The Laboratory Diagnosis of von Willebrand Disease: current insights

ECAT participants meeting, November 2010 Leiden

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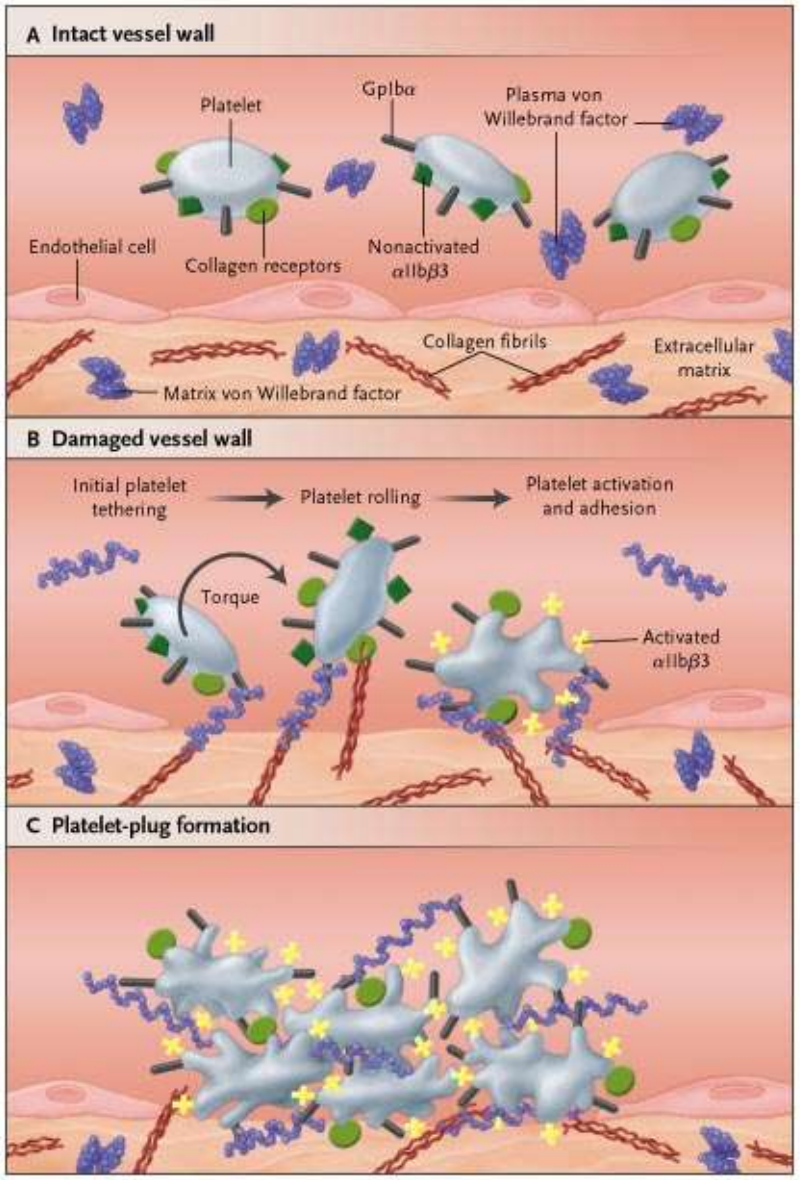
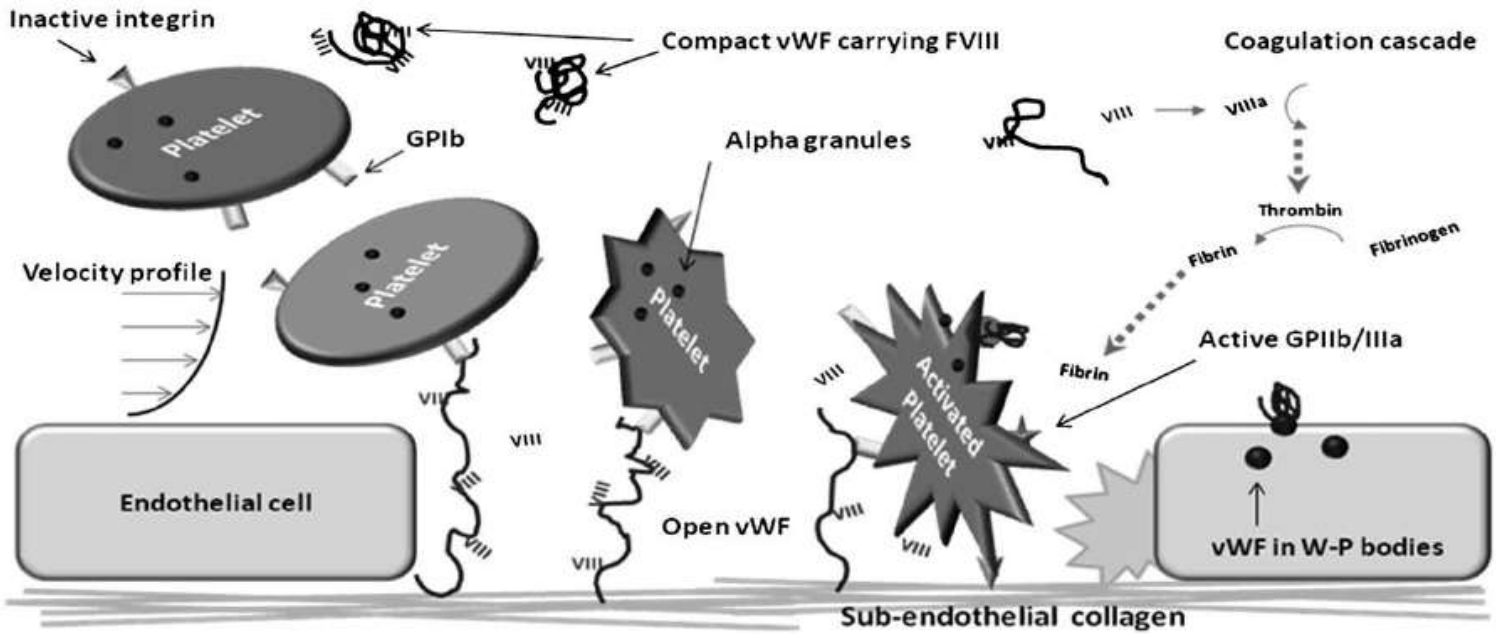
Functions of Von Willebrand factor



1. Adhesion of platelets
2. Aggregation of platelets
3. Carrier of factor VIII

Mannucci PM, NEJM 2004


Inactive integrin
Compact vWF carrying FVIII
Coagulation cascade
Platelet
GPIb
Alpha granules
VIII
VIIIa
Thrombin
Fibrin
Fibrinogen
Velocity profile
Platelet
Platelet
Activated Platelet
Active GPIIb/IIIa
Fibrin
Endothelial cell
Open vWF
vWF in W-P bodies
Sub-endothelial collagen

FINSKA LÄKARESÄLLSKAPETS HANDLINGAR. BAND LXVII. N:O 2.

ORIGINALARTIKLAR.

(Från Diakonissjukhusets i Helsingfors medicinska avdelning.
Docent E. A. v. WILLEBRAND.)

Hereditär pseudohemofili.

Av

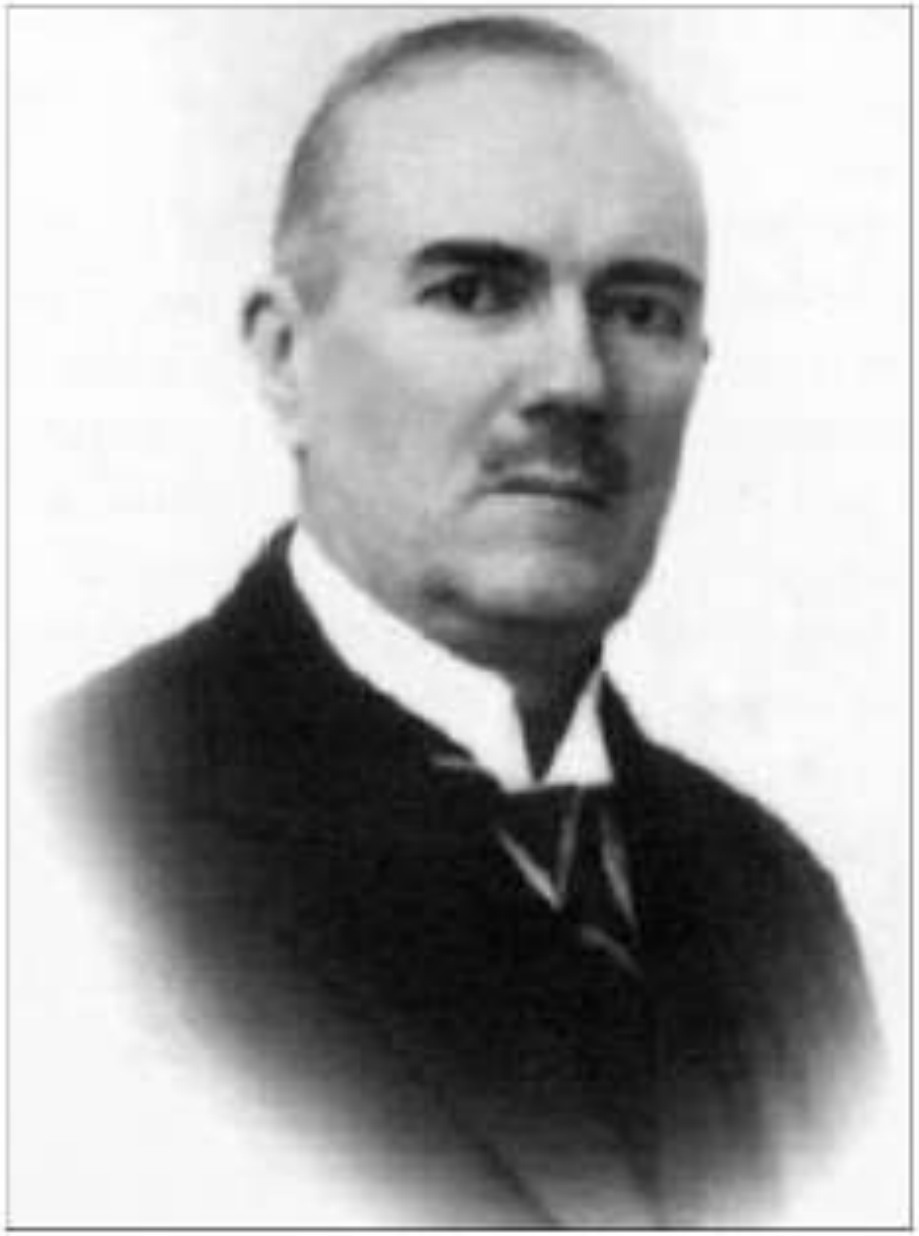
E. A. v. Willebrand.

(Med 3 figurer i texten.)

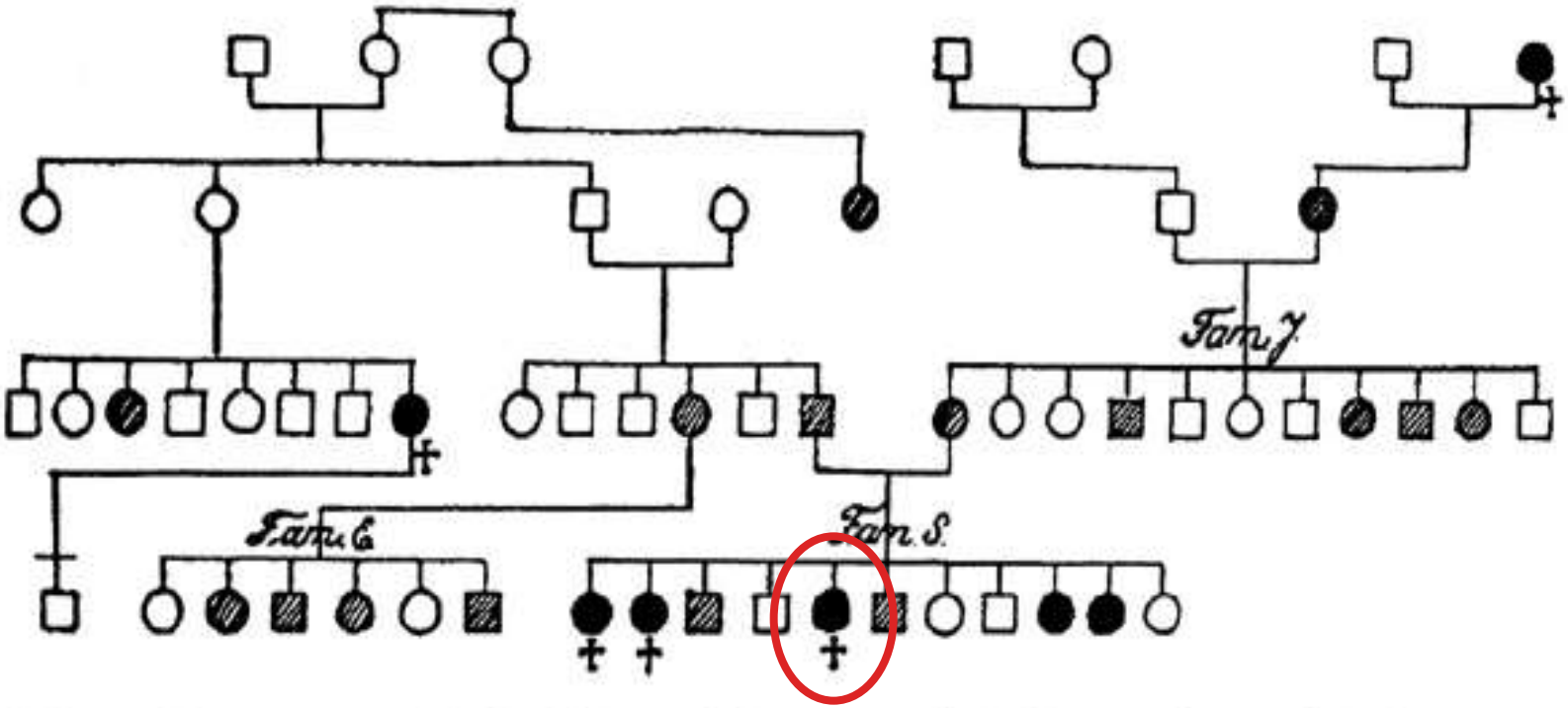
1. Sjukdomsbegrepp. Tidigare observerade fall.

I sitt nya stora arbete över de hemorragiska diateserna framhåller E. FRANK (Breslau), att den klassiska hemofilien är en så exkvisit hereditär—familjär anomali, att det kan ifrågasättas, huruvida över huvud sporadiska fall av sjukdomen existera. Däremot är, säger han, den klassiska trombopenien så utpräglat sporadisk, att man kan diskutera, om en familjär form av densamma alls förekommer. Med trombopeni avses här den sjukdom, som sedan gammalt bär namnet morbus maculosus WERLHOFI eller purpura haemorrhagica och som på senaste tid av FRANK och en del andra forskare betecknats såsom essentiell trombopeni.

Hittills har man velat betrakta ärftlig blödaresjukdom och hemofili såsom synonyma begrepp. Men om man genomögnar hithörande litteratur, skall man finna, om ock i ett fåtal fall, beskrivningar över en familjär form av hemorragisk diates, som redan därigenom skiljer sig från äkta 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 skarp gräns mellan ifrågavarande familjära lidande och hemofilien. Därom mera längre fram i kap. 6 om diagnosen.



Åländsk blödaresläkt



□man ○kvinna icke-blödare. ▨man ◍kvinna med lindrig blödaresjuka. ●kvinna med svår blödaresjuka † död av förblödning

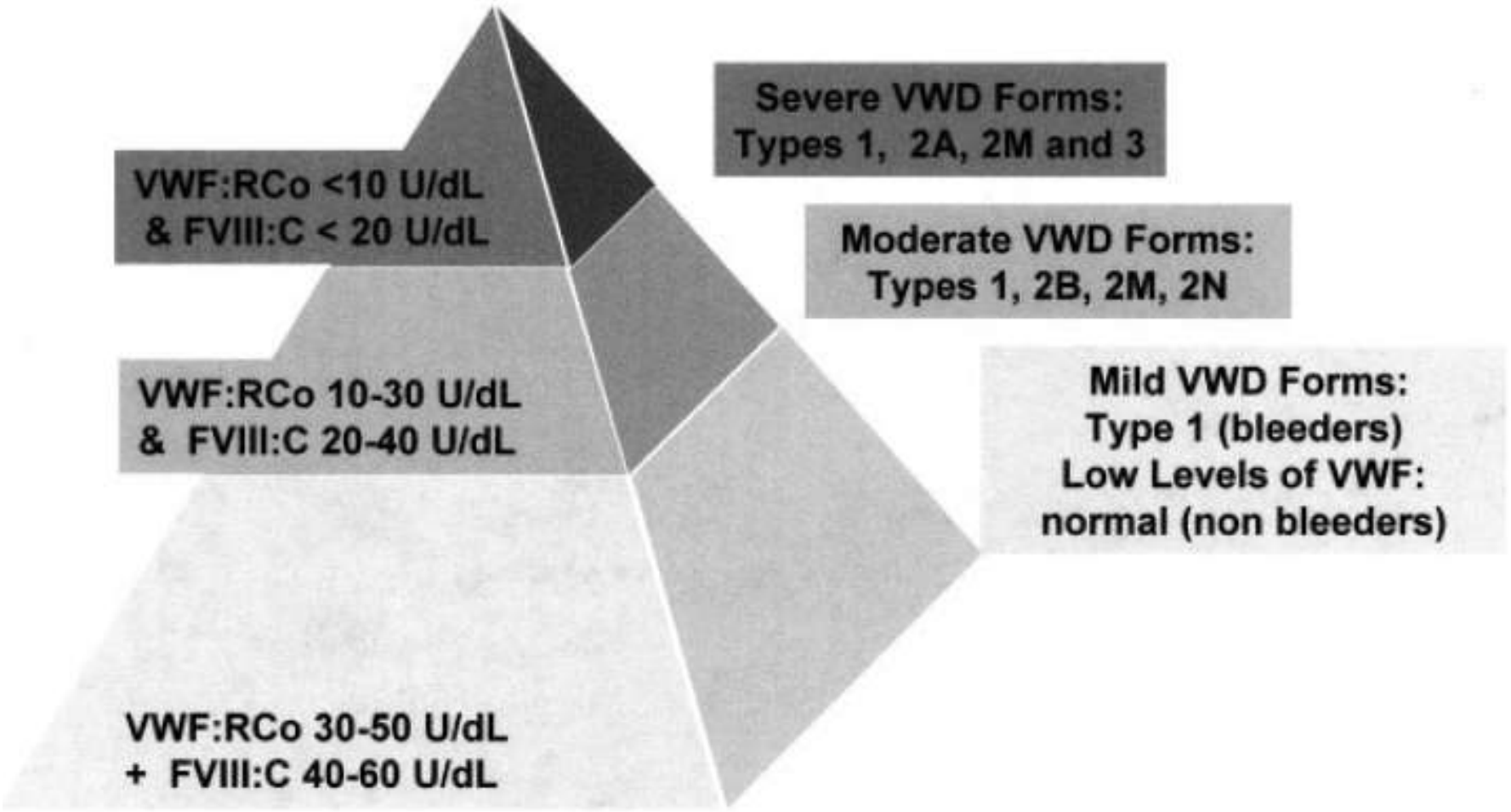
Von Willebrand Disease (VWD)

- Most common inherited bleeding disorder
- Mucocutaneous bleeding symptoms
- Prevalence: 0.5-1%
- Autosomal dominant / recessive
- Caused by a deficiency / abnormality of von Willebrand Factor (VWF)

von Willebrand disease: Symptoms

- Mucosal bleedings
  - Nose bleeds
  - Gingiva bleeds
  - Menorrhagia
- Haematomas
- Bleeding after surgical interventions
  - dental extractions
  - Adeno tonsillectomy
- Bleeding after delivery (type 2/3)

Severity of Von Willebrand disease



Federici A, et al. STH 2006

Von Willebrand Disease (VWD)

- Various types of VWD
  - Type 1: quantitative disorder, reduced level of normal VWF
  - Type 2: qualitative disorder, abnormal VWF molecule
  - Type 3: quantitative disorder, no detectable VWF in circulation

Von Willebrand disease classification

Type	Description
1	Partial quantitative defect of VWF
2	Qualitative defect of VWF
2A	Reduced platelet dependent vWF function, with a reduction of high molecular weight multimers
2B	Increased vWF platelet-dependent vWF function, with a reduction of high molecular weight multimers. Characterized by thrombocytopenia, increased affinity to GP1b-IX-V complex
2M	Reduced platelet-dependent vWF function, with normal multimer pattern
2N	Reduced affinity of vWF for factor VIII
3	Total absence of VWF

Sadler JE, et al. JTH 2006;4:2103-14

von Willebrand disease: diagnosis

- History: patient and family

- Use a validated questionnaire to quantify the bleeding severity

Tosetto Bleeding Score

Table 1 Assigned score for each bleeding symptom

	Score					
Symptom	−1	0	1	2	3	4
Epistaxis	–	No or trivial (less than 5)	> 5 or more than 10′	Consultation only	Packing or cauterization or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Cutaneous	–	No or trivial (< 1 cm)	> 1 cm and no trauma	Consultation only		
Bleeding from minor wounds	–	No or trivial (less than 5)	> 5 or more than 5′	Consultation only	Surgical hemostasis	Blood transfusion or replacement therapy or desmopressin
Oral cavity	–	No	Referred at least one	Consultation only	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Gastrointestinal bleeding	–	No	Associated with ulcer, portal hypertension, hemorrhoids, angiodysplasia	Spontaneous	Surgical hemostasis, blood transfusion, replacement therapy, desmopressin, antifibrinolytic	
Tooth extraction	No bleeding in at least two extraction	None done or no bleeding in one extraction	Referred in < 25% of all procedures	Referred in > 25% of all procedures, no intervention	Resuturing or packing	Blood transfusion or replacement therapy or desmopressin
Surgery	No bleeding in at least two surgeries	None done or no bleeding in one surgery	Referred in < 25% of all surgeries	Referred in > 25% of all procedures, no intervention	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Menorrhagia	–	No	Consultation only	Antifibrinolytics, pill use	Dilatation and currettage, iron therapy	Blood transfusion or replacement therapy or desmopressin or hysterectomy
Postpartum hemorrhage	No bleeding in at least two deliveries	No deliveries or no bleeding in one delivery	Consultation only	Dilatation and currettage, iron therapy, antifibrinolytics	Blood transfusion or replacement therapy or desmopressin	Hysterectomy
Muscle hematomas	–	Never	Post trauma no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Hemarthrosis	–	Never	Post trauma no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring Surgical intervention or blood transfusion
Central nervous system bleeding	–	Never	–	–	Subdural, any intervention	Intracerebral, any intervention

Tosetto A, et al. JTH 2006

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Oral cavity	–	No	Referred at least one	Consultation only	Surgical hemostasis	Blood transfusion
					currettage, iron therapy	or replacement therapy or desmopressin or hysterectomy
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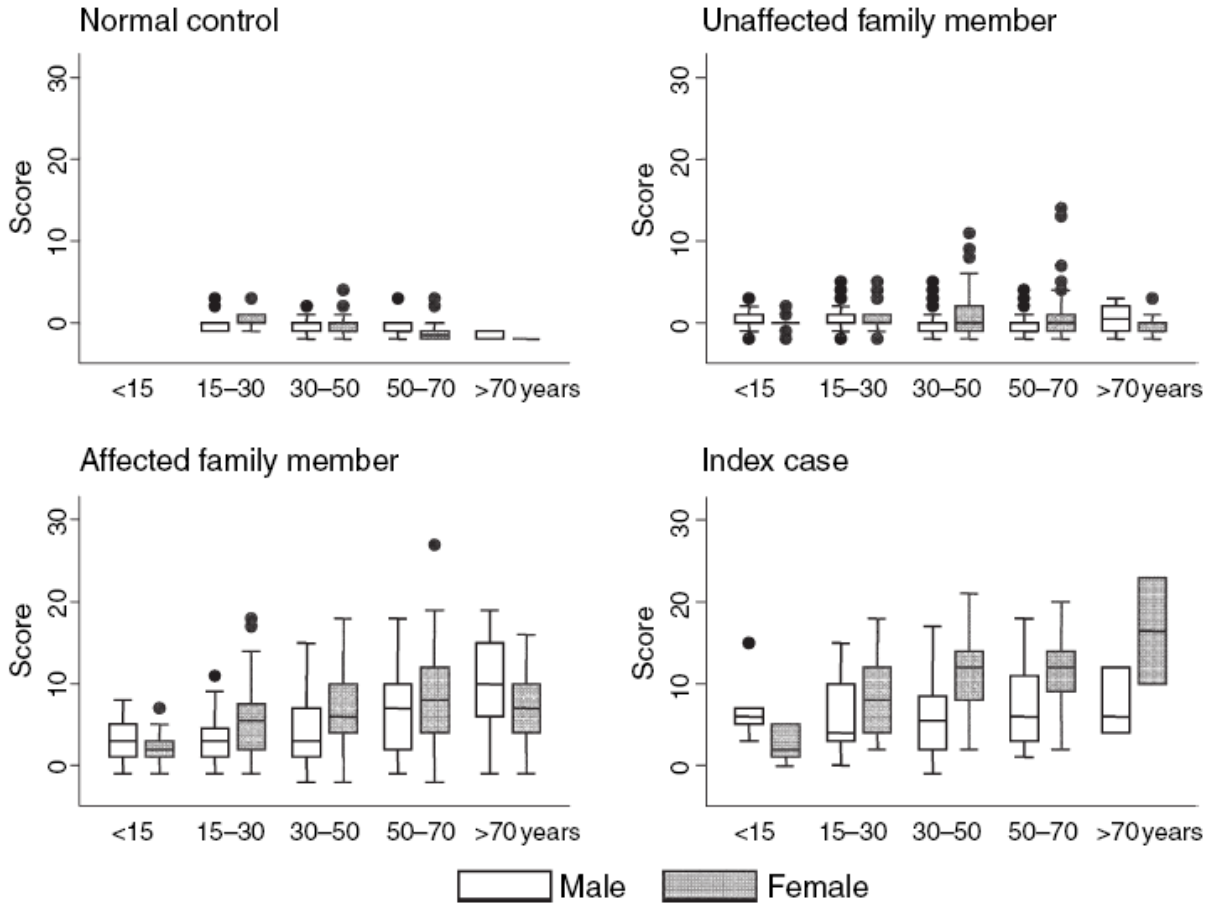
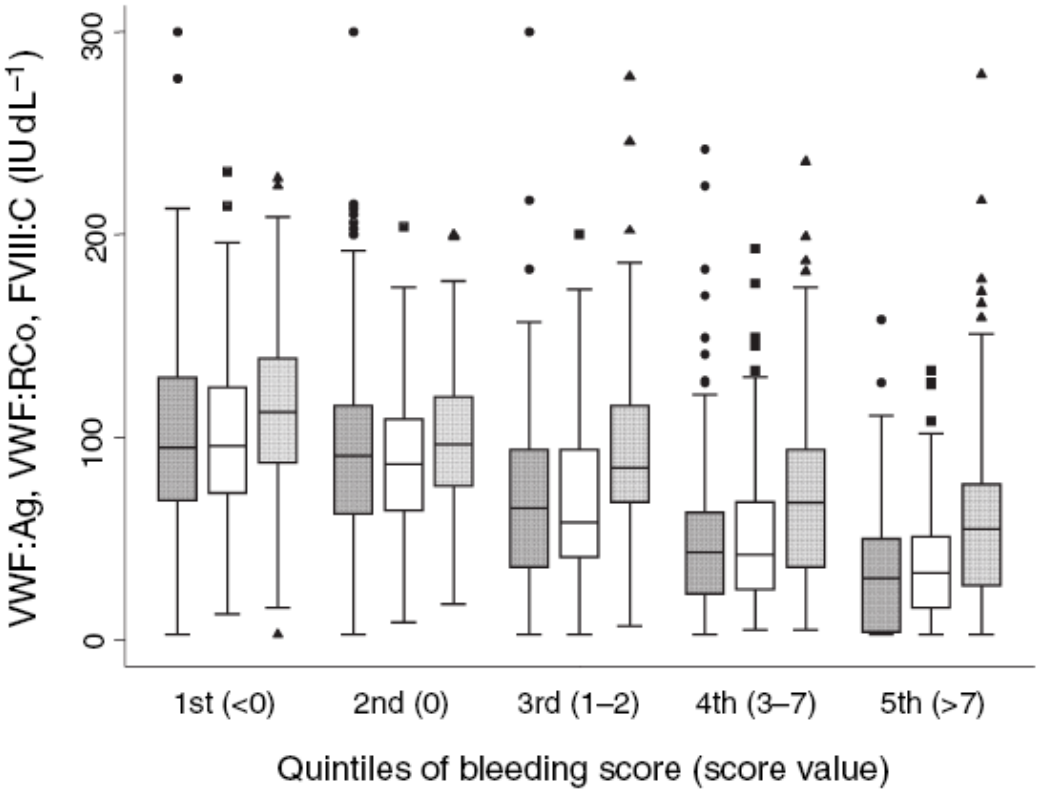
Tosetto A, et al. JTH 2006;4:766-773.


Normal control
Unaffected family member
Affected family member
Index case
Score
0
10
20
30
<15
15–30
30–50
50–70
>70 years
Male
Female


VWF:Ag, VWF:RCo, FVIII:C (IU dL^{-1})
0
100
200
300
1st (<0)
2nd (0)
3rd (1–2)
4th (3–7)
5th (>7)
Quintiles of bleeding score (score value)

National study of moderate and severe von Willebrand disease in the Netherlands
WiN study

- National study including children and adults
- Moderate and severe Von Willebrand Disease

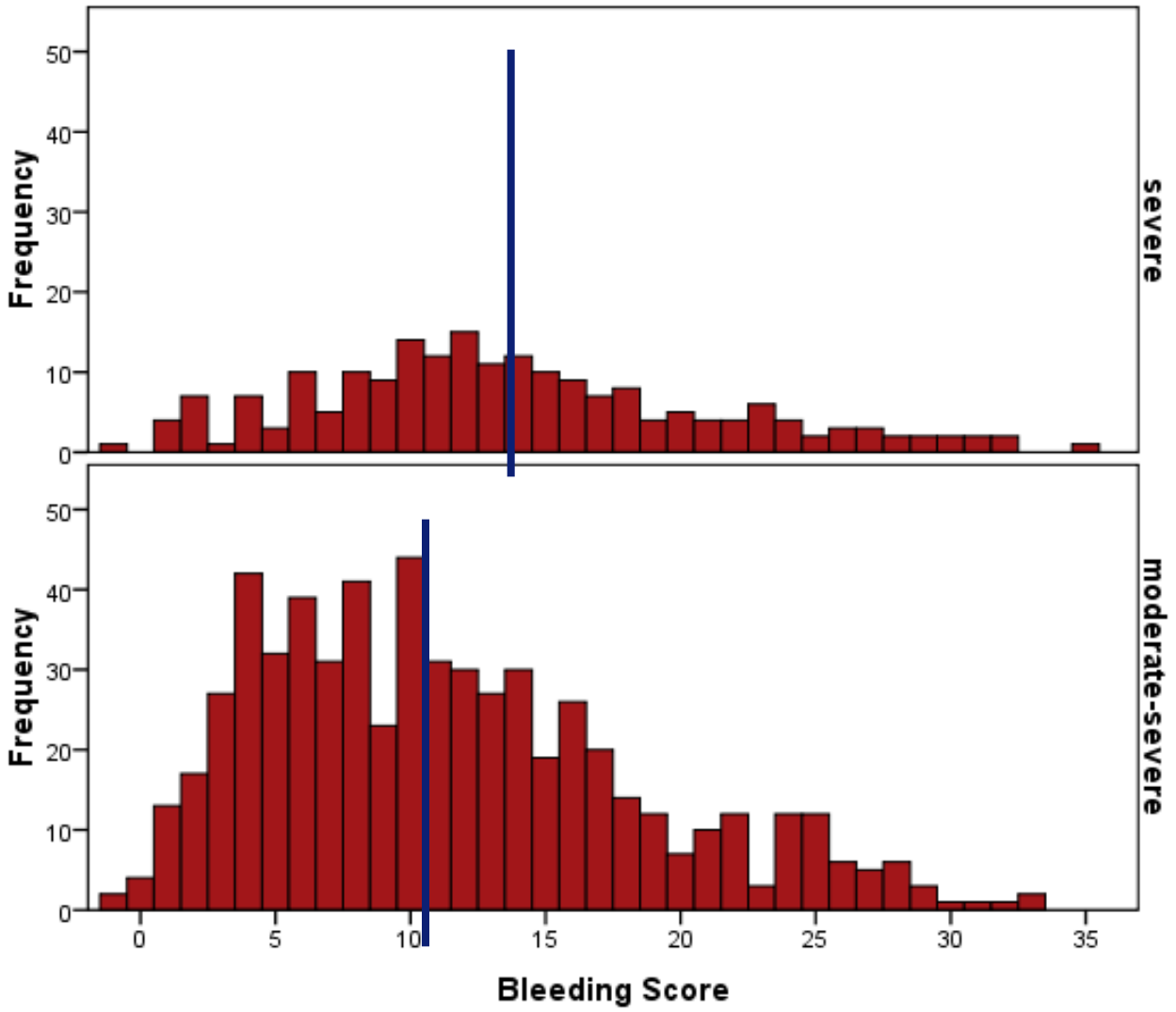
  moderate: VWF 10-30 IU/dL or FVIII 20-40 IU/dL

  severe: VWF < 10 IU/dL or FVIII < 20 IU/dL
- All types of VWD
- Questionnaire and blood sample for plasma and DNA

Patients' characteristics WiN study

		total n=806	
sex	males (n,%)	325	40%
	females (n,%)	481	60%
age	males (median, range)	37	0-84
	females (median, range)	44	0-87
VWD severity	severe VWD	201	40%
	moderate VWD	605	60%
VWD type	1 (n,%)	460	57%
	2 (n,%)	293	36%
	3 (n,%)	37	5%

Bleeding score in moderate and severe VWD



de Wee, submitted

von Willebrand disease: laboratory diagnosis

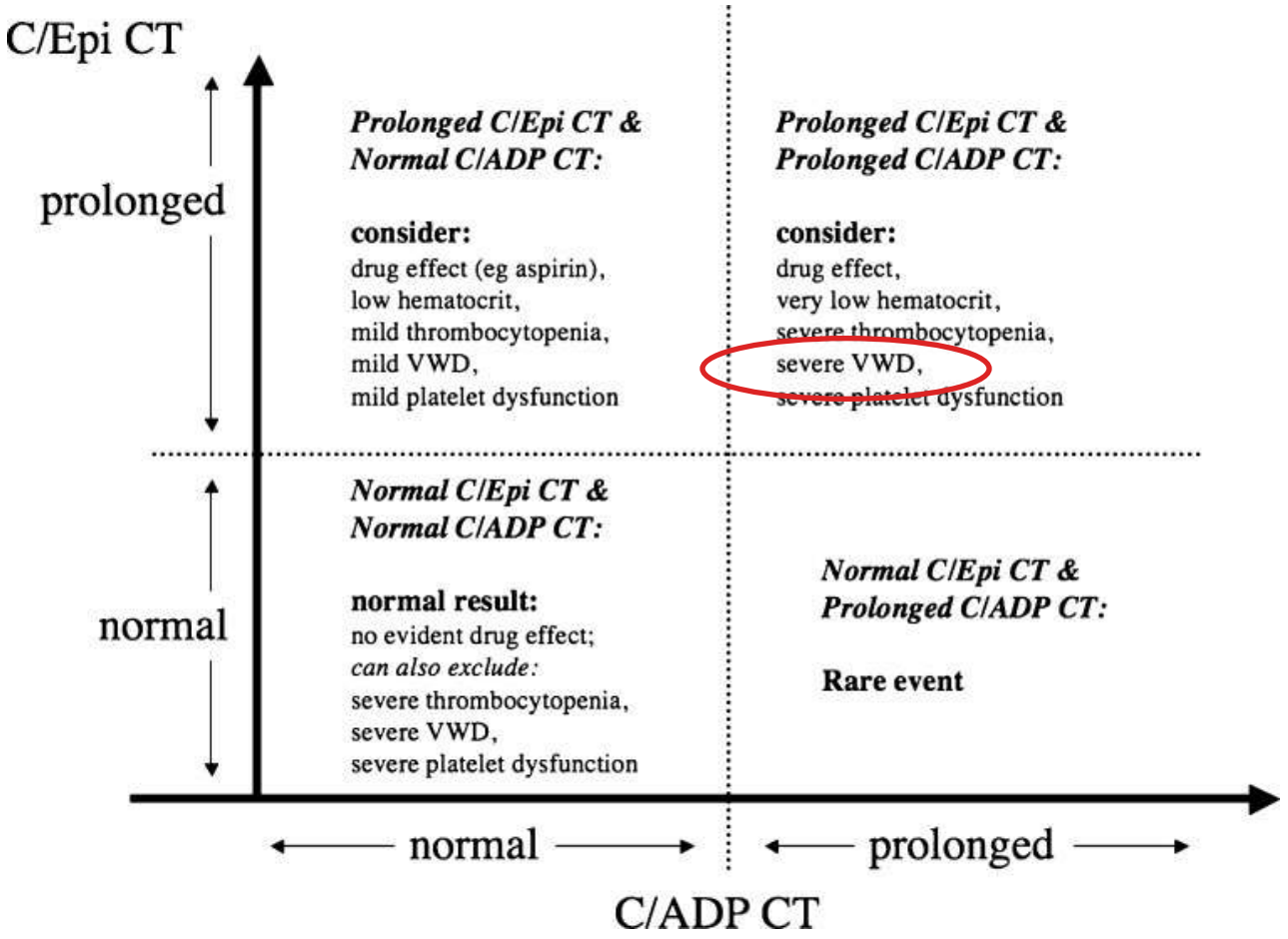
- Screening tests
  - Bleeding time
  - Closure time (PFA-100®)
  - APTT, PT, fibrinogen, platelet count
- Routine laboratory tests
  - Factor VIII:C
  - VWF:antigen
  - VWF activity (ristocetin cofactor activity, collagen binding activity)
- Specialized laboratory tests
  - multimer pattern
  - RIPA
  - DDAVP testing
  - VWF propeptide
  - FVIII binding assay
  - DNA analysis

Screening test for VWD

- Bleeding time
  - Lack of standardization, low reproducibility
  - Not sensitive enough for mild cases of VWD
  - Not predictive of bleeding tendency

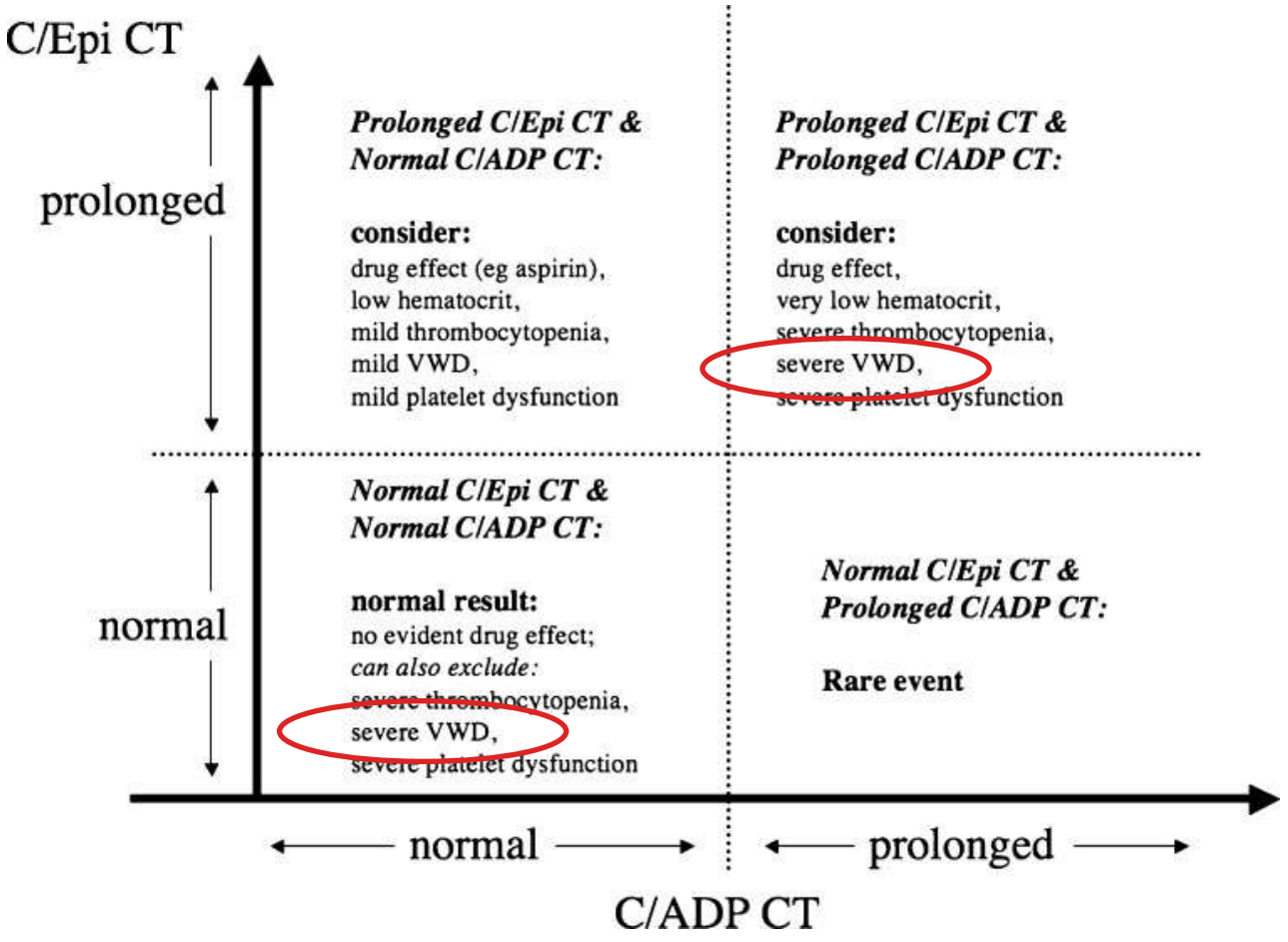
- PFA-100®
  - High sensitivity for severe VWD
  - Sensitivity as low as 50% in mild VWD
  - High variability up to 20%

Interpretation of PFA



Favaloro 2008

Interpretation of PFA



Favaloro 2008

Sensitivity and specificity of PFA 100® for VWD

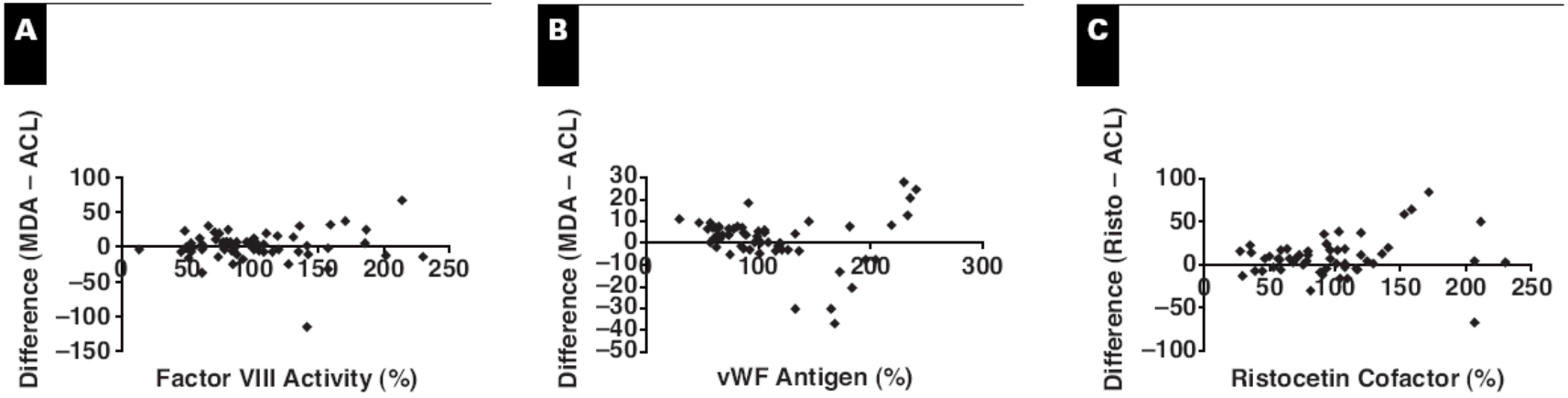
	Fressinaud et al.			Cattaneo et al.			Leebeek et al.		
	CT-Epi	CT-ADP	BT	CT-Epi	CT-ADP	BT	CT-Epi	CT-ADP	BT
Overall sensitivity	96 %	100 %	66 %	87 %	88 %	65 %	70 %	51 %	25 %
Overall specificity	96 %	99 %	-	95 %	95 %	-	63 %	67 %	76 %
PPV	93 %	98 %	-	96 %	96 %	-	85 %	88 %	77 %
NPV	98 %	100 %	-	84 %	86 %	-	41 %	23 %	24 %

FVIII and VWF assays

- FVIII:C
- VWF:Ag
  - Immunoassay with high precision
- VWF:RCo (functional assay)
  - Measures the capacity of VWF to interact with GP1b/IX complex in the presence of ristocetin
  - Plasma VWF agglutinates formalin fixed reagent platelets
    - Several commercial kits
    - High inter and intra laboratory variability
    - Not precise at low levels of RCo activity
- VWF:CB
  - Measures the ability of VWF to bind to collagen
    - A3 domain of VWF
    - High molecular weight multimers
    - Results depend on type of collagen used (I or III)

VWF activity (HemosIL)

- Assay based on latex particles conjugated to MAb directed against VWF GPIb binding site



Salem RO et al. Am J Clin Pathol 2007

FVIII and VWF assays

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- VWF:Ag
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- VWF:CB
  - Measures the ability of VWF to bind to collagen
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    - High molecular weight multimers
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Additional VWF assays

- RIPA assay
  - Low dose (<0.5 mg/ml) ristocetin induced platelet aggregation assay (type 2B or platelet type VWD)

- VWF: FVIII binding assay
  - To measure the ability of VWF to bind FVIII (type 2)

- VWF multimer analysis

Multimer analysis

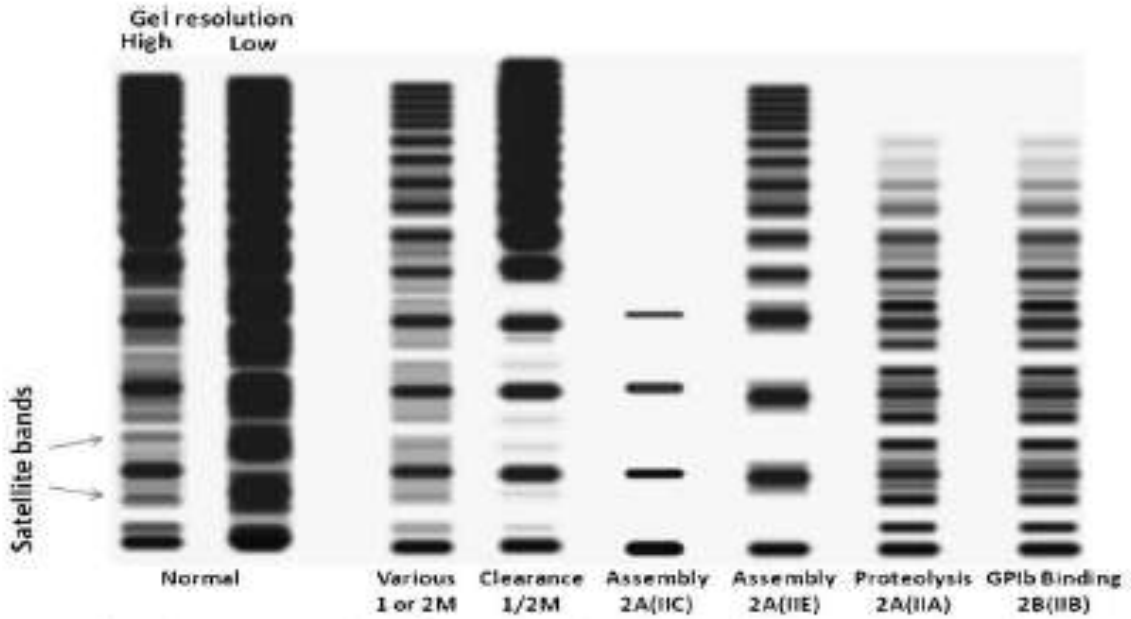
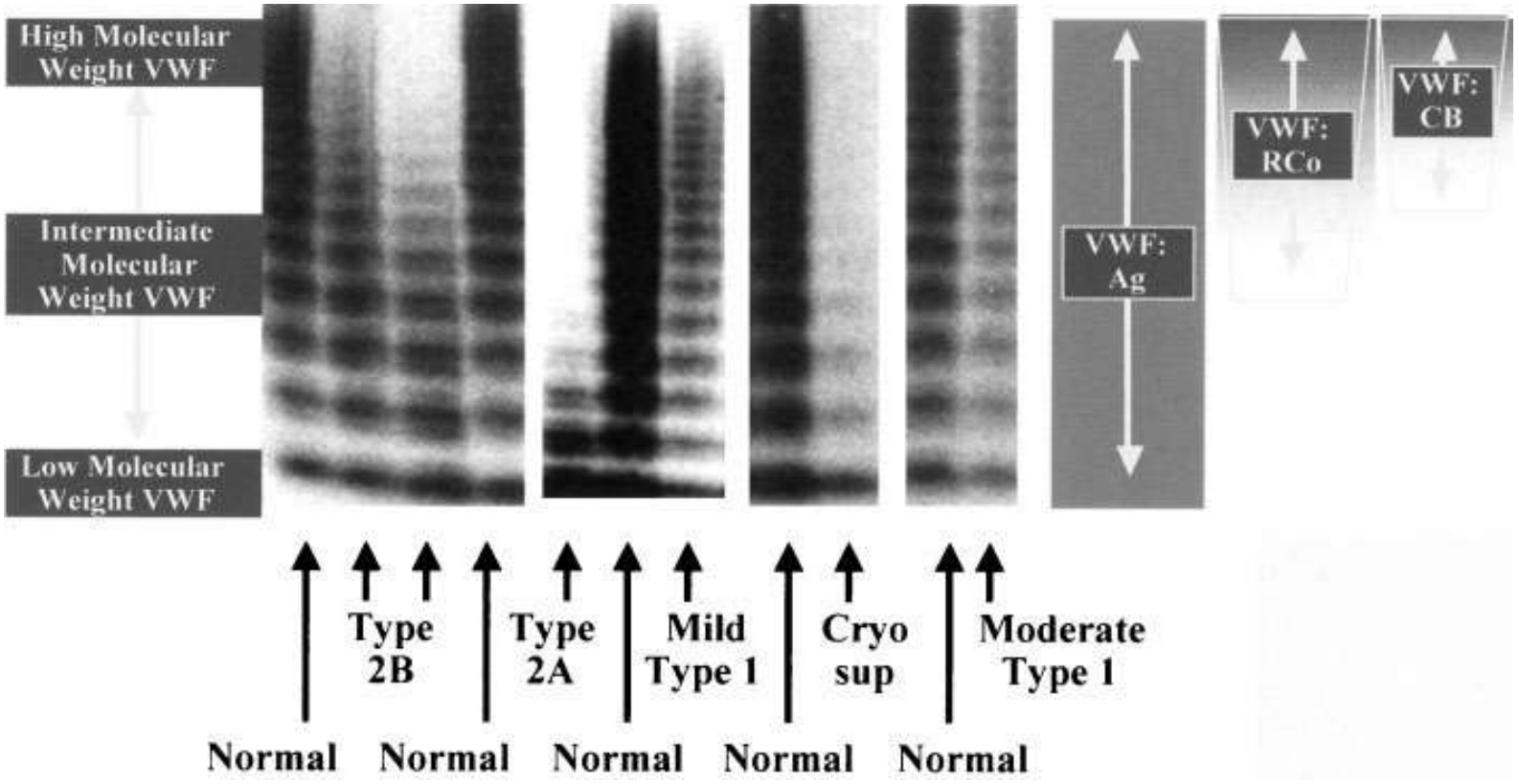


Fig. 3. Schematic representation of representative vWF multimer gels. Low-resolution gels show a distribution of multimers and are able to resolve broad patterns of small, intermediate, and large multimers. Higher resolution gels are needed to visualize satellite bands representing degradation products and flank main multimers. Various patterns are characterized predominantly by the main features of total intensity, distribution of sizes, and abnormalities of the satellite bands corresponding to different molecular mechanisms as discussed in the text.

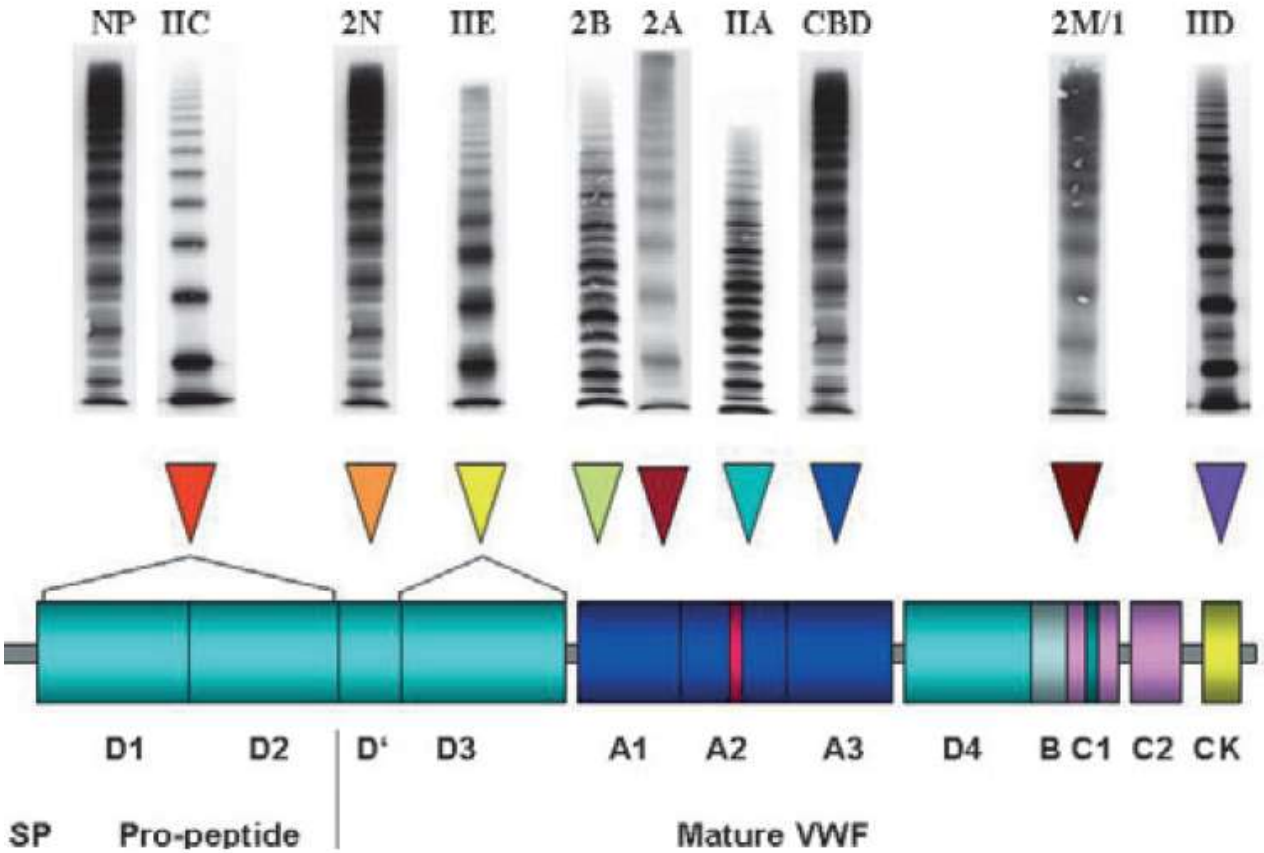
Torres 2009

Multimer analysis



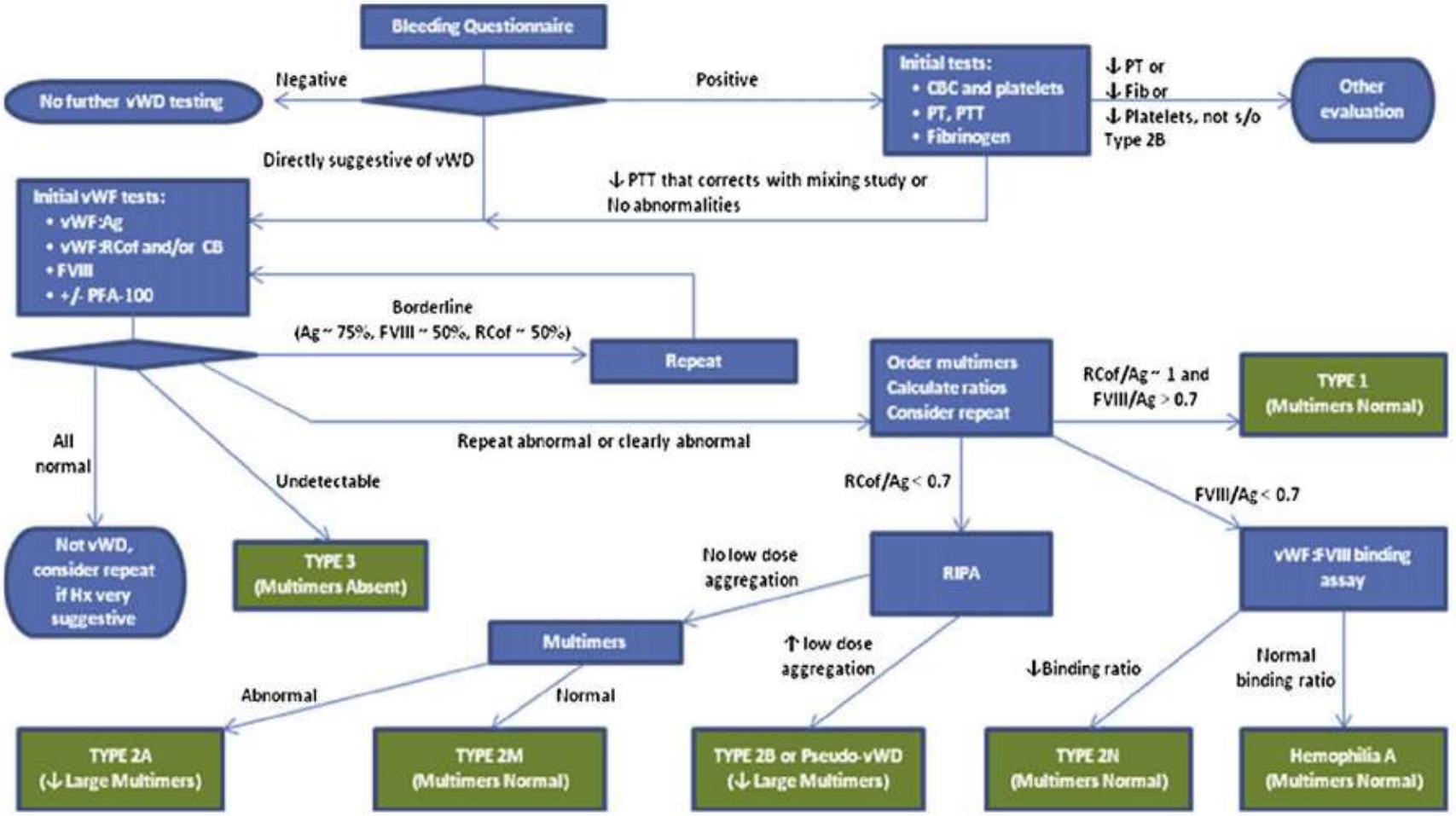
Favaloro 2006

Multimer analysis



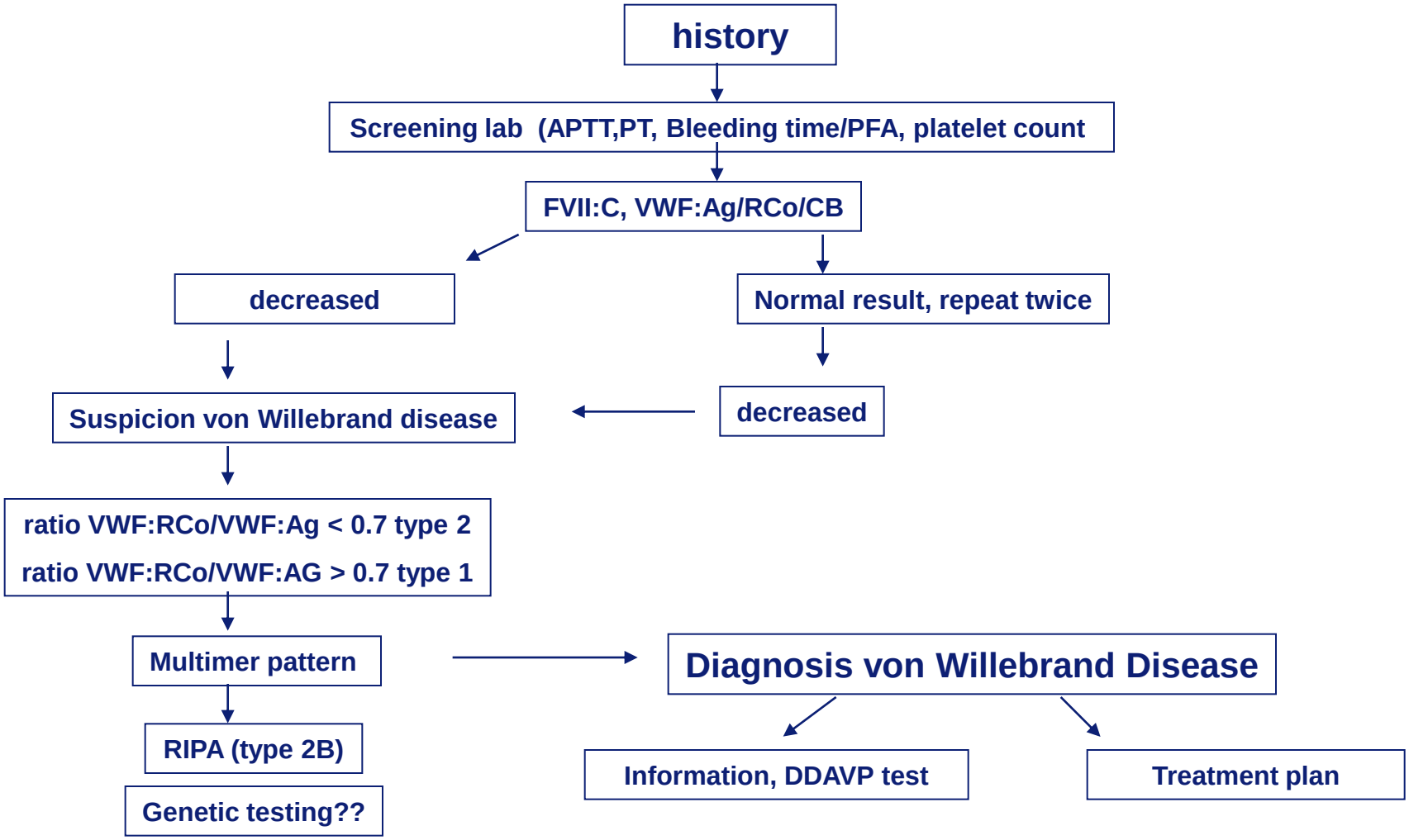
Budde U, Haemophilia 2008;14s:27-38

Diagnostic Algorithm



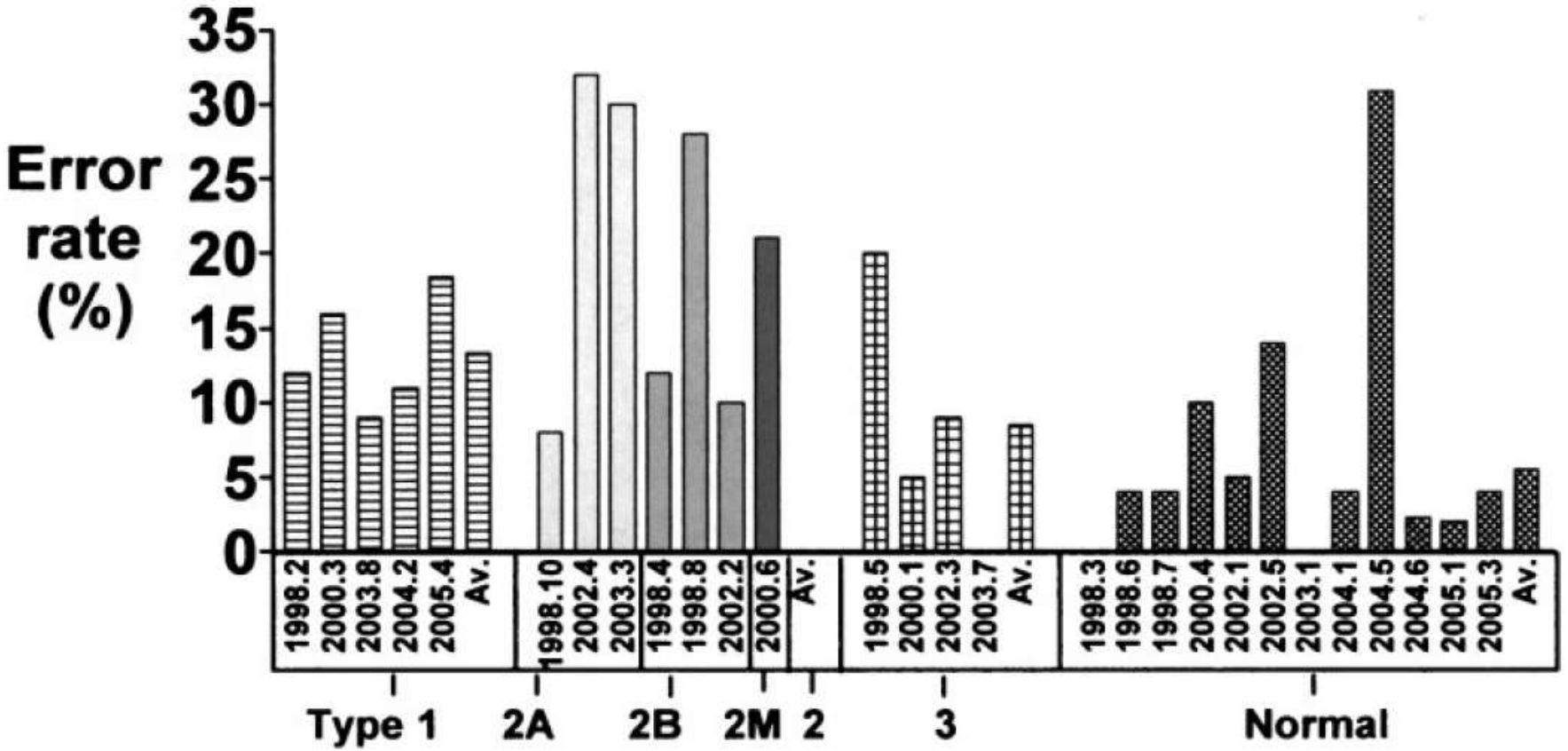
Torres 2009

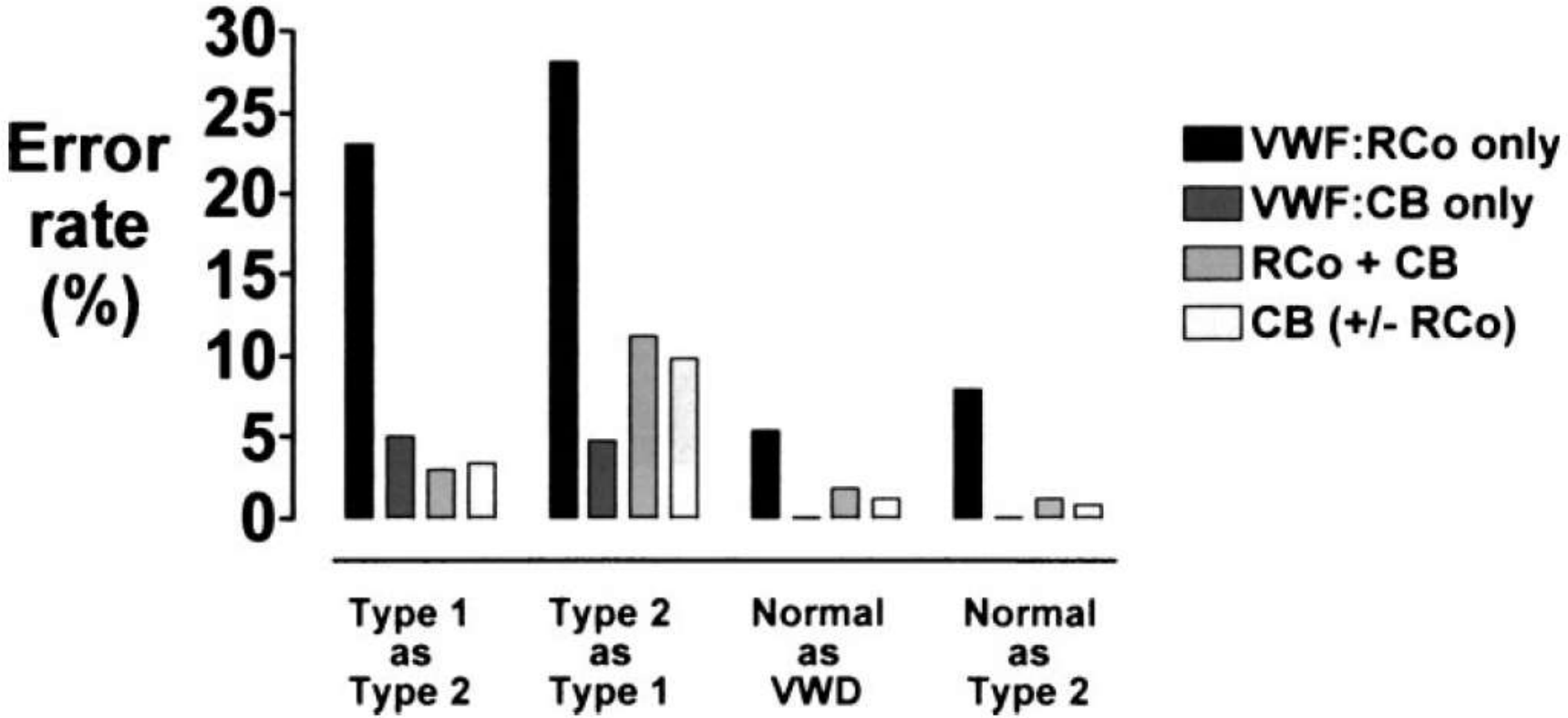
von Willebrand disease: algorithm diagnosis



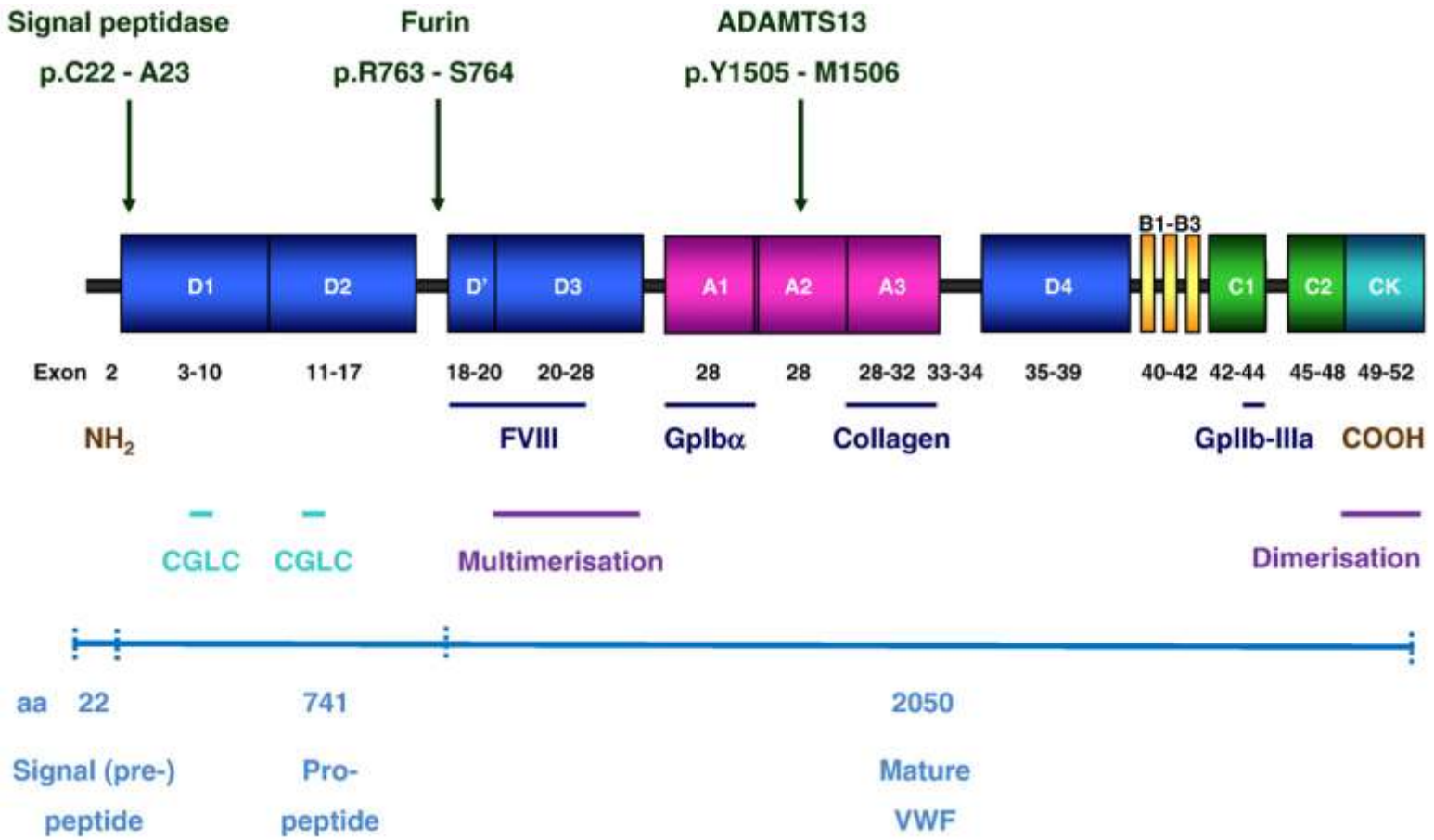
Reducing Errors in Identification of von Willebrand Disease: The Experience of the Royal College of Pathologists of Australasia Quality Assurance Program

Emmanuel J. Favaloro, Ph.D., M.A.I.M.S.,[1] Roslyn Bonar, B.Sc.,[1] Geoff Kershaw, B.Sc., F.A.I.M.S.,[1] John Sioufi,[1] Ross Baker, M.D.,[1] Mark Hertzberg, M.D., Ph.D.,[1] Alison Street, M.D.,[1] and Katherine Marsden, M.D.[1] (on behalf of the RCPA QAP in Haematology)


Error rate (%)
35
30
25
20
15
10
5
0
1998.2
2000.3
2003.8
2004.2
2005.4
Av.
1998.10
2002.4
2003.3
1998.4
1998.8
2002.2
2000.6
Av.
1998.5
2000.1
2002.3
2003.7
Av.
1998.3
1998.6
1998.7
2000.4
2002.1
2002.5
2003.1
2004.1
2004.5
2004.6
2005.1
2005.3
Av.
Type 1
2A
2B
2M
2
3
Normal

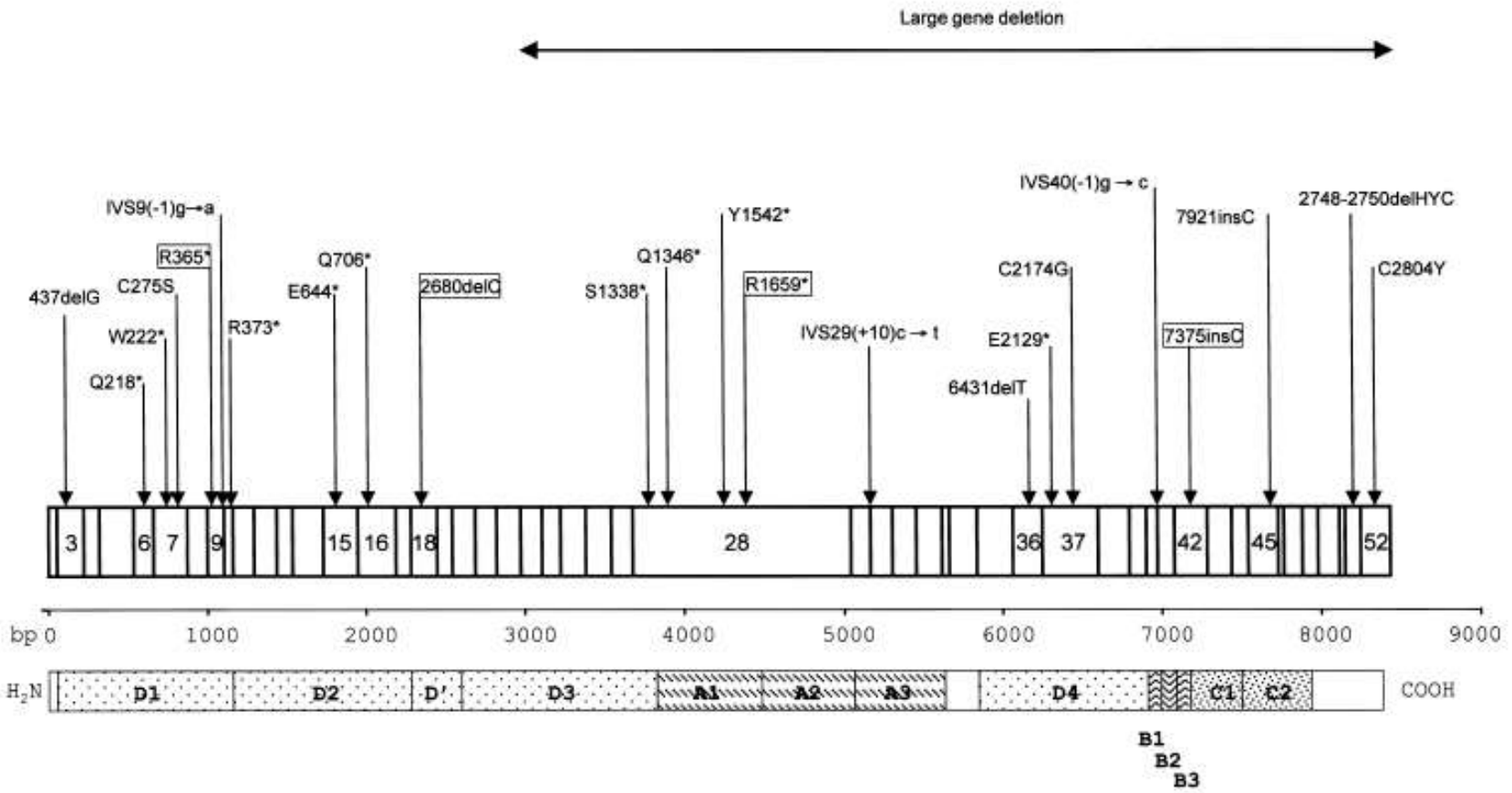

Error rate (%)
30
25
20
15
10
5
0
Type 1 as Type 2
Type 2 as Type 1
Normal as VWD
Normal as Type 2
VWF:RCo only
VWF:CB only
RCo + CB
CB (+/- RCo)

VWD type 3: genetic background



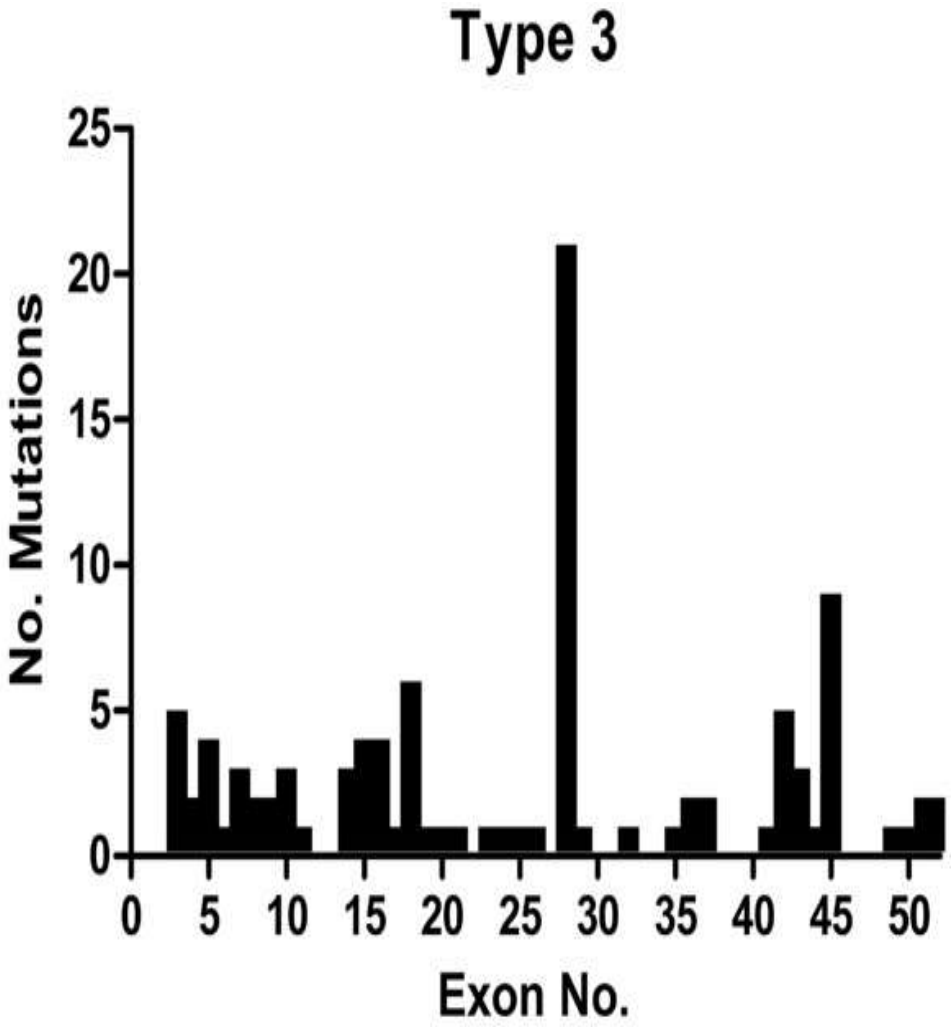
Goodeve AC. Blood Reviews 2010 Epub on line

VWD type 3: mutations in Italian patients



Baronciani L, et al. Thromb Haemostas 2000

VWD type 3: mutation frequency in Sheffield VWD database



Goodeve AC, Blood Reviews, 2010 Epub on line

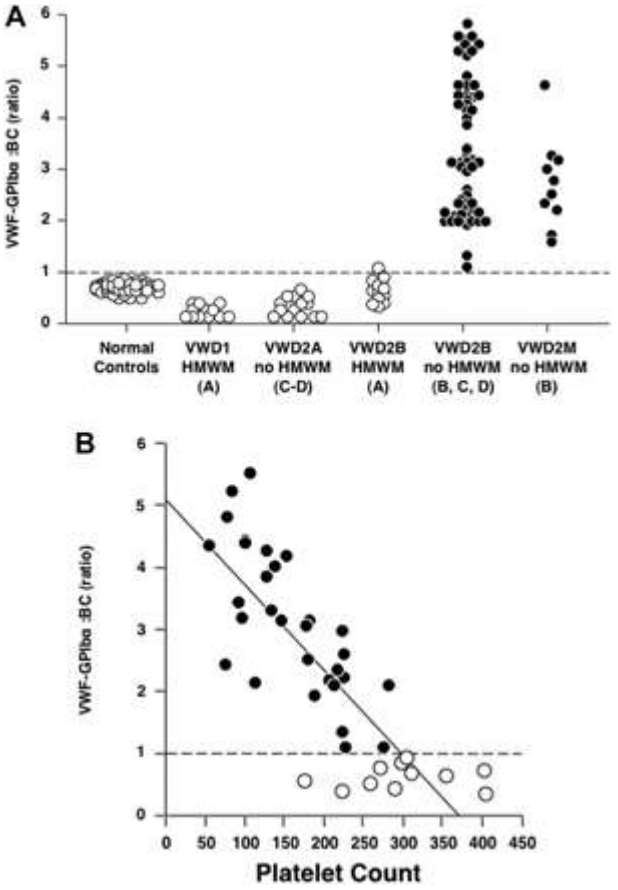
VWF propeptide

- VWF PP has a short half-life (2-3 hours)
- Determined by ELISA
- High ratio of PP:VWF Ag is seen in patients with high clearance of VWF

Active VWF

- Uses nanobodies against the active site of VWF

- Determines VWF in its active GPIb-a-binding conformation



Federici A, et al. Blood 2009

Conclusions

- VWD is a heterogeneous disorder with varying bleeding phenotype
- Laboratory diagnosis is difficult and consists of both screening tests and more specialized tests
- Use of algorithm is necessary for correct diagnosis
- New test are in development, but need to be validated