

The slide features a decorative arrangement of five circles. Three circles are positioned in the upper half, and two are in the lower half. The top row consists of a white circle with a light blue outline on the left, a solid light blue circle in the middle, and another solid light blue circle on the right. The bottom row consists of a solid light blue circle on the left and a white circle with a light blue outline on the right. The text is centered horizontally and partially overlaid by these circles.

LABORATORY DIAGNOSIS OF VON WILLEBRAND DISEASE

Chris Watson, Leicester Royal Infirmary

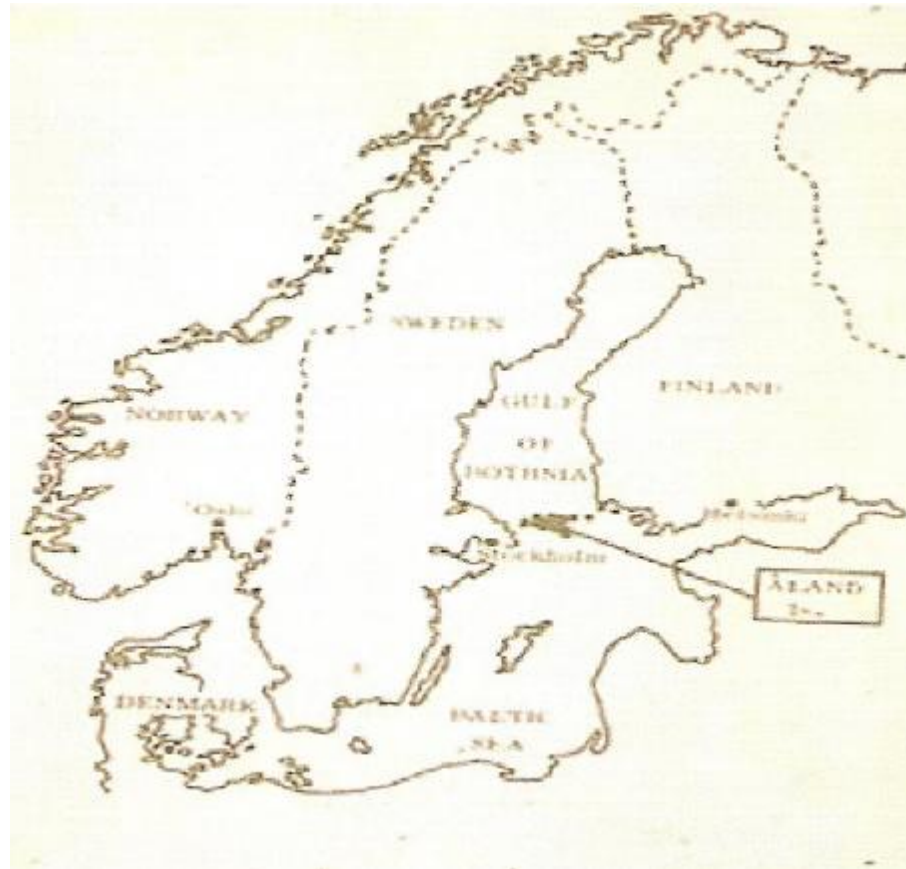
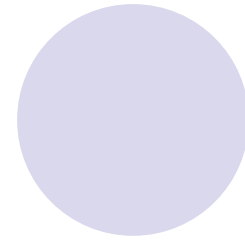
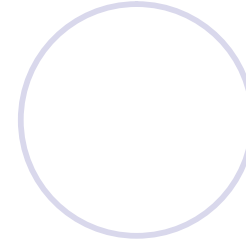
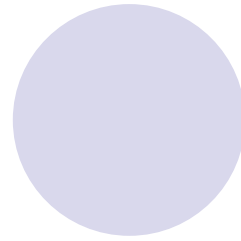
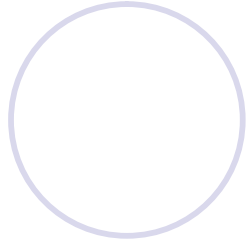
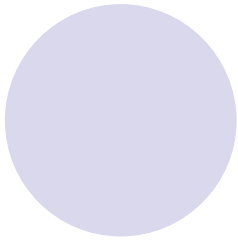
LABORATORY DIAGNOSIS OF VON WILLEBRAND DISEASE

- | **History**
- | **von Willebrand Factor**
- | **von Willebrand Disease**
- | **Classification**
- | **Laboratory diagnosis**
- | **Diagnostic Issues**
- | **Recommendations**
- | **Discussion**

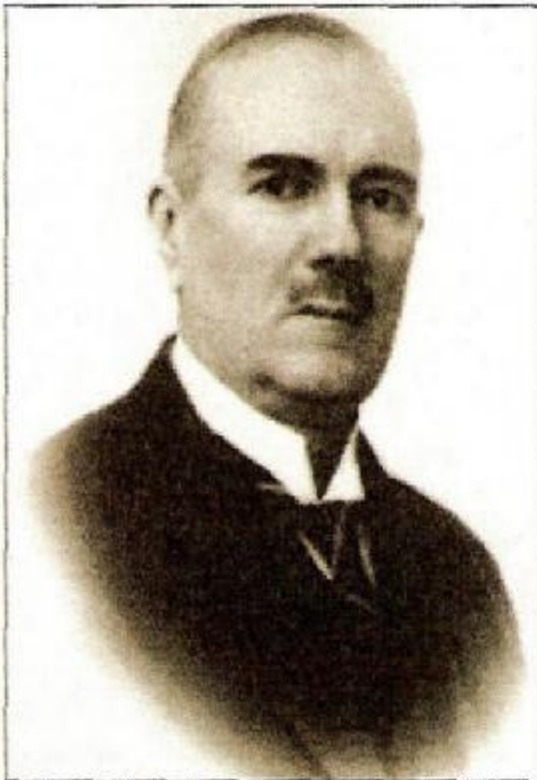


History

- | **In 1895 Hutchinson described familial bleeding from nose, mouth and uterus**
- | **Minot and Lee described bleeds with normal platelets and slightly prolonged clotting time (1920)**
- | **In 1926 Erik von Willebrand published a description of a bleeding disorder**
- | **Propositus was a 7 year old girl (Hjördis) from the Åland Islands**
- | **Hjördis had severe bleeding episodes and died at the age of 13**
- | **von Willebrand studied 66 family members of which 23 suffered bleeding symptoms**



The Åland Islands



**FINSKA LÄKARESÄLLSKAPETS
HANDLINGAR**

REDAKERAD AV
PROF. RICHARD SIEVERS
BAND LXXVIII

1926 FEBRUARI 1926

INNEHÅLL:

Originalartiklar.

E. A. v. Willebrand, Hereditär pseudohemofili. (Falls Diakrotojaktiska ämnen i blodet är medicinska anmärking. Docent E. A. v. Willebrand. (Med 2 figurer i texten)..... 67

T. W. Kallunki, Hjältid och spurt..... 113

Armas Gohlbach, Trene fall av enterit. (Falls II Kliniska studier i Helsingfors, prof. E. F. Lills, och Vidensk. Meddel. från, prof. E. W. Granberg. (Med 2 figurer i texten)..... 130

Praktiska Notiser.

Översikter.

Armas Gohlbach, Tergentliga strålar..... 142

Sjörörelsemedelstämman och referat.

Erikur Gustafson, Löwy's metod för bedömning av de olika blod kroppens viskositet. (Falls II Medicinska studier i Helsingfors. (Med 2 figurer i texten)..... 152

Ernst Zetterlin, Medicinska studier. (Med av Ernst Zetterlin)..... 157

Knut Zetterlin, Tuberkulos i Danmark. (Med av E. Zetterlin)..... 164

Öfversättningar.

H. Schramm och P. Seltman, Die perniciöse Anämie. (Med av Ernst Zetterlin)..... 178

Armas Gustafson, Vid betydelse för blodets viskositet för vår kropp och sjuk. (Med av E. A. v. Willebrand)..... 179

Totalt sidor 1946.

HILSINGBORG 1926
AMMONTONS TRYCKERI ÅSTERGATAN

FINSKA LÄKARESÄLLSKAPETS HANDLINGAR. BAND LXXVIII. NO 2.

ORIGINALARTIKLAR.

(Falls Diakrotojaktiska i Helsingfors medicinska studier.
Docent E. A. v. WILLEBRAND.)

Hereditär pseudohemofili.

AV
E. A. v. Willebrand.
(Med 2 figurer i texten.)

1. Sjukdomsbegrepp. Tidigare observerade fall.

I sitt nya stora arbete över de hemorragiska diateserna framhåller E. FRANK (Breslau), att den klassiska hemofilien är en så sakvisit hereditär-familjär anomal, att det kan frågasättas, huruvida över huvud sparsidiska fall av sjukdomen existera. Däremot är, säger han, den kliniska trombofopen en utpräglad sparsidisk, att man kan diskutera, om en familjär form av densamma alls förekommer. Med trombofopen avses här den sjukdom, som sedan gammalt har nämnts morbus maculosis Walsburi eller purpura haemorrhagica och som på senaste tid av FRANK och en del andra forskare betecknats såsom essentiell trombofopen.

Hittills har man velat betrakta årlig blodresorptions och hemofili såsom synonyma begrepp. Men om man genomgår rörörande litteratur, skall man finna, om och i ett fåtal fall, beskrifningar över en familjär form av hemorragisk diates, som sedan därigenom skiljer sig från likta hemofili att den även förekommer bland kvinnor och, såsom det tyckes, t. o. m. oftare än bland män. Men även i andra avseenden kan man draga en stor grans mellan tillräckande familjär lidande och hereditäritet. Till sist återkommer vi till kap. 6 om diagnosen.

Finska H. S. v. Willebrand i Helsingfors 1926.

Erik Adolf von Willebrand (1870-1949)

“the trait seems to be seen among women”

“genital haemorrhage in connection with menstruation and delivery is the second most common cause of death”

“in female bleeders, the diathesis becomes manifest in a milder and graver form, whereas the males show only the mild form and five deaths have occurred in women”

History



- | von Willebrand concluded that the disorder was due to a “disturbed function of thrombocytes and a general lesion of the capillary wall and called it hereditary pseudothaemophilia
- | In 1929 further description from Minot
- | Cases reported by Griffin (1928), Rothman & Nixon (1928), Little & Ayers (1928) and Rosling (1929)
- | Described by Jürgens as ‘constitutional thrombopathy’ (1933)
- | Greiger and Evans (1938) – ‘hereditary haemophiloid purpura’
- | Revol (1951) – ‘pseudothaemophilia’

History



- | **1953 – Patients with vWD lacked factor VIII (Alexander & Goldstein)**
- | **1957 – Nilsson et al studied 15 members of the original family**
- | **1959 – Nilsson demonstrated infusion of Haem A plasma into vWD patient increased factor VIII**
- | **1960 – Borchgrevink demonstrated reduced platelet adhesion**
- | **1963 – Salzman reported reduced platelet adhesion corrected by normal/Haem A plasma**
- | **1971 – Howard & Firkin demonstrated the effects of ristocetin**
- | **1971 – Zimmerman demonstrated a protein similar to Factor VIII absent in vWD patients**

History



- | **1972 - Owen & Wagner separated the antihaemophilic globulin into 2 proteins**
- | **1972 – Holmberg & Nilsson demonstrated that some vWD patients had normal plasma concentrations of vWF, but a structural abnormality**
- | **1980 - Ruggeri described a group of patients with increased responsiveness to ristocetin**
- | **1984 – First classification of vWD**
- | **1985 – vWF gene was cloned**
- | **1994 – Revised classification of vWD**
- | **2006 – Recommendations for updated classification of vWD**



von Willebrand Factor

- | **von Willebrand Factor (vWF) is a large glycoprotein consisting of a series of multimers ranging from molecular weight of 500 to >10000kDa**
- | **vWF gene located on chromosome 12 and spans 178kb comprising 52 exons**
- | **Primary product is a 2813 amino acid (AA) pre-pro vWF molecule synthesised in the endothelial cells and megakaryocytes**
- | **Pre-pro vWF molecule comprises a 22 AA peptide, a 741 AA pro-peptide and mature vWF molecule (2050 AA)**
- | **This precursor translocates to the endoplasmic reticulum to form pro-vWF dimers**



von Willebrand Factor

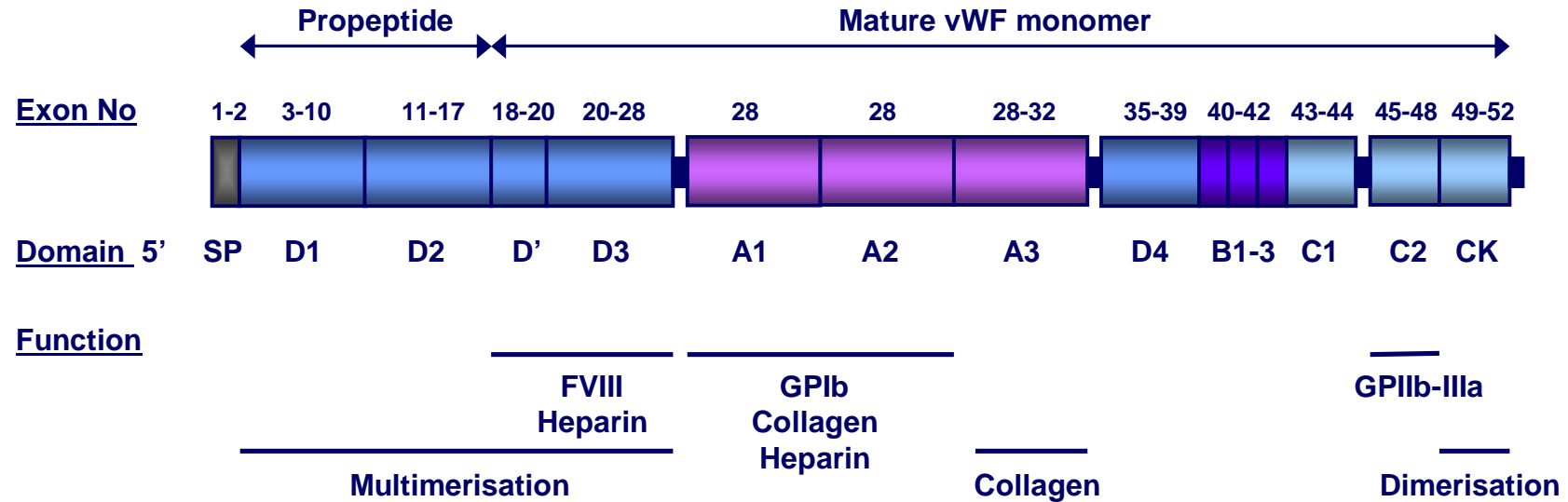
- | Transported to the Golgi apparatus where multimerisation occurs
- | Pro-peptide is cleaved off and stored with mature vWF in Weibel-Palade bodies within endothelial cells, megakaryocytes and platelet α -granules
- | Secreted from cells along a constitutive and regulated pathway
- | Large multimers undergo proteolytic cleavage by a metallo-protease – ADAMTS 13
- | vWF plasma levels correspond to synthesis and release of vWF and its clearance



von Willebrand Factor

- | **Plasma concentration is 10µg/ml**
- | **Half life is 12 – 20 hours**
- | **Levels influenced by blood group, race, age, pregnancy, pathological conditions, exercise, stress and trauma**
- | **Ligands – Factor VIII, Collagen, platelet glycoprotein Ib/IX, platelet glycoprotein IIb/IIIa, sulphatides and heparin**
- | **Mutations of the vWF gene give rise to vWD**

Structure and function relationship of vWF gene and protein



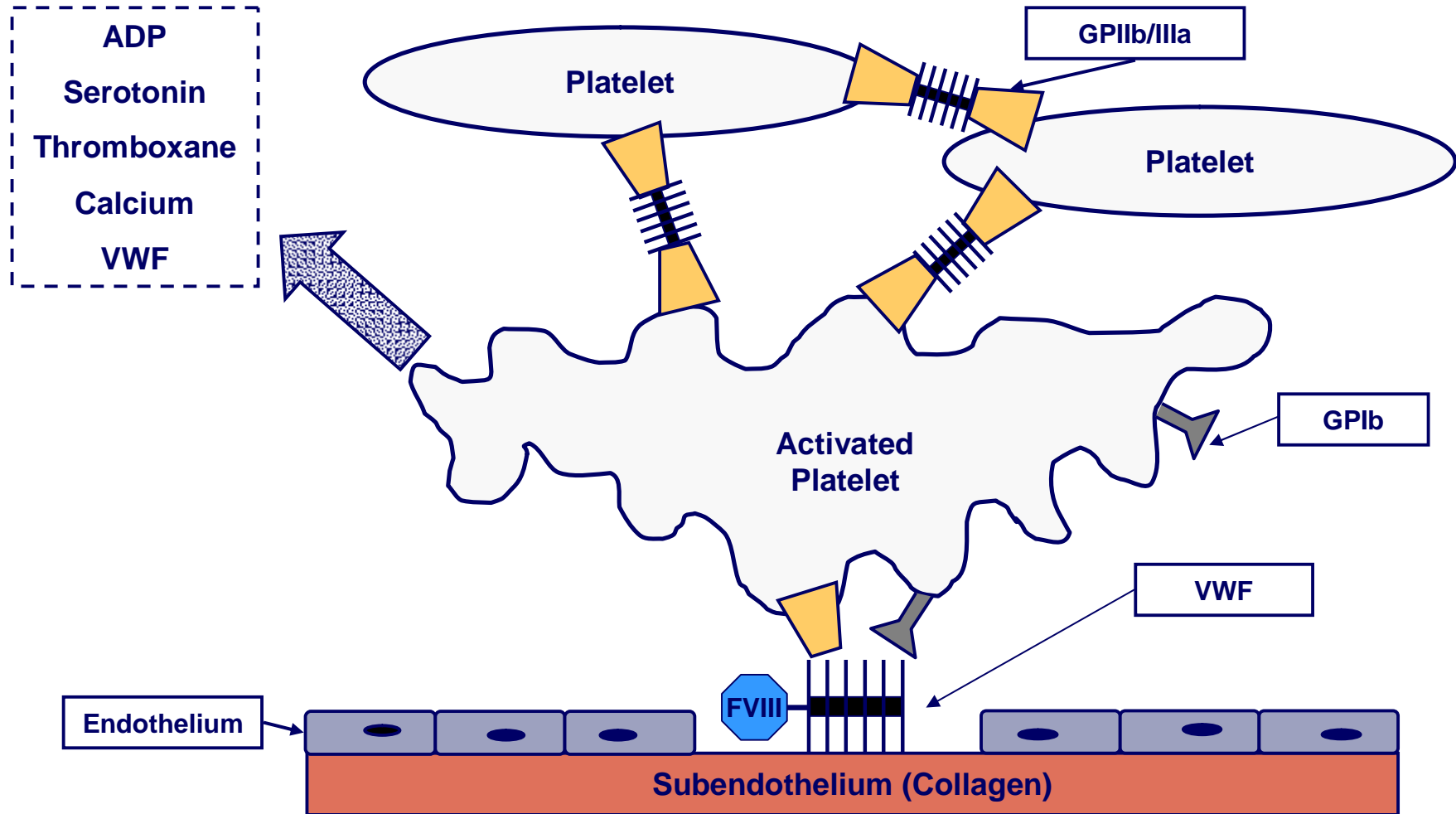


von Willebrand Factor

| Two important functions:

- (1) Required for adhesion of platelets to injured vessels**
- (2) Acts as a carrier for factor VIII protecting it from proteolytic degradation**

Role of von Willebrand Factor





von Willebrand Disease

- | Heterogeneous bleeding disorder caused by inherited defects in concentration, structure or function of vWF**
- | The most common inherited disorder in humans with or without bleeding manifestations**
- | Worldwide distribution and is common in other animal species including dogs and pigs**
- | Prevalence in epidemiological studies has been as high as 1%, estimates generally from 1/100 to 1/1000**
- | Italy: 8000 per million have inherited defects in vWF function (mostly asymptomatic)**



von Willebrand Disease

- | **Estimated as many as 17 million of the world population could have vWF gene defects (Sadler 2000)**
- | **Severe forms estimated to be between 1 and 3 per million;**
 - **Scandinavia 2.5 – 3 per million**
 - **Europe/Middle East 0.1 – 1.6 per million**
 - **USA/Canada 1.5 per million**
- | **Symptoms of bleeding vary greatly between individuals**



Clinical Features

- | **Involves mucous membranes, commonly easy bruising, nose bleeds, heavy menstrual periods and prolonged bleeding from wounds**
- | **Can be mild or moderate, some individuals unaffected**
- | **Bleeding can occur following serious injury, dental work or surgery**
- | **Bleeding into joints or muscles is rare, but may occur in severe forms of vWD (similar to Haemophilia)**



Classification

- | **First official classification was in 1984 based upon multimer patterns**
- | **Over 22 subtypes were described**
- | **Revised classification in 1994 (Sadler)**
- | **Based on differences in pathophysiology and that all vWD is caused by mutations within the vWF gene**
- | **vWD classified as a quantitative or qualitative disorder**

Classification



I Primary classification:

- Type 1; Quantitative deficiency of vWF
- Type 2; Qualitative deficiency of vWF
- Type 3; Virtually complete deficiency of vWF

I Secondary classification:

- Type 2A; Variants with decreased platelet dependent function with loss of HMW multimers
 - Type 2B; Variants with increased affinity (gain of function) for platelet glycoprotein Ib (GPIb)
 - Type 2M; Variants with decreased platelet dependent function NOT associated with loss of HMW multimers
 - Type 2N; Variants with decreased affinity for Factor VIII

Type 1



- | **Most common form of vWD, approximately 70 – 80% of all cases**
- | **Typically autosomal dominant in inheritance**
- | **Genetic basis poorly understood**
- | **vWF protein functionally normal with normal multimers**
- | **Clinical presentation variable and influenced by other genetic and environmental factors**
- | **Severity of bleeding symptoms usually correlate with vWF levels**
- | **Desmopressin (DDAVP) treatment of choice**

Type 2A



- | **Reduced platelet dependent function of vWF due to decreased HMW multimers**
- | **10-15 % of all cases of vWD (Type 2 in total 20 – 25% of all cases)**
- | **Inheritance generally autosomal dominant some cases recessive**
- | **2 pathophysiological mechanisms:**
 - **Impaired biosynthesis of vWF (Group 1 mutations)**
 - **Normal vWF assembly and secretion but increased degradation (Group 2 mutations)**
- | **20 mutations within Exon 28 (A2 domain) and 4 missense mutations within the A1 domain**
- | **vWF concentrates usually used in treatment, but DDAVP trial may be performed**

Type 2B



- | All qualitative variants with increased affinity for platelet GPIb
- | Approximately 5% of all cases of vWD
- | HMW multimers usually absent
- | Variable thrombocytopenia
- | Autosomal dominant inheritance
- | All mutations occur in Exon 28 within the A1 domain
- | 4 mutations account for approximately 90% of 2B
- | DDAVP may cause severe thrombocytopenia

Type 2M



- | Qualitative variants with reduced platelet dependent function that is not associated with an absence of HMW multimers
- | Structural or functional defect of vWF
- | Multimer distribution is normal, but the triplet structure of these patients may show an absence of sub-bands
- | Autosomal dominant inheritance
- | Mutations mainly occur in Exon 28 within the A1 domain
- | vWF concentrates usually used in treatment, but DDAVP trial may be performed



Type 2N

- | All qualitative defects with reduced affinity for factor VIII
- | vWF platelet dependent function normal
- | Phenotype similar to mild or moderate Haemophilia A
- | 'N' stands for Normandy, France
- | Autosomal recessive inheritance
- | Missense mutations mainly in the N terminal binding region of vWF gene (D'/D3 domain)
- | Usually homozygous for 2N or compound heterozygous 1/2N
- | vWF concentrates usually used in treatment

Type 3

- | **Less than 5% of all cases of vWD**
- | **Severe bleeding disorder resulting from a markedly reduced or absent vWF**
- | **Usually autosomal recessive**
- | **Plasma multimers absent**
- | **DDAVP ineffective, vWF concentrates required for treatment**



Platelet pseudo vWD

- | Not due to a defect in vWF
- | Mutations in platelet glycoprotein Ib/IX receptor causing an increase in platelet – vWF binding
- | Rare autosomal dominant disorder
- | Mild thrombocytopenia is often seen
- | Laboratory presentation similar to Type 2B
- | Requires different therapeutic intervention to Type 2B

Acquired vWD



- | Also known as **Acquired von Willebrand Syndrome (AVWS)**
- | Occurs in a small population of patients (**0.04 – 0.15% of general population**)
- | **Antibodies against vWF molecule**
- | **Clinical presentation variable**
- | **Diseases associated include lymphoproliferative, myeloproliferative and autoimmune diseases**



Diagnosis of vWD

- | **Diagnosis of vWD requires three components:**
 - (1) Personal history of excessive mucocutaneous bleeding**
 - (2) Laboratory results consistent with vWD**
 - (3) Family history of bleeding**
- | **Important to obtain accurate vWF levels since this determines haemorrhagic risk and subsequent clinical management**



vWD screening tests

- | PROTHROMBIN TIME – Insensitive to deficiencies of factor VIII or vWF abnormalities**
- | ACTIVATED PARTIAL THORMBOPLASTIN TIME (APTT):**
 - Sensitive to reduced factor VIII level, e.g. severe Type 1, 2N or Type 3**
 - May be normal for Type 1 and 2A, 2B and 2M**
 - Normal APTT will not exclude vWD**
- | Coagulation screening tests are of limited use for detecting vWD and of more use in excluding other coagulation factor deficiencies**

vWD screening tests

I Full Blood count/Morphology:

- Platelet count should be normal, except in Type 2B or pseudo vWD where mild thrombocytopenia may be present
- Platelet count and morphology may also be useful in defining other platelet related haemostatic disorders

I Bleeding Time:

- Originally the Duke Bleeding time, Ivy modified method, Template method
- Time taken for standardised incision on the forearm to stop bleeding
- Normal range usually < 9/10 minutes





vWD screening tests

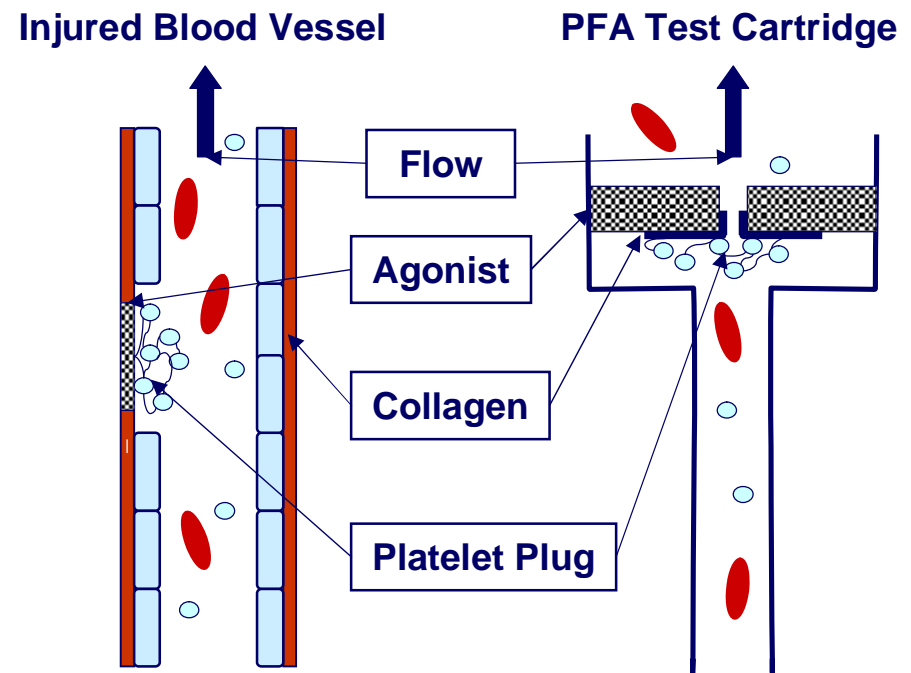
I Bleeding Time (continued):

- Prolonged by severe Type 1/2s and Type 3, but may be insensitive to mild Type 1
- Also prolonged by low platelet count, functional platelet disorders and collagen disorders
- Poor reproducibility and high operator error
- Not recommended for exclusion of vWD

vWD screening tests

I PFA 100:

- Simulates primary haemostasis by mimicking high shear stress conditions that occur after vessel injury
- Whole blood is aspirated through an aperture in a cartridge coated with collagen and Adrenaline or ADP
- Subsequent platelet adhesion and aggregation will occlude the aperture (Closure Time)



vWD screening tests



I PFA 100 (Continued):

- Maximum closure time is 300 seconds
- Sensitivity to vWD Type 2A, 2B, 2M and 3 is >98%, but overall sensitivity to vWD Types 1, 2A, 2B, 2M and 3 is ~ 85% (Favaloro, 2006)
- Global assessment of primary haemostasis so not specific for vWD
- Normal closure time does not exclude vWD
- May have a role in DDAVP monitoring
- Advantages; Rapid result, easy to operate, more sensitive than bleeding time
- Disadvantages; Detection of platelet defects, sensitive to reduced platelet numbers and anaemia, expensive and potential cartridge instability/variation



vWD Diagnostic Tests

- | Factor VIII levels (FVIII:C)**
- | vWF antigen levels (vWF:Ag)**
- | vWF 'activity' measurement**
- | Ristocetin induced platelet aggregation (RIPA)**
- | Factor VIII binding assay (vWF:FVIII B)**
- | Assessment of multimeric profile**



Factor VIII:C measurement

- | **One stage (APTT based), two stage or chromogenic based assays**
- | **Essential for diagnosis of vWD**
- | **Reduced in severe Type 1, Type 3 and 2N vWD**
- | **The lower the factor VIII:C, the more severe the vWD and subsequent haemorrhagic risk**
- | **Normal factor VIII:C does not exclude vWD and abnormal factor VIII:C does not necessarily indicate vWD**

vWF antigen measurement

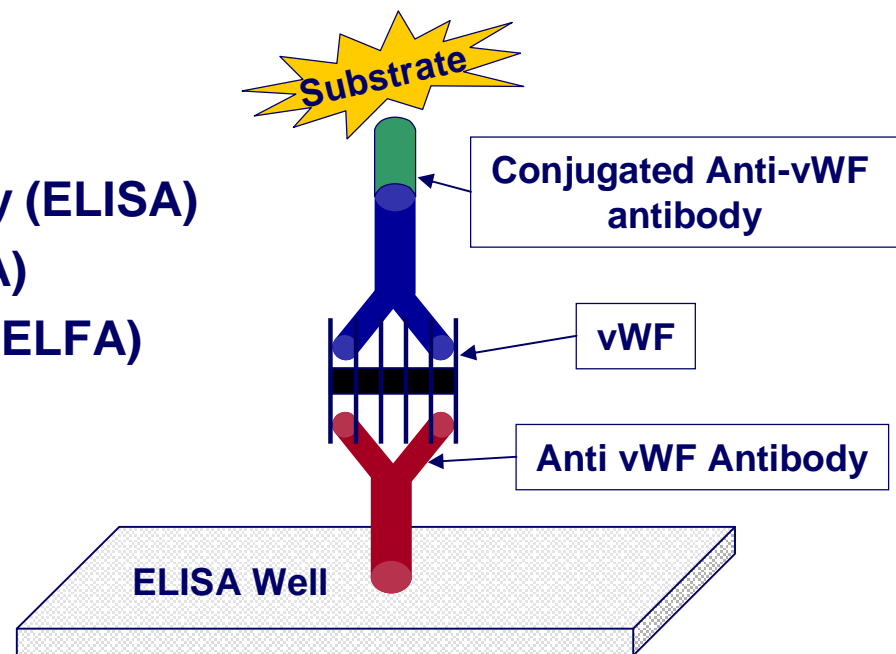
- Quantifies the total level of vWF protein but not an assessment of vWF function
- Various methodologies in use;

Electro-immunodiffusion (EID)

Enzyme linked immunosorbant assay (ELISA)

Latex immunoturbimetric assays (LIA)

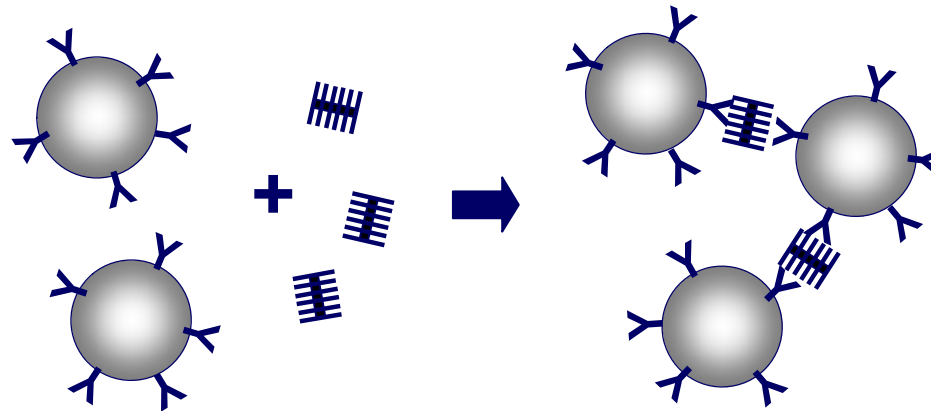
Enzyme linked fluorescence assays (ELFA)



ELISA Principle

vWF antigen measurement

- | Original EID methods rarely used now and ELISA users among UK NEQAS participants has decreased from 48% (Dec 01) to 26% (May 05)
- | LIA usage among UK NEQAS participants has increased from 14% to 61% in the same period (Kitchen et al, 2006)
- | LIA methods measure the degree of agglutination of anti vWF coated latex particles



- | LIA advantages, single random access testing and improved intra/inter assay performance
- | However, potential interference of rheumatoid factor may lead to overestimation of vWF:Ag



vWF antigen measurement

- | Increased use of ELFA techniques - mean CV for ten centres was 7.1% (Kitchen et al, 2006)**
- | vWF:Ag levels will be reduced in most Type 1s and Type 3 vWD**
- | vWF:Ag levels may be normal in some Type 2s**
- | No information on the subtype**

vWF 'activity' measurement

- | To diagnose and classify vWD a measurement of vWF functionality or assessment of multimer presence is necessary**
- | Ristocetin co-factor assay (vWF:Rco)**
- | vWF 'activity' assay**
- | Collagen binding activity assay (vWF:CB)**



Ristocetin co-factor assay

- | Original 'functional' assay performed using platelet agglutination methods – visual and aggregometer (Weiss et al, 1973)
- | Assay measures agglutination of normal fixed (or fresh) platelets in dilutions of test plasma containing a fixed concentration of ristocetin
- | Ristocetin is a glycopeptide synthesised by *Nocardia lurida* and was previously used as an antibiotic
- | Ristocetin forms dimers to both vWF and platelet GPIb
- | Assay has the capacity to detect HMW forms of vWF and assess defects in platelet adhesion



Ristocetin co-factor assay

- | In Type 1 vWF:Rco will be reduced concordantly with vWF:Ag and will be < 5% in Type 3 vWD
- | vWF:Rco will be lower than vWF:Ag in Types 2A, 2B and 2M vWD
- | However, the original vWF:Rco has poor reproducibility, poor agreement between centres (CV 40 – 50%), poor lower limit of detection, time consuming and difficult to perform
- | An ELISA for vWF:Rco using rGPIb α fragment in the presence of ristocetin was developed by Vanhoorelbeke et al (2000)



Ristocetin co-factor assay

- | **ELISA has improved reproducibility and correlated well with Type 1 and Type 2 vWD**
- | **Federici et al (2003) described a similar ELISA in which vWF binds to a different recombinant fragment**
- | **Development of automated techniques using lyophilised platelets measuring change in absorbance caused by agglutination**
 - **Miller et al (2002); Within run/between run CV 8.1/10.5%**
 - **Raedelli et al (2005); Within run/between run CV 6.9/10.3%**
- | **vWF:Rco is the assay of choice for the monitoring of treatment**



vWF 'activity' assay

- | An ELISA using a monoclonal antibody directed against GPIb in the A1 domain of vWF**
- | First described by Goodhall et al (1985) and Murdock et al (1997) showing good discrimination of vWD subtypes**
- | Assay was commercially marketed by Shield**
- | Fischer (1998) showed good correlation of vWF multimers with vWF:Rco but not with ELISA**
- | Preston (1998) reported higher median activity using a Type 2A sample in the UK NEQAS survey**

vWF 'activity' assay



- | Favaloro et al (2000) also demonstrated that the activity ELISA did not discriminate Types 2A and 2B from Type 1
- | Proposed that problems may be related to binding affinity and avidity properties
- | Shield launched a Mark II version (2000) with an improved detection system
- | Kitchen et al (2006) reported that results obtained with vWF:Rco and ELISA were very similar for Type 1 and Type 2M.
- | LIA using a specific anti-vWF monoclonal antibody directed against GPIb of vWF has been developed

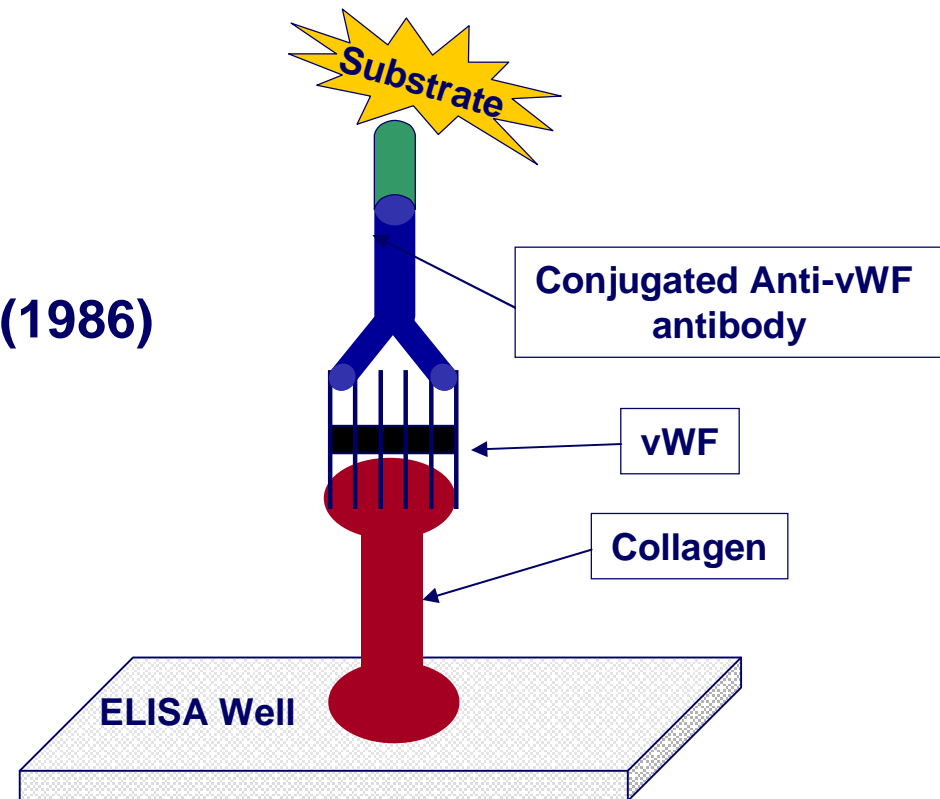
vWF 'activity' assay



- | **Between centre CV for LIA was 27% for UK NEQAS participants (Kitchen et al, 2006)**
- | **Good correlation with the Shield kit ($r > 0.94$)**
- | **Pinol et al (2007) reported 98.6% sensitivity to vWD for LIA as a screening test used alongside the vWF:Ag**
- | **Sensitivity for LIA was 94.7% for Type 2 vWD compared with 100% for vWF:Rco**
- | **Budde (2004) reported reduced sensitivity of 55% for Type 2A using LIA**
- | **Potential interference of rheumatoid factor may lead to overestimation of vWF activity using LIA**
- | **? Rapid results - may have a role in monitoring**

Collagen binding activity assay

- | An ELISA method which measures the amount of vWF binding to immobilised collagen
- | Modified from a procedure described by Brown & Bosak (1986)
- | Alternative measurement of vWF function assessing adhesive properties and able to selectively detect HMW multimers





Collagen binding activity assay

- | **Type I and Type I/III collagen mixture preparations (equine or bovine) detect HMW multimers better than Human Type I or III**
- | **Purified human derived Type III bind vWF very well so lower concentration must be used**
- | **Many reports indicate vWF:CB is more sensitive to the presence and absence of HMW multimers than vWF:Rco**
- | **Favaloro (2005) has also reported greater sensitivity for confirming Types 1 and 3 vWD**
- | **Advantages; Improved within/between run CV%, more sensitive at lower levels, easy to perform**

Collagen binding activity assay

- | **Disadvantages; Batch testing, no rapid results, commercial kits expensive, ? most sensitive collagen, not sensitive to platelet GPIb defects**
- | **Riddell et al (2002) demonstrated the vWF:CB was not sensitive to Type 2M defects**
- | **However, a '2M' due to a collagen binding defect has been described (Ribba et al, 2000)**
- | **A Ser968Thr substitution in the A3 domain was reported in two members of a family with vWD**
- | **Still limited use in the U.K.**
- | **Coleman (2006) reported the use of the vWF:CB to detect vWF autoantibodies**



Ristocetin Induced Platelet Aggregation

- | **Assesses the patient's platelet rich plasma (PRP) for sensitivity to ristocetin at various concentrations**
- | **Uses at least three ristocetin concentrations ranging from 0.5mg/ml to 1.5mg/ml**
- | **Normal individuals will have platelet aggregation at 1.0mg/ml to 1.25mg/ml**
- | **Severe Type 1 will have absent or reduced responses at 1.5mg/ml**
- | **No aggregation response for Type 3 vWD**
- | **Type 2A and 2M will have reduced responses**

Ristocetin Induced Platelet Aggregation

- | In contrast there is an increased response for Type 2B and platelet-type pseudo vWD, typically at 0.5mg/ml
- | RIPA is required specifically for identification of 2B and platelet-type pseudo vWD
- | Differentiation of 2B and platelet-type pseudo vWD by plasma/platelet mixing studies;
 - Normal PRP/Type 2B plasma – enhanced RIPA
 - Normal PRP/pseudo vWD plasma – normal RIPA
 - Type 2B PRP/cryoprecipitate – no aggregation
 - pseudo vWD PRP/cryoprecipitate – Spontaneous Agg
- | Favaloro (2006) reported some cases of aggregation in Type 2B with cryoprecipitate

Factor VIII Binding Assay

- | Assay used to assess the ability of an individual's vWF to bind to factor VIII and discriminates mild Haemophilia A from Type 2N vWD
- | Typically performed as an ELISA and involves a chromogenic assay step
- | Initial step involves the capture of vWF onto a plate coated with anti-vWF antibody
- | Any vWF bound factor VIII is removed using a high concentration of calcium chloride
- | A known quantity of factor VIII is added and interacts with the bound vWF
- | ELISA bound vWF and FVIII measured by an ELISA and chromogenic assay respectively
- | Ratio of bound FVIII to bound vWF is calculated and <0.6 is suggestive of Type 2N

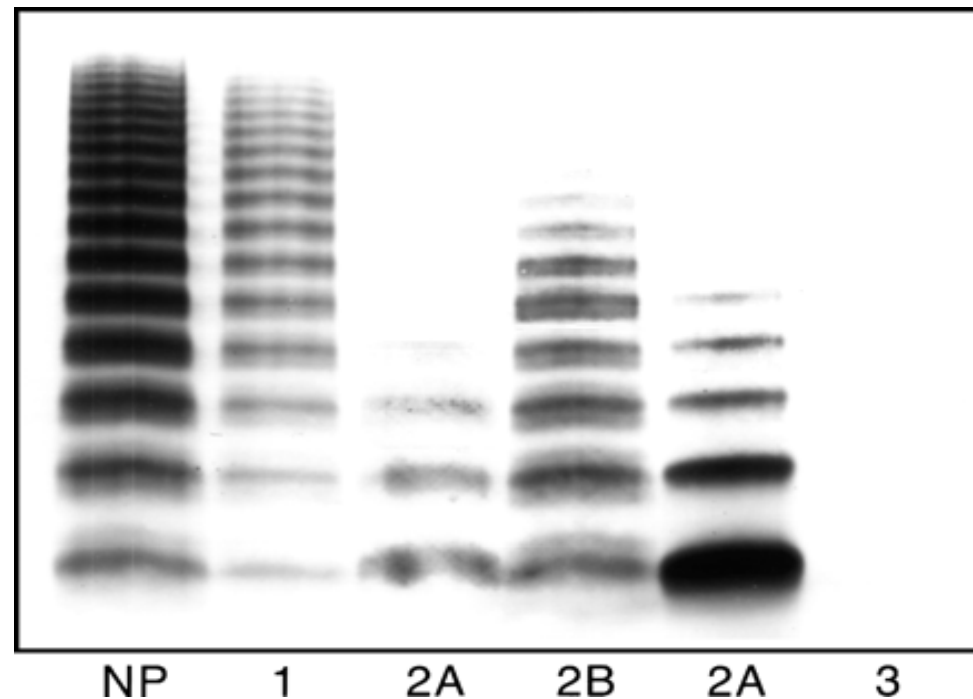


Multimer Analysis

- | **Analysis of the multimeric composition of vWF to establish the presence of differing molecular weight multimers and any structural abnormalities**
- | **Involves electrophoresis of vWF into its multimeric components using non reducing agarose gels in the presence of Sodium Dodecyl sulphate**
- | **Visualised by Iodine labelled vWF, enzyme linked antibodies or luminescence**
- | **Low resolution gels used as first line method as it differentiates between loss of largest multimers, presence of supranormal multimers and normals**

Multimer Analysis

- Higher gel concentrations discriminate smaller multimers and reveal the presence of the triplet structure of individual multimers
- Labour intensive, technically demanding and expensive method





Sulphatide binding assays

- | Sulphatides bind to vWF with high affinity**
- | Favaloro (2000) described an ELISA assay based upon sulphatide binding**
- | Assay of no diagnostic use for vWD detection**

Typical Results

	FVIII	vWF:Ag	Rcof	vWF:CB	Rcof/Ag	CBA/Ag	RIPA	Multimers
Type 1	N / ↓	↓	↓	↓	> 0.7	> 0.7	N / ↓	Normal
Type 2A	N / ↓	↓ / N	↓	↓	< 0.7	< 0.7	↓	HMW Absent
Type 2B	N / ↓	↓ / N	↓	↓	< 0.7	< 0.7	↑	HMW Absent
Type 2M	N / ↓	↓ / N	↓	↓ / N	< 0.7	> 0.7	↓	Normal
Type 2N	↓	N / ↓	N / ↓	N / ↓	> 0.7	> 0.7	N	Normal
Type 3	↓↓	↓↓	↓↓	↓↓	N/A	N/A	↓	Absent



Diagnostic Issues

- | **Pre-analytical variables**
- | **Factors affecting vWF levels**
- | **Which is the best assay to measure vWF ‘function’?**
- | **Use of ratios to distinguish Type 1 and Type 2 vWD**
- | **What is Vicenza vWD?**
- | **Use of DDAVP or concentrates for diagnosis**
- | **Update on the classification of vWD**



Pre-analytical variables

- | **Poor collection techniques may lead to loss of HMW Multimers**
- | **Storage and transport of citrated whole blood at 4°C can lower vWF results (Favaloro, 2004)**
- | **Filtration of plasma can result in loss of HMW multimers**
- | **? Freezing of plasma samples can result in loss of HMW multimers**
- | **Diurnal variation**

Factors affecting vWF levels

- | **Genetic – vWF gene, blood group, ethnicity**
- | **Physiological and Environmental – Age, menstrual cycle, oral contraceptive pill, pregnancy, hormone replacement therapy, inflammatory/malignant disorders, thyroid disease, stress, exercise**
- | **Can make diagnosis of vWD difficult**



Factors affecting vWF levels

BLOOD GROUP

- | vWF:Ag is 25% lower for blood group O individuals compared to other blood groups
- | Group O range 36 – 157% (Gill et al, 1987)
- | Mean level of vWF:Ag – Normal group O is 75% and normal group AB is 123%
- | Large overlap between normal group O and vWD of other blood groups
- | Dosage effect with AA and AO
- | Secretor subtypes; SeSe secretors in Group O higher than Sese and sese non secretors
- | Lewis group has no effect on vWF levels



Factors affecting vWF levels

ETHNICITY

- | **Kadir et al (1999) and Miller et al (2001) both reported higher levels of factor VIII and vWF:Ag in African-American women compared with Caucasians**
- | **Miller et al (2003) demonstrated vWF:Ag levels were increased in 123 African-American women, but not for vWF:Rco**
- | **Probably due to ABO blood group**



Factors affecting vWF levels

AGE

- | Neonates have raised vWF levels which fall to baseline by 6 months
- | Diagnosis or exclusion of Type 1 difficult in early age
- | vWF levels increase slowly throughout adult life (10% per decade)



Factors affecting vWF levels

EXERCISE/STRESS

- | Maximal physical and mental exertion can lead to rises in vWF
- | Levels remain elevated for approximately 10 hours
- | Moderate exercise unlikely to lead to significant elevation
- | In children the trauma from crying may increase levels

Factors affecting vWF levels

MENSTRUAL CYCLE

- | Conflicting data regarding changes in vWF levels during the menstrual cycle**
- | Most data reported in non vWD women**
- | Blomback et al (1992) noted that days 5 - 7 were associated with the least interindividual variation**
- | Kadir (1999) reported reduced vWF:Ag and vWF:Rco during the first 3 days and peak levels in the luteal phase**
- | Miller (2002) reported that during the first 4 days of the menses vWF was lowest and highest in days 9 and 10**
- | Insufficient data to recommend testing during specific part of cycle (UKHCDO guidelines 2004)**



Factors affecting vWF levels

ORAL CONTRACEPTIVE PILL/HORMONE REPLACEMENT THERAPY

- | Causes a small rise in the level of vWF:Ag and vWF:Rco
- | HRT has no effect on levels of vWF

PREGNANCY

- | Factor VIII:C and vWF levels change during pregnancy
- | Levels increase 3 – 5 fold during pregnancy
- | Most, but not all women with Type 1 show a similar rise to normals
- | Type 2A and 2M changes are variable; increased FVIII:C and vWF:Ag are not paralleled by an increase in vWF:Rco
- | No increase in Type 3
- | Levels should be assessed at 34 weeks to document any rise (Strong, 2006)

Which is the best assay to measure vWF 'function'?

- | vWF:Rco is sensitive to loss of multimers and platelet GPIb defects
- | However, poor reproducibility and detection limits for vWF:Rco may lead to misclassification of vWD
- | vWF 'activity' ELISA/LIA have improved reproducibility but may miss Type 2 subtypes
- | vWF:CB has higher sensitivity to loss of HMW multimers and improved sensitivity for confirming Type 1 and 3s
- | Rapid assay required for monitoring of vWF function (e.g. automated vWF:Rco or LIA)

Which is the best assay to measure vWF 'function'?

- | vWF:CB is less sensitive to platelet GPIb defects (i.e. 2M defects)

Type	n	vWF: Ag	vWF: Rco	vWF: CB
2A	6	22.5	<5	5
2B	1	32	17	5
2M	25	27	5	35

(Riddell et al, 2002)

- | Use of both vWF:Rco/vWF 'activity' assays AND vWF:CB required for diagnosis and classification of vWD
- | vWF:CB also required for detection of potential collagen binding abnormalities

Use of ratios to distinguish Type 1 and Type 2 vWD

- | Calculation of vWF function to vWF:Ag
- | First evaluated by Federici (2000) who reported that ratios of < 0.7 distinguished Type 1 from Type 2 vWD
- | Favaloro (2001) reported a cut off of < 0.5
- | Casonato et al (2001) reported that Type 2A and 2B patients had vWF:CB/vWF:Ag and vWF:Rco/vWF:Ag ratios of < 0.3 and < 0.7 respectively
- | Riddell et al (2002) demonstrated that the vWF:Rco/vWF:Ag and vWF:CB/vWF:Ag was < 0.7 for Types 2A and 2B. In 2M only the vWF:Rco/vWF:Ag was < 0.7
- | UKHCDO guidelines (2004) reported a cut off of < 0.7

Use of ratios to distinguish Type 1 and Type 2 vWD

- | Adcock et al (2006) reported the vWF:CB/vWF:Ag as < 0.5 and vWF:Rco/vWF:Ag < 0.7 for all Type 2A and 2B studied
- | If vWF:Rco/vWF:Ag was < 0.5 then 20% of Type 2s would have been missed
- | Ratios tended to be lower for 2A than 2B
- | Cut off could be dependent on different sources of collagen and platelets within assay systems
- | Lower ratios may indicate a poor response to DDAVP
- | Use of ratios may be less accurate for lower vWF:Ag levels

What is Vicenza vWD?

- | First described in a family from Northern Italy
- | Autosomal dominant inheritance pattern characterised by reduced vWF:Ag, reduced vWF function, normal platelet vWF content and the presence of larger than normal (supranormal) vWF multimers
- | First classified as Type 2M 'Vicenza', now considered Type 1
- | Casonato (2002) proposed that because the vWF platelet GPIb interaction was normal before and after DDAVP it should not be classified as 2M
- | Normally synthesised, stored and released but reduced in plasma
- | Characterised by vWF:Ag levels 6 – 12% and half life of vWF reduced 4.4 fold after DDAVP
- | Heterozygous mutation Arg1205His in the D3 domain
- | Casonato (2006) has reported a discrepancy between platelet vWF and plasma vWF levels

Use of DDAVP or concentrates for diagnosis

- | Favaloro (2006) considered the potential use of DDAVP or factor concentrates to aid diagnosis
- | Michiels et al (2005) reported on the differentiation of severe Type 1 vWD
- | Recessive Severe Type 1 can be distinguished from Type 3 by DDAVP challenge
- | Type 1 Vicenza – good response to DDAVP but half life times are short
- | Type 2M – good response of FVIII, vWF:Ag and vWF:CB, but poor response with vWF:Rco

Update on the classification of vWD

- | Report of the SSC on vWF – October 2006 (Sadler et al)
- | Progress in the understanding of pathophysiology of vWD
- | 1994 classification restricted vWD to mutations within the vWF gene
- | European and Canadian Type 1 vWD studies have indicated ~ 30% of cases had no vWF mutation
- | Canadian study revealed 6% of families had abnormal multimer results and vWF:Rco/vWF:Ag ratios < 0.6



Update on the classification of vWD

- | In the European study 41% had abnormal multimers
- | Type 1 can be caused by reduced secretion of functionally normal vWF with nearly normal multimer distribution
- | Mutations can (i) reduce secretion by impairing intracellular transport, (ii) accelerate clearance
- | Type 2A encompasses several physiological mechanisms
- | Reduction of HMW multimers is due to a defect in multimer assembly or increased sensitivity to ADAMTS-13

Update on the classification of vWD

- | **Type 2A also includes variants formally classified as IIC, IID and IIE**
- | **Mutations in vWF propeptide prevent multimerisation and give a multimer pattern that is devoid of satellite bands – originally IIC, Budde & Scheppenheim have now classified as 2C**
- | **Defects in the multimer assembly due to mutations in C terminal CK domain prevent dimerisation and small multimers are secreted – originally IID, Budde & Scheppenheim have now classified as 2D**



Update on the classification of vWD

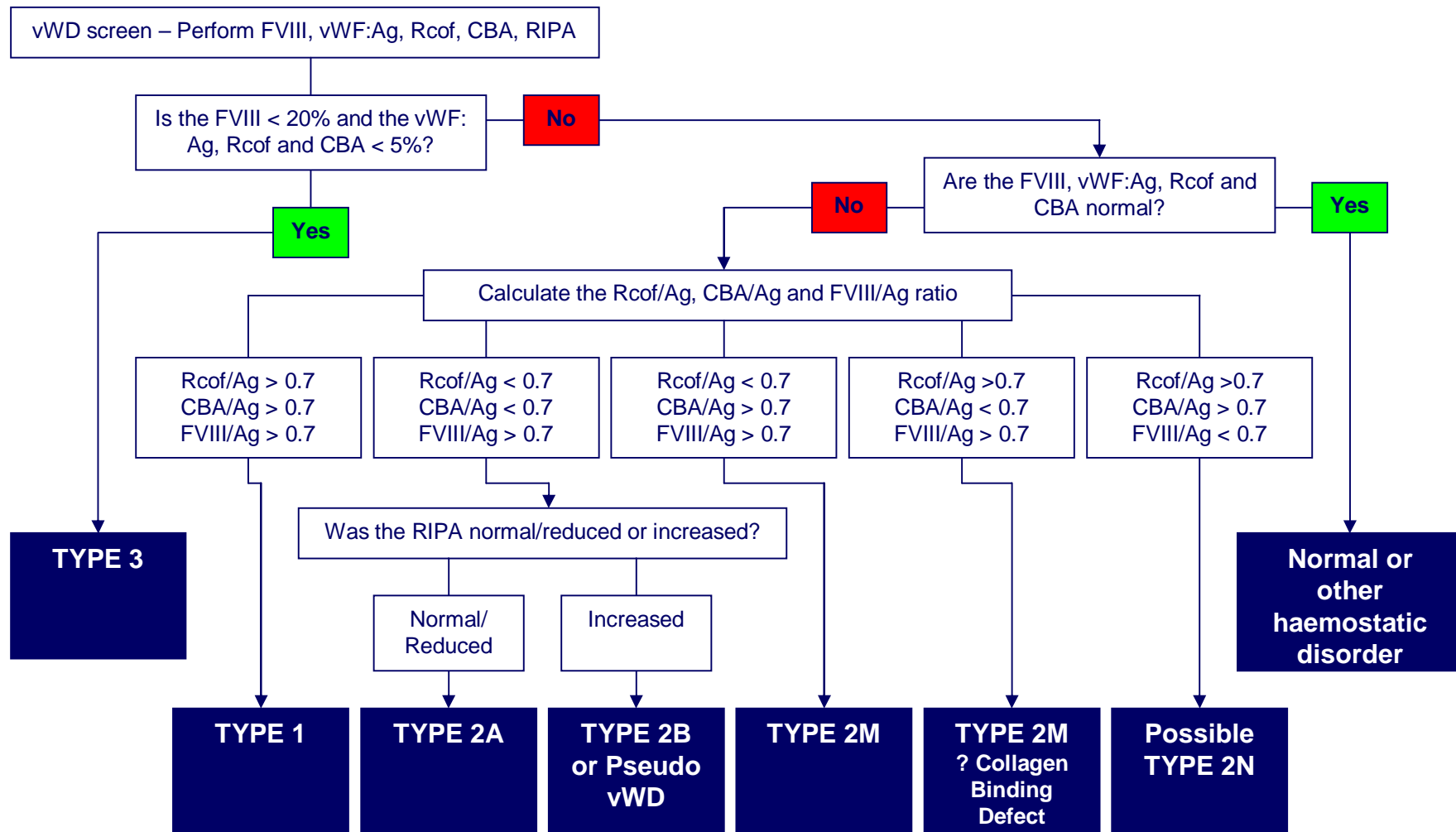
- | **Mutations in D3 domain interfere with intersubunit disulphide bond formation and produce a 'smeary' multimer pattern originally IIE, Budde & Scheppenheim have now classified as 2E**
- | **Additional studies needed to investigate effect of Type 2M mutations on platelet binding, multimer distribution and subunit degradation**
- | **Clinical significance of this heterogeneity is under investigation which may support future subdivision of Types 1 and 2A**

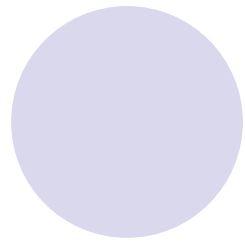
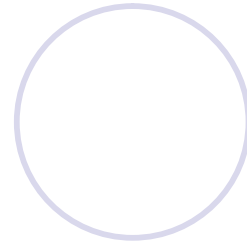
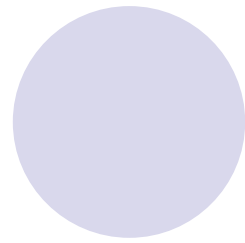
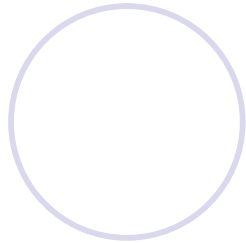
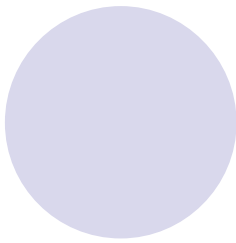


RECOMMENDATIONS

- | **Assessment of vWD requires both clinical evaluation and appropriate laboratory testing**
- | **Haemostatic screening tests do not exclude vWD but should be used to exclude other haemostatic disorders**
- | **If vWD is suspected by clinical history, the FVIII:C, vWF:Ag and a measure of vWF function should be performed**
- | **vWF should be assessed by assays sensitive to HMW multimer defects and platelet GPIb/collagen binding defects**
- | **Further subtyping should be done using the RIPA, Factor VIII binding assays and multimers**
- | **Interpretation of results must take into account physiological and environmental factors**
- | **All abnormal results must be repeated at least once to confirm original findings**

vWD Testing Algorithm





E.A. von WILLEBRAND
von Willebrands sjukdom

EUROPA

230

MARKET