inflammation, and has a proposed [8,9] link with angiogenesis. Patients with VWD may have reduced cardiovascular mortality due to a hypocoagulation and decreased risk of thrombosis although atherogenesis seems to be in the same order as hemostatically normal people [10-12]. The influence of VWD on cardiovascular events has been studied in a few reports and lower rates of cardiovascular disease in patients with VWD have been described. It should be noted that in these studies no individually matched controls have been included [13]. Sanders et al. [14] presented a cross-sectional study of 635 patients with VWD observed between 2007 and 2009 and compared the prevalence of arterial thrombosis with two reference groups from the general Dutch population. Authors found a decreased prevalence of arterial thrombosis among those with VWD. Using discharge data, Seaman et al. [11] reported a decreased prevalence of CVD among a large cohort of patients with VWD in the USA during a three-year period compared to patients without VWD. Both studies had a short observation time and lacked individually

matched controls. The question therefore remains unanswered as to whether patients with VWD truly are protected against arterial thrombotic events.

In Sweden, all subjects with VWD are diagnosed at one of three hemophilia comprehensive care centers and are registered nationally. The aim of the present study was to generate a nation-wide estimate of the absolute and comparative burden of cardiovascular disease morbidity and mortality among persons with VWD compared to a group of birthdate- and sex-matched individuals from the general population in Sweden.

Methods

Study design, setting, and participants

This retrospective cohort study was part of a larger research project entitled Congenital Bleeding Disorders in Sweden (CBDS). Persons with VWD regardless of the type and severity, diagnosed by a medical doctor, and who lived in Sweden for some time during an observation period between 1987 through 2008 were included. VWD participants were included from two sources: HTCs and national patient register. Of note that the latter group, despite receiving a VWD diagnosis from a medical doctor at a hospital, have not necessarily been referred to an HTC. For each participant with VWD, up to five randomly-selected birthdate- and sex-matched persons were included as comparisons. Comparisons were free from congenital bleeding disorders. Observation began at birth or in January 1987 and ended for each participant upon emigration, death or January 2009.

Registers and data

In this study, we used several national registers available through the National Board of Health and Welfare (NBHW) and Statistics Sweden. The national patient register (NPR) was the main source of data on CVD and bleeding outcomes. The NPR covers inpatient care visits from 1987 and outpatient

hospital visits from 2004. It has been validated for research purposes [15]. Using International Classification of Disease (ICD) codes (ICD-9 for 1987-1996, and ICD-10 for 1997 and onwards), primary and secondary diagnoses (up to 11 codes) are registered in the NPR for each admission. The NPR also includes surgical procedures (with up to seven surgeries for each admission). We also used a cancer register containing data from 1958, a medical birth register, an emigration register, and a cause of death register, as sources of data on comorbidities, start and end of observation, and the cause of death for this study. More details on the registers used in this research have been published elsewhere [16]. Of note is that details about VWD, such as factor levels or subtypes, were not available in these registries.

Outcomes and statistical methods

The primary study outcomes included hospitalization and mortality due to selected cardiovascular diseases (CVD). We defined CVD as any of the following diagnoses: coronary heart disease (CHD), pulmonary embolism, stroke, and deep venous thrombosis (DVT). In addition, we estimated incidence of diabetes as a potential underlying cause of CVD. For evaluating incidence and hazard rates of CVD hospitalizations we limited the assessment to those who were born before 1969 and thus aged 40 years or older by the end of observation. The incidence of study outcomes was estimated per 1,000 person years. We used Cox regression to estimate the hazard rate ratio (HR) of hospitalization for CVD. Repeated hospitalizations were ignored. All estimates were adjusted for birthdate, sex, diabetes, and cancer. Adjustment for the latter was done as it is known that malignancies may cause bleeding as well as thromboembolic disease. We only adjusted for diabetes and cancer diagnoses which were registered prior to participant's first registered CVD hospitalization. In view of the lack of information on the type and severity of VWD in this study, we used inpatient and outpatient hospital admissions to detect participants with a phenotypic bleeding diagnosis at the hospitalization event. We used this variable to categorize participants with VWD based on their bleeding tendency and to stratify the analysis accordingly. For analysing the second study outcome, CVD mortality, we included the entire cohort of subjects with VWD born between 1895-2008. Using data from cause of death register, we estimated

hazard ratios for both all-cause and CVD mortality. A list of all ICD codes used for extracting data on bleeding diagnosis, cancer diagnosis and CVD hospitalizations is presented in Appendix 1.

Ethical considerations

The CBDS register project received approval from the regional ethics committee of Lund, Sweden (registration number 241/2008). Individual informed consent was not required and, instead, the opt-out method was used. In this method, following public announcements through mass media in Sweden, individuals who do not wish to be enrolled in the study may contact the investigator and request to be excluded. Authors received de-identified data for analysis.

Results

Overall, this study included 2,790 participants with VWD including 888 male and 1,902 female individuals living in Sweden (Table 1) and a comparison group of 13,938 participants individually matched for birthdate and sex to those with VWD. Death and emigration were the only reasons for the loss of follow-up. During 22 years of observation 1987 through 2008, a total of 302 (10.8%) and 1482 (10.6%) deaths (due any reasons) were observed among those with VWD and the comparison group, respectively. Persons with VWD were less likely to emigrate compared to the sample from the general population (1.5% vs. 7.1%). In assessing the CVD-related hospitalizations, only 2,735 participants with VWD and 12,948 comparisons contributed with person-time of observation and remained in the analysis. This analysis accrued 304,712 person-years of observation: 53,722 for participants with VWD and 250,990 for individuals in the comparison group.

Age at first hospitalization for CVD

Figure 1 presents age at first hospitalization for CVD among study participants. Hospitalizations began around the age of 40 and more rapidly increased among male participants. The Kaplan-Meier curves indicated that both men (panel a) and women (panel b) with VWD were hospitalized for CVD earlier

than their counterparts from the general population. However, the hospitalization gap was, in numeric terms, larger among male participants. Fifty percent of male and female participants with VWD were hospitalized by the age of 79 (86 for comparisons) and 85 (90) years, respectively.

Overall hazard ratios of hospitalization for CVD

The hazard of CVD-related hospitalization was 1.6-fold (95% CI: 1.3, 2.1) among participants with VWD with a bleeding diagnosis at hospitalization (Table 2). This finding was consistent in both sex groups, with males and females having 1.9-fold and 1.6-fold hazard ratios for hospitalization, respectively. However, among those without a bleeding diagnosis (as registered in the NPR), the hazards of CVD-related hospitalizations were not statistically significantly different compared to controls from the general population.

Cause specific incidence rates and hazard ratios of hospitalization for CVD

Table 3 presents the overall and sex-stratified incidence rates (per 1000 person-years) and hazard ratios of hospitalization for CVD among study participants born between 1895-1968. Overall, CHD and stroke were the most common CVD-related causes for hospitalization among both participants with VWD and comparisons. Our results indicated an overall 1.3-fold (95% CI: 1.1, 1.5) hazard of CVD among persons with VWD than comparisons, adjusting for birthdate, sex, diabetes, and cancer. Among men, those with VWD had a borderline statistically significantly different hazard of hospitalization for CHD than comparisons. Men with VWD also had a statistically significant 3.5-fold higher hazard of DVT-related hospitalization than comparisons. Among women, however, only the hazard of CHD-related hospitalizations was statistically significant greater among those with VWD compared to comparisons. Men and women with VWD had an overall 1.4-fold (95% CI: 1.0, 1.9) and 1.3-fold (95% CI: 1.0, 1.5) hazard of hospitalization than comparisons, respectively.

In the stratified analysis based on the history of bleeding among participants with VWD, we investigated any differences between subgroups of VWD with higher and lower bleeding tendency (Appendix 2-4). The hazard of CHD-related hospitalization was consistently 1.4-fold among those

with VWD than comparisons in both sex groups. However, persons with VWD had a 1.9-fold hazard of hospitalization due to DVT (95% CI: 1.1, 3.5). Stroke-related hospitalizations did not differ significantly between those with VWD and comparisons in any of the sex groups and the overall sample.

CVD-related mortality

Participants with VWD born between 1895-2008 had a similar hazard of all-cause mortality to the comparison group after adjusting for birthdate and sex (Figure 2). Overall, however, they had a 0.4-fold (95% CI: 0.3, 0.6) hazard of CVD-related mortality compared to sample from the general population. This result was consistent in both male and female groups. Thus, despite having higher hospitalization rates, persons with VWD seemed to have a lower mortality caused by CVD.

Discussion

This study investigated the CVD-related mortality and hospitalization among persons with VWD compared to a reference population. The results indicated a higher burden of CVD morbidity in VWD, especially among men. The CVD mortality, on the other hand, was lower among persons with VWD than a sample from the general population. To the authors' knowledge, this study, for the first time, has quantified the absolute and comparative CVD morbidity among persons with VWD. National coverage, and population-based registries as the source of outcome data with over 20 years of observation were among the unique features and strengths of this study. Five gender- and age-matched controls per study subject further improve the robustness of the investigation.

Sweden has a long tradition of maintaining a variety of registers with complete data for many diagnoses. In our study, the additional CVD morbidity among participants with VWD can hypothetically be explained by the ease of access to a high level of healthcare resources. In general, we did not find that a VWD diagnosis protects against CVD as measured by number of

hospitalizations. If anything, the opposite was observed, but the lower mortality rate may indicate a more benign disease in VWD and/or prompt detection and therefore better care from an early stage of the disorder. This would be in line with other studies showing a similar extent of atherosclerosis measured as intima thickness [11,17] among patients with congenital bleeding disorders. In the large cross-sectional WiN study, Sanders and colleagues studied 635 adult patients aged 16-85 years with VWF levels at or below 30 %, and compared the prevalence of arterial thrombosis to two reference groups from the general Dutch population adjusted for age and sex. Twenty-nine arterial thrombotic events occurred. The prevalence of all arterial thrombotic events combined (acute myocardial infarction, ischemic stroke and coronary heart disease) was 39% and 63% lower than in the reference populations. Seaman and colleagues [18] investigated cross-sectional discharge data for 7,556 patients with VWD over a three-year period compared with discharge data from 19,918,970 individuals without VWD. Those with VWD were significantly younger (49. 7 vs. 57.3 years) and differed in distribution of genders (24. 6 vs. 40.4% males). Prevalence of CVD was 15% and 26%, respectively. Both of these large studies came to the same conclusion as our study but were cross-sectional, had shorter or no follow up and did not enrol individually matched controls. The relative importance of VWF and FVIII cannot be elucidated by our data. The higher hospitalization rates may seem paradoxical but also fit with the previous observation of unchanged development of atherosclerosis in people with clotting factor deficiencies and may be explained by an increased alertness among health care providers, as many persons with VWD are well connected to hemophilia comprehensive care centers.

We found higher rates of diabetes and cancer among males with VWD in our study. One hypothesis related to excess diabetes is that persons with VWD perform less physical activity due to a higher bleeding risk but, if that was the case, it is difficult to explain why it is not different among women with VWD compared to controls as well. Cancer is likely detected earlier among patients with VWD due to the higher occurrence of bleeding caused by the malignancy. We think that our study highlights the fact that CVD remains an important source of morbidity and mortality among patients

with VWD and we must be vigilant with respect to prevention, treatment and follow up. Prospective studies are needed for further evaluation and preparation of specific guidelines for the care of these patients.

Limitations

We did not have access to risk factors for CVD such as smoking, obesity and family history so we could not adjust for them. Another weakness was that we did not have the details of the subtypes of VWD. We know from current hemophilia center data, however, that approximately 75% of patients with VWD have a mild type 1 form of the disease. This figure should reflect the composition of patients included in the national long-term registries as well. We attempted to address the lack of data on VWD subtype and VW/FVIII levels in the hospitalization data by stratifying those having a bleeding diagnosis as sign of a more severe bleeding phenotype.

Conclusions

In this nation-wide, long-term register study with individually matched controls we have been able to show that persons with VWD have a higher hospitalization rate due to CVD events. However, the mortality rates appear lower than in the control population. The latter finding is consistent with previous studies and indicates a protective effect of the clotting factor deficiency inherited with VWD.

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Disclosures

None of the authors report any conflict of interests.

References

1. Castaman G, Goodeve A, Eikenboom J, European Group on von Willebrand D. Principles of care for the diagnosis and treatment of von Willebrand disease. *Haematologica* 2013; **98**(5): 667-74.

2. Leebeek FW, Eikenboom JC. Von Willebrand's Disease. *The New England journal of medicine* 2016; **375**(21): 2067-80.

3. Shahidi M. Thrombosis and von Willebrand Factor. *Adv Exp Med Biol* 2017; **906**: 285-306.

4. Blann AD, McCollum CN, Lip GY. Relationship between plasma markers of endothelial cell integrity and the Framingham cardiovascular disease risk-factor scores in apparently healthy individuals. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis* 2002; **13**(6): 513-8.

5. Holme PA, Combescure C, Tait RC, et al. Hypertension, haematuria and renal functioning in haemophilia - a cross-sectional study in Europe. *Haemophilia : the official journal of the World Federation of Hemophilia* 2016; **22**(2): 248-55.

6. Meade TW, Cooper JA, Stirling Y, Howarth DJ, Ruddock V, Miller GJ. Factor VIII, ABO blood group and the incidence of ischaemic heart disease. *British journal of haematology* 1994; **88**(3): 601-7.

7. Spiel AO, Gilbert JC, Jilma B. von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes. *Circulation* 2008; **117**(11): 1449-59.

8. Starke RD, Ferraro F, Paschalaki KE, et al. Endothelial von Willebrand factor regulates angiogenesis. *Blood* 2011; **117**(3): 1071-80.

9. Randi AM, Laffan MA, Starke RD. Von Willebrand factor, angiodysplasia and angiogenesis. *Mediterr J Hematol Infect Dis* 2013; **5**(1): e2013060.

10. Franchini M, Coppola A. Atherothrombosis in von Willebrand disease: an analysis of the literature and implications for clinical management. *Seminars in thrombosis and hemostasis* 2012; **38**(2): 185-99.

11. Seaman CD, George KM, Ragni M, Folsom AR. Association of von Willebrand factor deficiency with prevalent cardiovascular disease and asymptomatic carotid atherosclerosis: The Atherosclerosis Risk in Communities Study. *Thrombosis research* 2016; **144**: 236-8.

12. Willeit P, Thompson A, Aspelund T, et al. Hemostatic factors and risk of coronary heart disease in general populations: new prospective study and updated meta-analyses. *PloS one* 2013; **8**(2): e55175.

13. Sanders YV, Giezenaar MA, Laros-van Gorkom BA, et al. von Willebrand disease and aging: an evolving phenotype. *Journal of thrombosis and haemostasis : JTH* 2014; **12**(7): 1066-75.

14. Sanders YV, Eikenboom J, de Wee EM, et al. Reduced prevalence of arterial thrombosis in von Willebrand disease. *Journal of thrombosis and haemostasis : JTH* 2013; **11**(5): 845-54.

15. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011; **11**: 450.

16. Lovdahl S, Henriksson KM, Baghaei F, et al. Incidence, mortality rates and causes of deaths in haemophilia patients in Sweden. *Haemophilia : the official journal of the World Federation of Hemophilia* 2013; **19**(3): 362-9.

17. Sramek A, Bucciarelli P, Federici AB, et al. Patients with type 3 severe von Willebrand disease are not protected against atherosclerosis: results from a multicenter study in 47 patients. *Circulation* 2004; **109**(6): 740-4.

18. Seaman CD, Yabes J, Comer DM, Ragni MV. Does deficiency of von Willebrand factor protect against cardiovascular disease? Analysis of a national discharge register. *Journal of thrombosis and haemostasis : JTH* 2015; **13**(11): 1999-2003.

Table 1. Characteristics of participants with VWD born 1895-2008 and a birthdate- and sex-matched comparison group

	Male		Female		Both sexes	
	VWD, n (%)	**Comp., n (%)**	**VWD, n (%)**	**Comp., n (%)**	**VWD, n (%)**	**Comp., n (%)**
Birth cohort						
1888-1939	157 (17.7)	785 (17.7)	316 (16.6)	1,579 (16.6)	473 (17.0)	2,364 (17.0)
1940-1949	99 (11.1)	495 (11.2)	255 (13.4)	1,273 (13.4)	354 (12.7)	1,768 (12.7)
1950-1959	95 (10.7)	474 (10.7)	308 (16.2)	1,539 (16.2)	403 (14.4)	2,013 (14.4)
1960-1968	101 (11.4)	503 (11.3)	283 (14.9)	1,414 (14.9)	384 (13.8)	1,917 (13.7)
1969-2008	436 (49.1)	2,177 (49.1)	740 (38.9)	3,699 (38.9)	1,174 (42.1)	5,876 (42.2)
All	888	4,434	1,902	9,504	2,790	13,938
Emigrated	10 (1.1)	313 (7.1)	33 (1.7)	674 (7.1)	43 (1.5)	987 (7.1)
Deceased[¥]	127 (14.3)	556 (12.5)	175 (9.2)	926 (9.7)	302 (10.8)	1,482 (10.6)

(Number of participants: 2,790 with VWD and 13,938 comparisons)
[*] The maximum age at the study start was 92 years.
[†] The maximum age at the end of observation was 101 years.
[¥] The earliest death was recorded in 1969.

Table 2. Raw frequency and cox regression-based hazard ratios (HR) of inpatient hospitalization for CVD among persons with VWD born 1895-1968 and sex- and birthdate-matched comparisons in Sweden

	VWD with a bleeding diagnosis*		**VWD without a bleeding diagnosis***		**Overall sample**	
	Frequency among vWD/comparisons	**HR (95% CI)**	**Frequency among vWD/comparisons**	**HR (95% CI)**	**Frequency among vWD/comparisons**	**HR (95% CI)**
Male	32/78	1.9 (1.3 , 2.9)	42/145	1.2 (0.9 , 1.67)	74/223	1.4 (1.1 , 1.8)
Female	78/242	1.5 (1.2 , 1.9)	43/185	0.9 (0.7 , 1.3)	121/427	1.3 (1.0 , 1.5)
Both sexes*	110/320	1.6 (1.3 , 2.0)	85/330	1.0 (0.8 , 1.3)	195/650	1.3 (1.1 , 1.5)

(All HR estimates were adjusted for birthdate, diabetes and cancer.)

* We obtained the information on bleeding diagnosis from inpatient hospitalization records.

**We additionally adjusted for sex in this group.

Table 3. Cause-specific incidence rate (IR) per 100 person-years and hazard ratio (HR) of inpatient hospitalizations for cardiovascular diseases (CVD) among participants with VWD born 1895-1968 and birthdate- and sex-matched comparisons from general population in Sweden

	Men			Women			Total		
	VWD, IR (95% CI)	**Comparisons, IR (95% CI)**	HR (95% CI)	**VWD, IR (95% CI)**	**Comparisons, IR (95% CI)**	HR (95% CI)	**VWD, IR (95% CI)**	**Comparisons, IR (95% CI)**	HR (95% CI)
CHD	5.8 (4.3 , 7.7)	4.0 (3.4 , 4.7)	1.3 (1.0 , 1.8)	3.4 (2.7 , 4.3)	2.3 (2.0 , 2.6)	**1.4 (1.2 , 1.8)**	4.0 (3.4 , 4.8)	2.3 (2.5 , 3.0)	**1.4 (1.1 , 1.6)**
Ischemic stroke	3.0 (2.0 , 4.4)	2.0 (1.6 , 2.5)	1.4 (0.9 , 2.2)	1.4 (1.0 , 1.9)	1.4 (1.2 , 1.7)	0.9 (0.6 , 1.3)	1.8 (1.4 , 2.3)	1.6 (1.4 , 1.8)	1.1 (0.8 , 1.4)
Pulmonary embolism	0.7 (0,3 , 1.6)	0.3 (0.2 , 0.6)	2.1 (0.9 , 5.1)	0.5 (0.3 , 0.8)	0.4 (0.3 , 0.5)	1.2 (0.6 , 2.3)	0.5 (0.3 , 0.9)	0.4 (0.3 , 0.5)	1.4 (0.9 , 2.3)
DVT	0.8 (0.4 , 1.7)	0.3 (0.2 , 0.6)	**3.5 (1.4 , 8.3)**	0.4 (0.2 , 0.8)	0.3 (0.2 , 0.4)	1.5 (0.8 , 2.9)	0.5 (0.3 , 0.9)	0.3 (0.2 , 0.4)	**1.9 (1.2 , 3.2)**
All above CVDs	9.1 (7.3 , 11.5)	6.1 (5.3 , 6.9)	**1.4 (1.1 , 1.8)**	5. 2 (4.3 , 6.2)	4.0 (3.6 , 4.4)	1.3 (1.0 , 1.5)	6.2 (5.4 , 4.2)	4.5 (4.2 , 4.9)	**1.3 (1.1 , 1.5)**

(The incidence rates were unadjusted, the sex-specific HR estimates were adjusted for birthdate, diabetes, and cancer. HR estimates in the total sample were additionally adjusted for sex.)

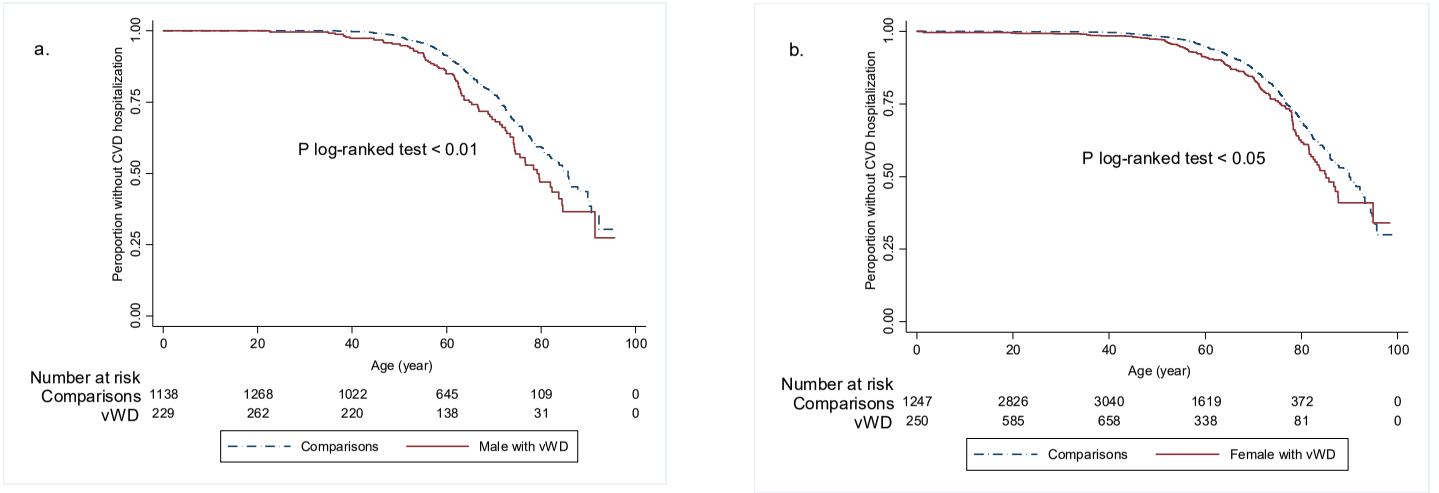

Figure 1. Kaplan-Meier estimates of age at first hospitalization for CVD among male (a) and female (b) participants with VWD born 1895-2008 and birthdate- and sex-matched comparisons from Sweden.

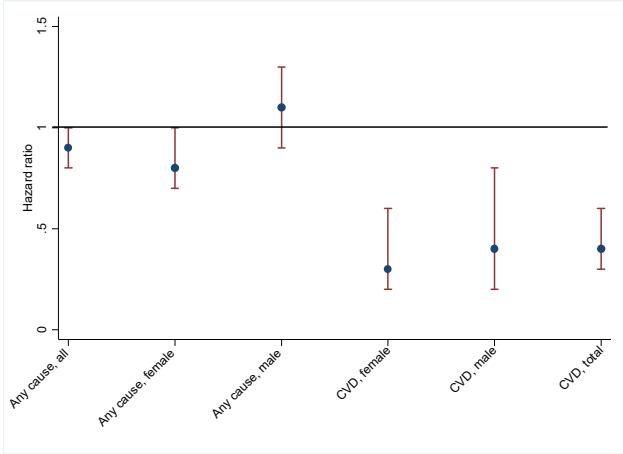

Figure 2. Hazard ratio for all-cause and CVD-related mortality among persons with VWD born 1895-2008 compared to birthdate- and sex-matched individuals from general population.

Appendix 1. Outcomes and ICD codes used in this study

Outcomes and conditions	ICD 9 (1987-1996)	ICD 10 (1997-2008)
Cardiovascular diseases (CVD)		
Coronary heart disease	410, 411, 412, 413, 414	I20, I21, I22, I23, I24
Pulmonary embolism	415	I26
Ischemic stroke	433, 434	I63, I64
Deep venous thrombosis (DVT)	451	I80
Diabetes	250	E10, E11, E12, E13, E14
Hemorrhagic conditions		
Purpura and other hemorrhagic conditions	280, 287	D69
Non-traumatic intracranial hemorrhage	430, 431, 432	I60, I61, I62
Excessive, frequent and irregular menstruation	626	N92
Gastrointestinal bleeding	569, 578	K62, K92
Hemorrhage from respiratory passages (epistaxis)	784	R04
Malignant neoplasms **		
Malignant neoplasms of digestive organs	150, 151, 152, 153, 154, 155, 156, 157, 158, 159	C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C25, C26
Lung cancer	162	C34
Breast cancer	174	C50
Malignant neoplasms of female genital organs	179, 180, 181, 182, 183, 184,	C51, C52, C53, C54, C55, C56, C57, C58
Malignant neoplasms of male genital organs	185, 186, 187	C60, C61, C62, C63
Malignant neoplasm of urinary tract	188, 189	C64, C65, C66, C67, C68
Malignant neoplasms of lymphoid, hematopoietic and related tissue	200, 201, 202, 203, 204, 205, 206, 207, 208, 209	C81, C82, C83, C84, C85, C86, C87, C88, C89, C90, C91, C92, C93, C94, C95, C96

* For malignant neoplasms, we also looked at ICD 8 codes. There was only one discrepancy between ICD 8 and 9. ICD 8 listing did not have code 179 as “malignant neoplasms of female genital organs”.

Appendix 2. Cox regression-based hazard ratio (HR) for CVD-related hospitalization among **men** with VWD born 1895-1968 and birthdate- and sex-matched comparisons in Sweden

	VWD without bleeding diagnosis*, HR (95% CI)	**VWD with bleeding diagnosis*, HR (95% CI)**	**All VWD, HR (95% CI)**
CHD	1.2 (0.9 , 1.8)	1.4 (0.8 , 2.4)	**1.3 (1.0 , 1.8)**
Stroke	1.0 (0.5 , 1.8)	**2.2 (1.1 , 4.4)**	1.4 (0.9 , 2.2)
Pulmonary embolism	2.0 (0.6 , 6.5)	NE**	2.1 (0.9 , 5.1)
DVT	**3.8 (1.3 , 11.2)**	3.6 (0.9 , 14.3)	**3.5 (1.4 , 8.3)**
All above conditions (CVD)	1.2 (0.9 , 1.6)	**1.9 (1.3 , 2.9)**	**1.4 (1.1 , 1.8)**

(Estimates were adjusted for birthdate, diabetes, and cancer.)

* Bleeding diagnoses included: purpura and other hemorrhagic conditions, non-traumatic intracranial haemorrhage, excessive, frequent and irregular menstruation, gastrointestinal bleeding, and hemorrhage from respiratory passages (epistaxis)

** Too few outcome events after adjusting for cancer.

Appendix 3. Cox regression-based hazard ratios (HR) of hospitalization for CVD among **women** with VWD born 1895-1968 and birthdate- and sex-matched comparisons in Sweden

	VWD without bleeding diagnosis*, HR (95% CI)	**VWD with bleeding diagnosis*, HR (95% CI)**	**All, HR All VWD, HR (95% CI)**
CHD	1.2 (0.8 , 1.7)	**1.5 (1.2 , 2.1)**	**1.4 (1.1 , 1.8)**
Stroke	**0.4 (0.2 , 0.8)**	1.4 (1.0 , 2.2)	0.9 (0.6 , 1.3)
Pulmonary embolism	1.8 (0.6 , 5.7)	0.9 (0.4 , 2.1)	1.2 (0.6 , 2.3)
DVT	1.4 (0.5 , 4.1)	1.6 (0.7 , 3.6)	1.6 (0.8 , 2.9)
All above conditions (CVD)	0.9 (0.7 , 1.3)	**1.5 (1.2 , 1.9)**	**1.3 (1.0 , 1.5)**

(Estimates were adjusted for birthdate, diabetes, and cancer.)

* Bleeding diagnoses included: purpura and other hemorrhagic conditions, non-traumatic intracranial haemorrhage, excessive, frequent and irregular menstruation, gastrointestinal bleeding, and hemorrhage from respiratory passages (epistaxis)

Appendix 4. Cox regression-based hazard ratio (HR) of hospitalization for CVD among participants with VWD born 1895-1968 and birthdate- and sex-matched comparisons in Sweden

	VWD without bleeding diagnosis*, HR (95% CI)	**VWD with bleeding diagnosis*, HR (95% CI)**	**All, All VWD, HR (95% CI)**
CHD	1.2 (0.9 , 1.6)	**1.5 (1.1 , 1.9)**	1.4 (1.1 , 1.6)
Stroke	0.6 (0.4 , 1.0)	**1.7 (1.2 , 2.4)**	1.1 (0.8 , 1.4)
Pulmonary embolism	2.0 (1.0 , 4.0)	1.1 (0.5 , 2.3)	1.4 (0.9 , 2.3)
DVT	2.1 (1.0 , 4.4)	1.8 (0.9 , 3.6)	**1.9 (1.2 , 3.2)**
All above conditions (CVD)	1.0 (0.8 , 1.3)	1.6 (1.3 , 2.0)	**1.3 (1.1 , 1.5)**

(Estimates were adjusted for birthdate, sex, diabetes, and cancer.)

* Bleeding diagnoses included: purpura and other hemorrhagic conditions, non-traumatic intracranial haemorrhage, excessive, frequent and irregular menstruation, gastrointestinal bleeding, and hemorrhage from respiratory passages (epistaxis)

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