



# XXIV Congresso Nazionale SISET

Abano Terme, 9-12 novembre 2016



## *Aspetti pratici nella gestione della malattia di von Willebrand*

### **Diagnosi della Malattia di von Willebrand**

***Augusto B. FEDERICI***

**Hematology and Transfusion Medicine**

**Luigi Sacco University Hospital, University of Milan**

**[augusto.federici@unimi.it](mailto:augusto.federici@unimi.it)**



# Disclosures for Augusto B. Federici, MD



- **Consultancy:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Honoraria:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Speakers Bureau:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Membership on an Entity's Board of Directors or Advisory Committee:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Discussion of Off-Label:** Not Applicable.



# VWD: Clinical and Lab Diagnosis *Background 2016 (1)*



VWD is the most common inherited bleeding disorder and is due to **quantitative** (VWD3 & VWD1) **and/or qualitative** (VWD2A, VWD2B, VWD2M, VWD2N) defects of VWF: in **severe forms** of VWD3, VWD1 & VWD2N FVIII is also reduced

Despite the complex and heterogeneous nature of the VWF defects, nowadays all VWD types can be managed efficiently in most patients.



# VWD: Clinical and Lab Diagnosis

## *Background 2016 (2)*



- **Correct VWD diagnosis and classification cannot be always available in several Centers to provide the best therapeutic approach.**
- **Differently from HA easily classified (severe, moderate, mild) by baseline FVIII levels, **clinical severity of different VWD forms** is not well defined within types so far.**



# List of Clinical and Laboratory Tools Used for VWD Diagnosis



## More Than One Test Always Needed

Basic Tests	Specific tests	Additional tests
<b>Patient &amp; Family History</b> <b>Bleeding Score</b> Bleeding Time PFA 100 PTT <b>FVIII:C</b>	<b>VWF:Ag</b> <b>VWF:RCo</b> <b>VWF:CB</b> <b>VWF:RCo/Ag</b> <b>VWF:CB/Ag</b> <b>VIII:C/VWF:Ag</b>	<b>RIPA test</b> <b>VWF:FVIII B</b> <b>Multimeric analysis</b> <b>Molecular genetics</b>



# Classification of VWD Types Based on Several Assays



	Normal	Type 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3
<b>VWF:Ag</b>	N	L, ↓ or ↓↓	↓ or L	↓ or L	↓ or L	N or L	absent
<b>VWF:RCO</b>	N	L, ↓ or ↓↓	↓↓ or ↓↓↓	↓↓	↓↓	N or L	absent
<b>FVIII</b>	N	N or ↓	N or ↓	N or ↓	N or ↓	↓↓	1-9 IU/dL
<b>RIPA</b>	N	often N	↓	often N	↓	N	absent
<b>LD-RIPA</b>	absent	absent	absent	↑↑↑	absent	absent	absent
<b>Platelet count</b>	N	N	N	↓ or N	N	N	N
<b>VWF multimer pattern</b>	N	N	abnormal	abnormal	N	N	absent

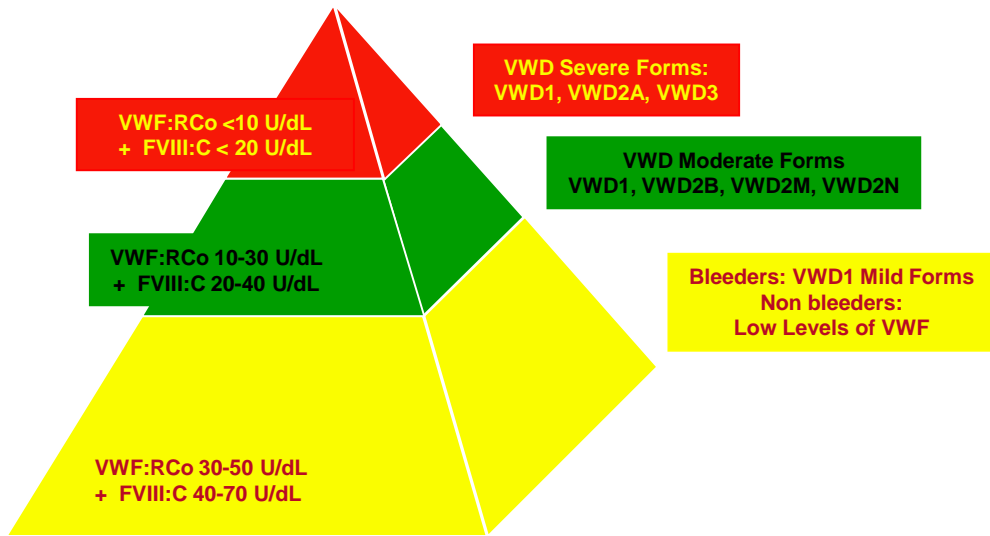


# Heterogeneity of VWD Patients Based on Cohort Studies



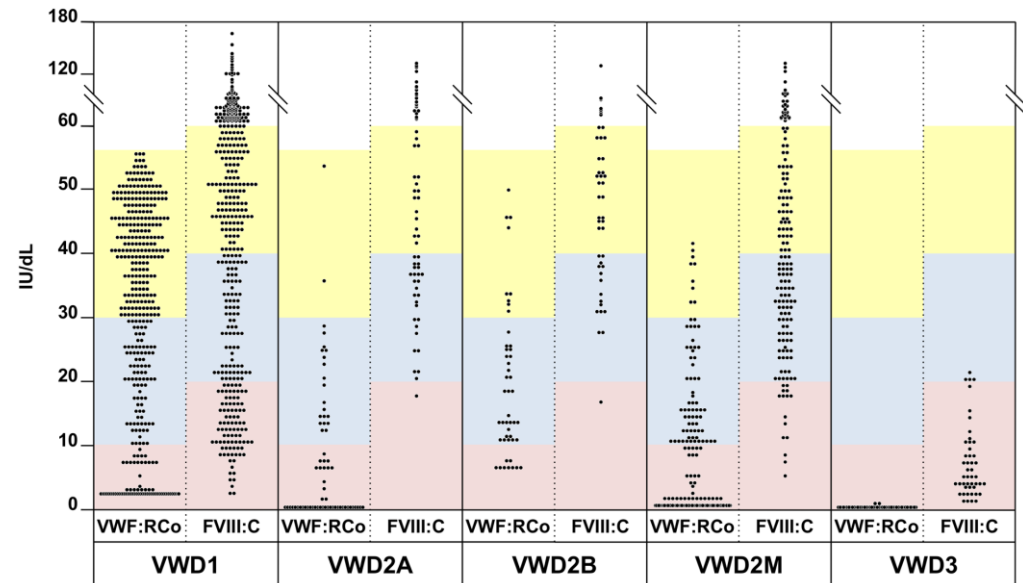
Ospedale Luigi Sacco  
AZIENDA OSPEDALIERA - POLO UNIVERSITARIO

## Bleeders versus Non Bleeders



*Diagnosed VWD: the tip of the iceberg?*

## Heterogeneous VWD Cohort: Italian Registries (RENAWI)



*Federici AB et al, Blood 2014; 123: 4037-44.*





# Criteria for Correct Diagnosis

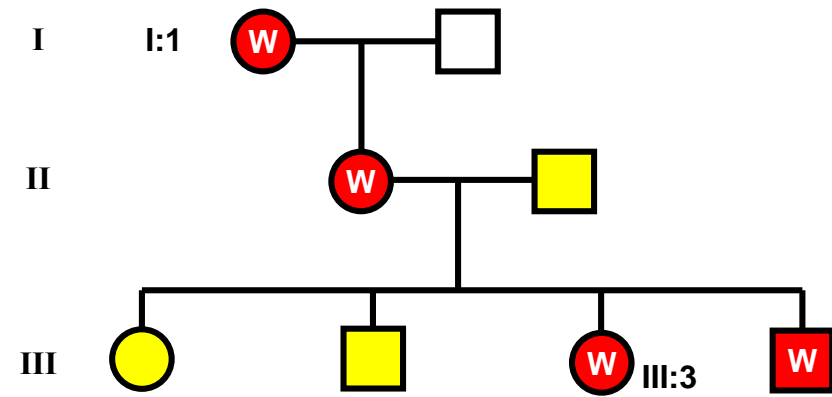
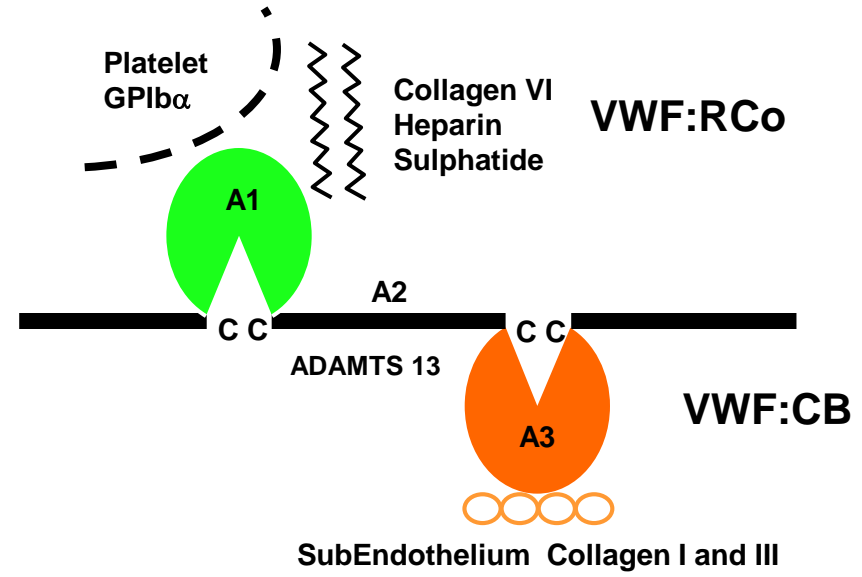
(Bleeding History, Low VWF Activity, Inheritance)



**Ospedale Luigi Sacco**  
AZIENDA OSPEDALIERA - POLO UNIVERSITARIO

Symptom	SCORE					
	-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 transf or Replacement therapy or Desmopressin
Cutaneous	-	No or trivial (<1 cm)	> 1 cm and no trauma	Consultation only		
Bleeding minor wounds	-	No or trivial (less than 5)	> 5 or more than 5'	Consultation only	Surgical hemostasis	Blood transf or Replacement therapy or Desmopressin
Oral cavity	-	No	Referred at least one	Consultation only	Surgical hemostasis or Antifibrinolytic	Blood transf or Replacement therapy or Desmopressin
GI bleeding	-	No	Associated with ulcer, portal hyp., hemorrhoids, angiodysplasia	Spontaneous	Surgical hemostasis, Blood transf, Replacement therapy, Desmopressin, Antifibrinolytic	
Tooth extraction	No bleeding in at least 2 extraction	None done or no bleed. in 1 extraction	Referred in <25% of all procedures	Referred in >25% of all procedures, no intervention	Resuturing or packing	Blood transf or Replacement therapy or Desmopressin
Surgery	No bleeding in at least two surgeries	None done or no bleed. in 1 surgery	Referred in <25% of all surgeries	Referred in >25% of all procedures, no intervention	Surgical hemostasis or Antifibrinolytic	Blood transf or Replacement therapy or Desmopressin
Menorrhagia	-	No	Consultation only	Antifibrinolytics, Pill use	D & C, Iron therapy	Blood transf or Replacement therapy or Desmopressin or Hysterectomy
Post-partum hemorrhage	No bleeding in at least two deliveries	No deliveries or no bleeding in 1 delivery	Consultation only	D & C, Iron therapy, Antifibrinolytics	Blood transf or Replacement therapy or Desmopressin	Hysterectomy

Tosetto et al JTH 2006







# Clinical and Lab Diagnosis of VWD

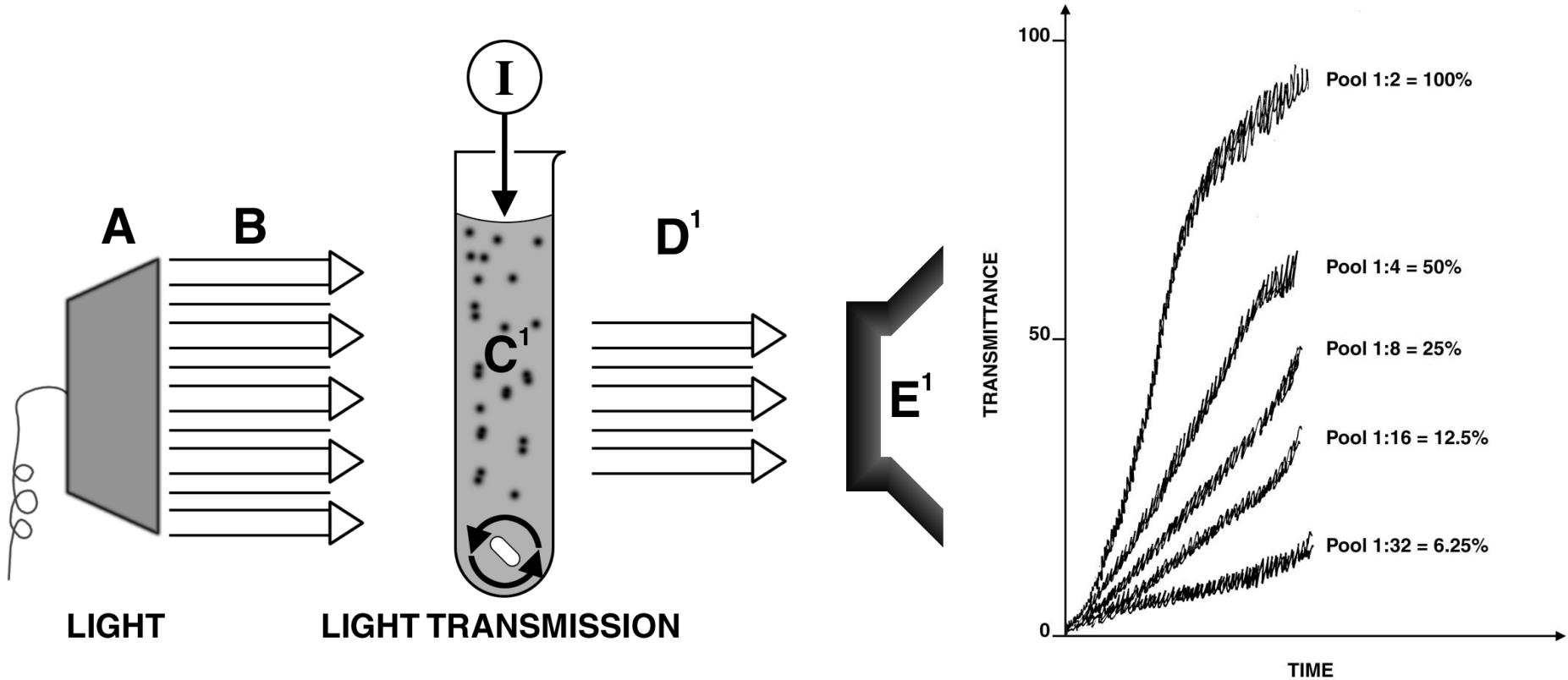
## Outlines



- Definitions and classification of VWD
- Clinical parameters for VWD
- **First-level laboratory tests**
- **Second-level laboratory tests**
- Additional and automatic assays
- Severe or mild VWD types: outcomes



# VWF:RCo (Platelet Aggregometric Method)



Normal Fixed Platelets + Patient Plasma Dilutions + **Ristocetin** [1 mg/mL]

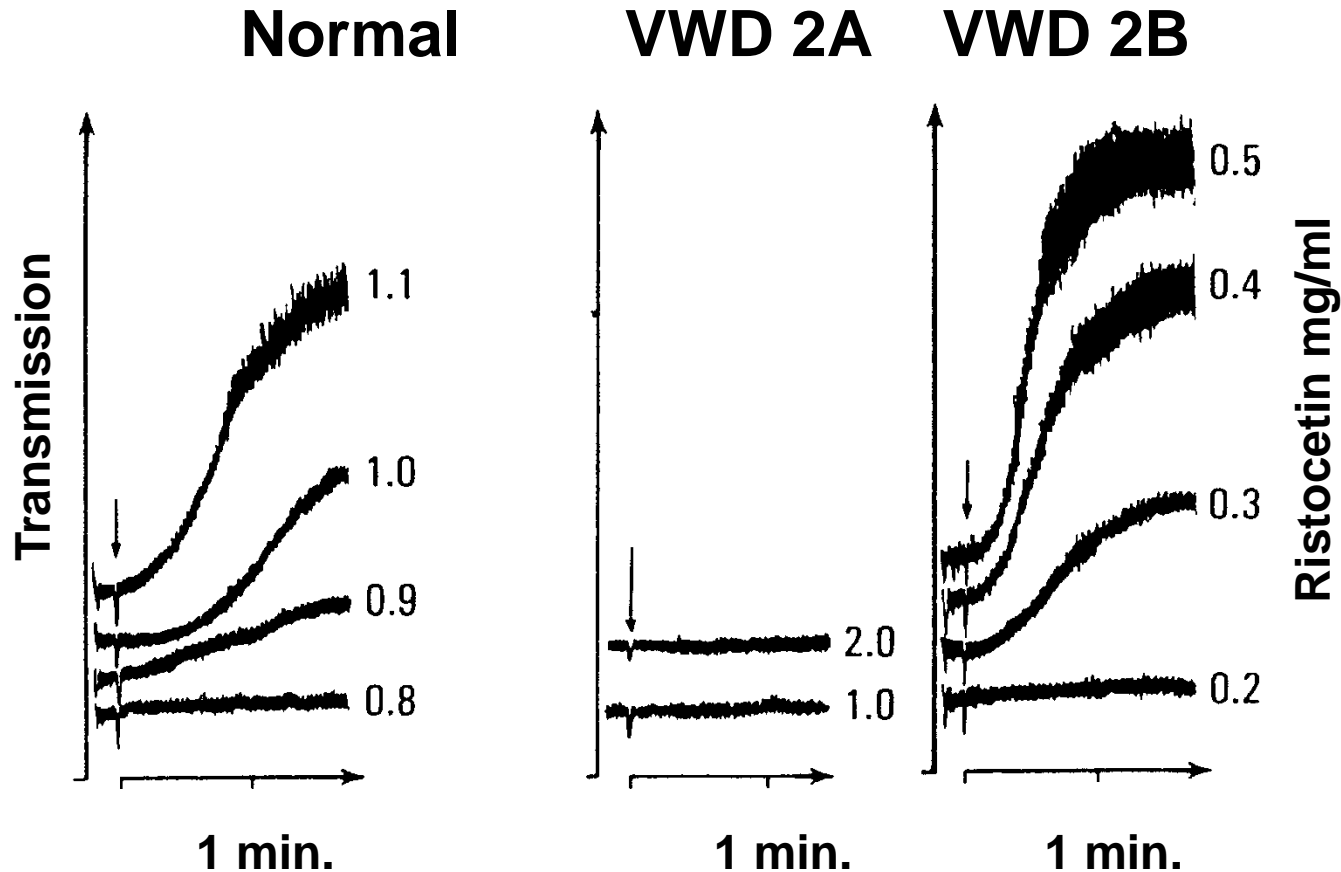
Parameters That Influence the VWF:RCo

- VWF:Ag
- Multimeric Pattern (**2A & 2B**)
- Mutations in the A1 Domain (**2M**)



# VWF:RIPA

(Ristocetin Induced Platelets Agglutination)

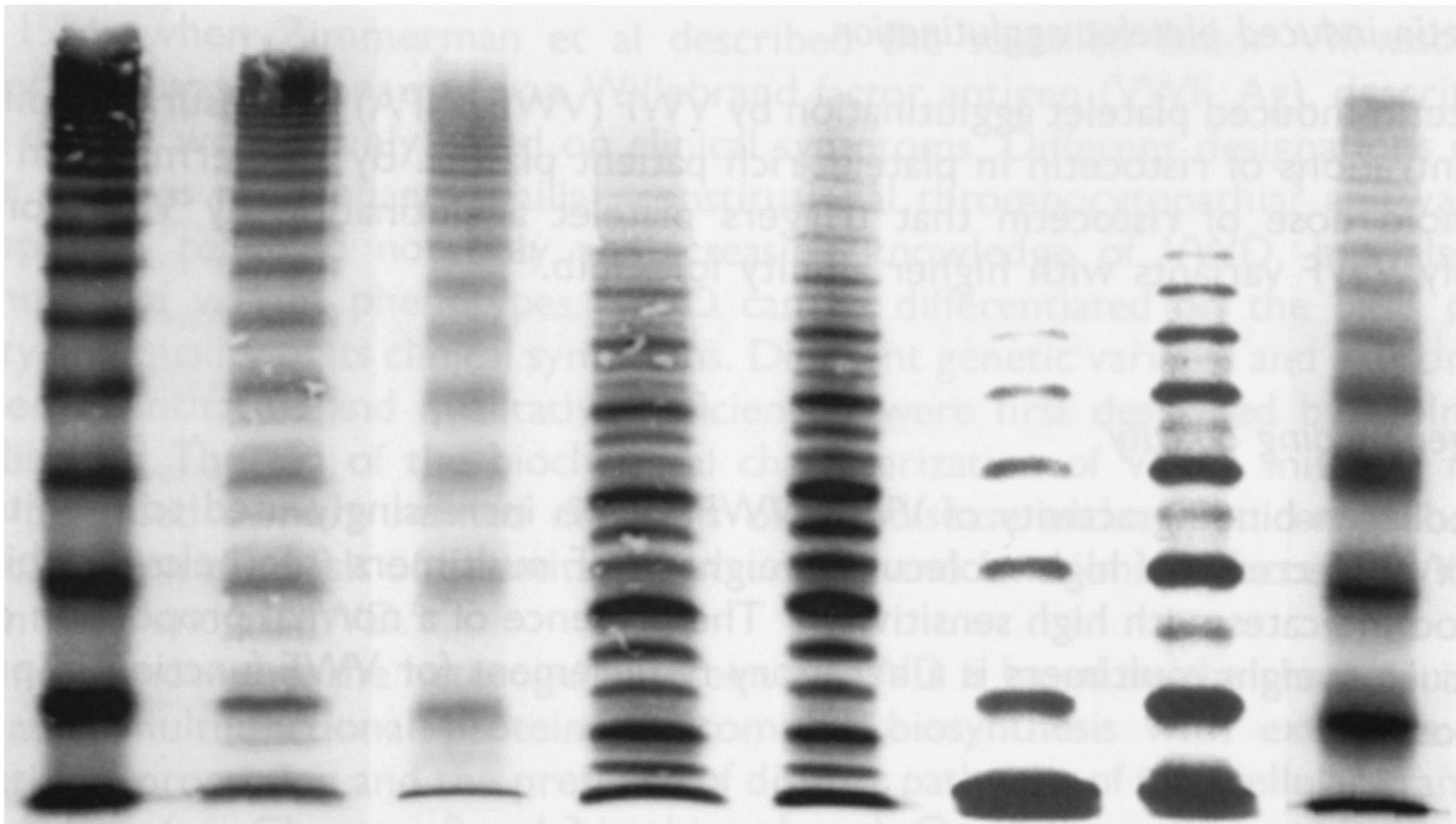


Platelet Rich Plasma from Patients + RISTOCETIN [0.2-2.0 mg/ml]

*Ruggeri ZM et al, JCI 1978*



# VWF Multimeric Analyses (Kindly Provided by U. Budde)

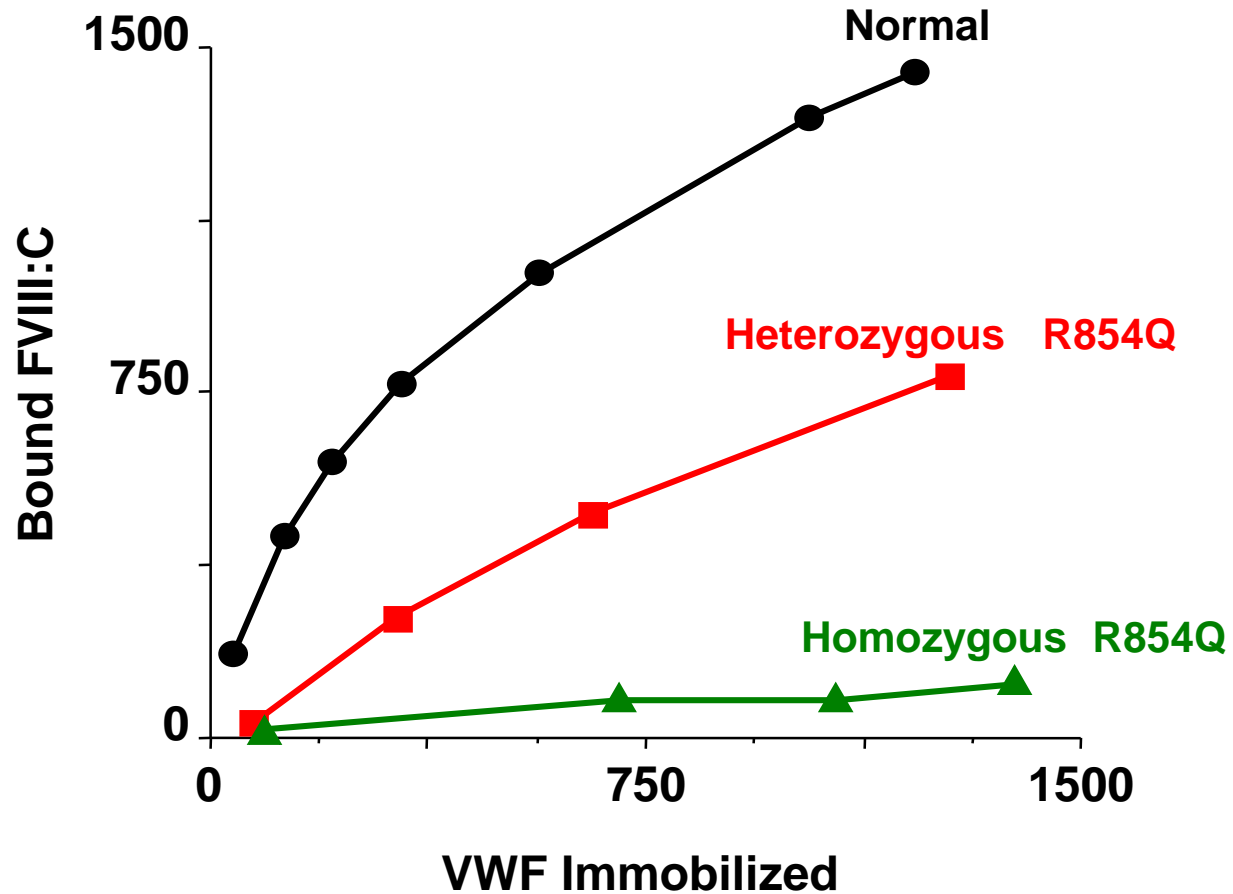


Plt	N	IB	IIA	IIB	IIC	IID	IIE/IIF
	N	2A	2A	2B	2C	2D	2E



# VWF:FVIII B

(Binding Assay - ELISA)





# VWF Pro-Peptide

## *Usefulness of this assay*



The assay for **VWF pro-peptide** measures in circulation the amount of protein cleaved from PRE-PRO-VWF synthesized in Endothelial Cells

Increased **VWF:pp/VWF:Ag ratio** identifies those patients with shortened VWF survival

Shortened VWF survival can also be observed during the infusion trial with DDAVP

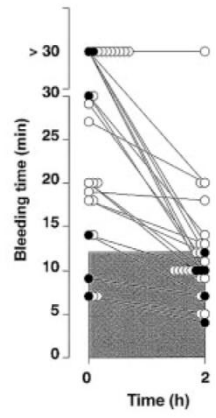
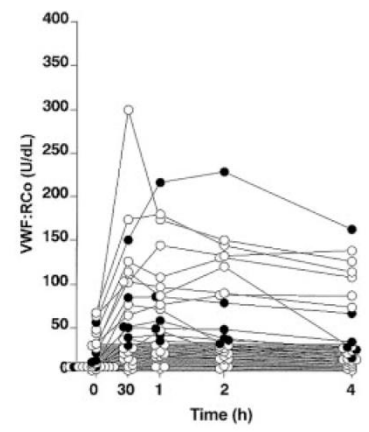
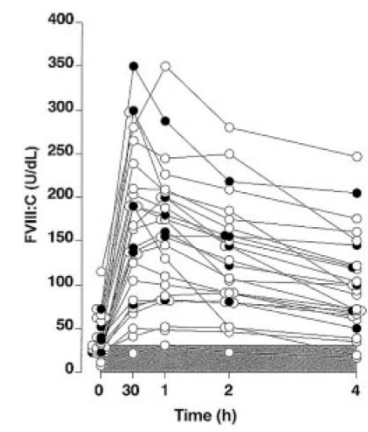
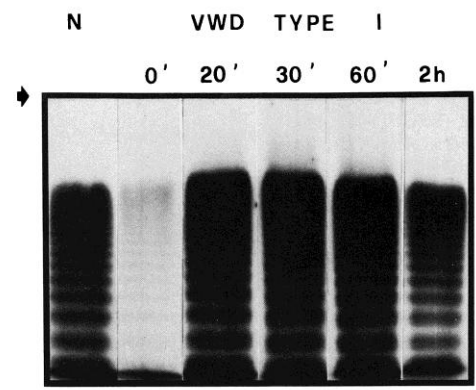
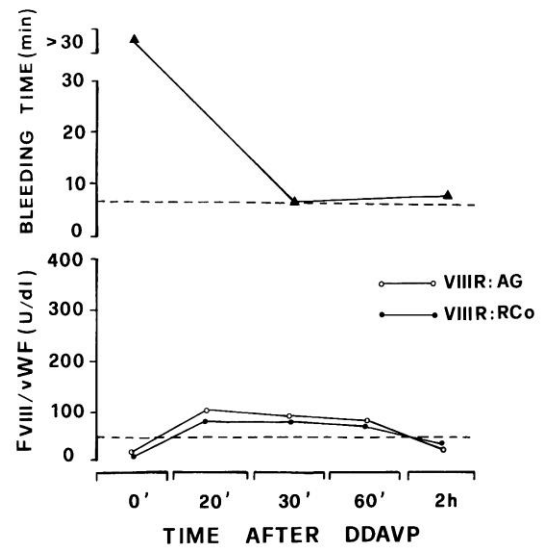




# DDAVP Challenge Test: An important assessment at diagnosis



**Ospedale Luigi Sacco**  
AZIENDA OSPEDALIERA - POLO UNIVERSITARIO



*Ruggeri et al, Blood 1982*

*Federici AB et al, Blood 2004; 103: 2032-2038*





# Clinical and Lab Diagnosis of VWD

## Outlines



- Definitions and classification of VWD
- Clinical parameters for VWD
- First-level laboratory tests
- Second-level laboratory tests
- **Additional and automatic assays**
- Severe or mild VWD types: outcomes



# VWF:CB

## (Collagen Binding Activity)

- **Evaluates VWF capability to bind to collagen**
  - Mimics VWF interaction with sub-endothelial collagen matrix at site of vascular injury
- **Dependent on VWF multimeric size**
  - Collagen binds more readily with HMWM
  - Studies show VWF:CB can serve as a **surrogate measure for presence of HMWM**
- **When tested with VWF:Ag and VWF:RCo can improve differentiation between **VWD types 1, 2A, 2B and 2M****



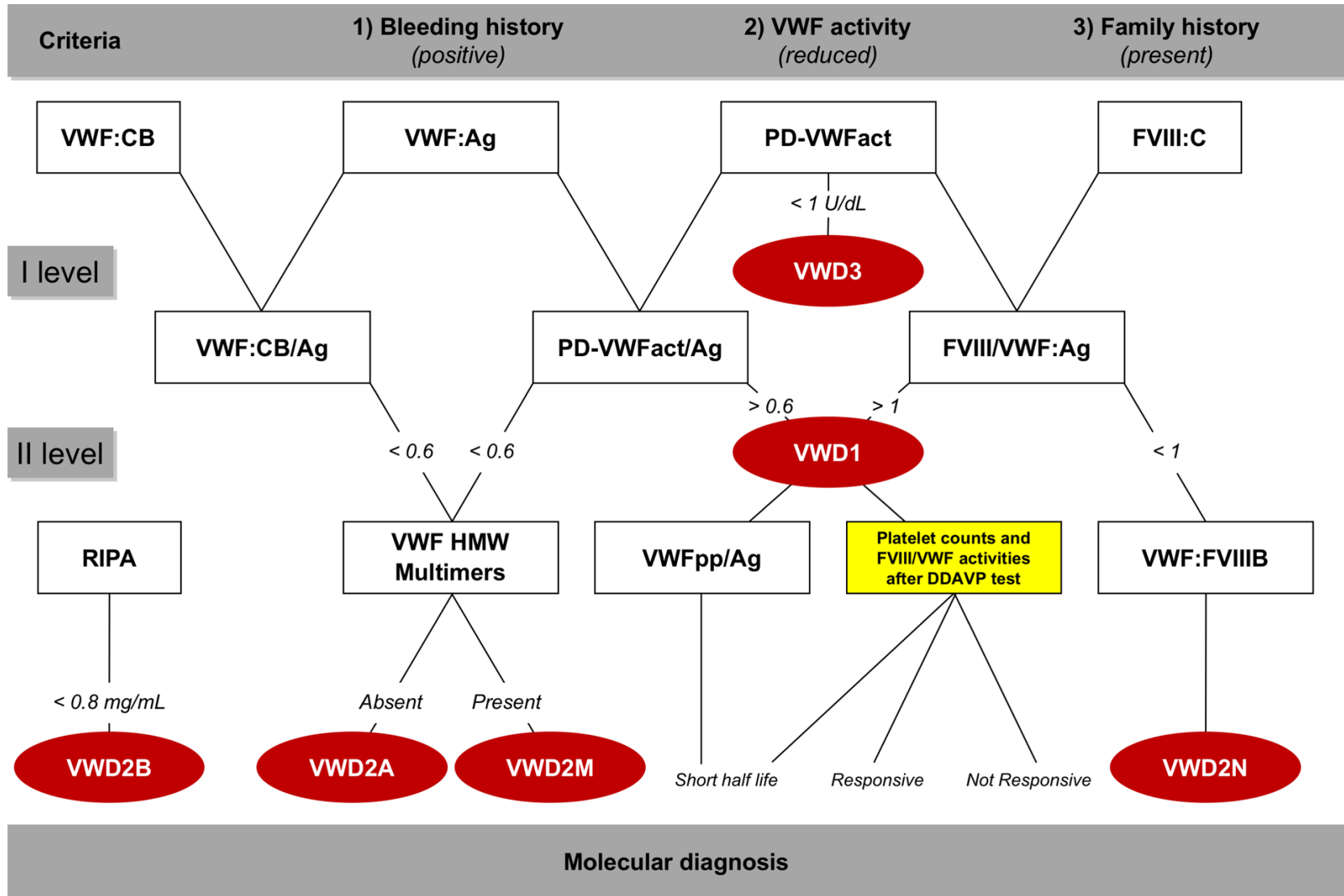
# Platelet Dependent-VWF Activity (Nomenclature and Methodology)



Abbreviation for VWF activity	Description
<b>VWF:RC<sub>o</sub></b>	<u>R</u> istocetin <u>C</u> ofactor activity: all assays that use platelets and <u>R</u> istocetin
<b>VWF:GPIbR</b>	All assays that are based on the <u>R</u> istocetin-induced binding of VWF to a recombinant WT <u>GPIb</u> fragment
<b>VWF:GPIbM</b>	All assays that are based on the spontaneous binding of VWF to a gain-of-function <u>M</u> utant <u>GPIb</u> fragment.
<b>VWF:Ab</b>	All assays that are based on the binding of a monoclonal antibody (m <u>Ab</u> ) to a VWF A1 domain epitope



# Flow chart for VWD Diagnosis Used in Italian Registry





# Clinical and Lab Diagnosis of VWD

## *Outlines*



- Definitions and classification of VWD
- Clinical parameters for VWD
- First-level laboratory tests
- Second-level laboratory tests
- Additional and automatic assays
- **Severe or mild VWD types: outcomes**



# Aims of the RENAWI-2



 Ospedale Luigi Sacco  
AZIENDA OSPEDALIERA - POLO UNIVERSITARIO



# blood

Prepublished online April 30, 2014;  
doi:10.1182/blood-2014-02-557264

## **The bleeding score predicts clinical outcomes and replacement therapy in adults with von Willebrand disease: a prospective cohort study of 796 cases**

Augusto B. Federici, Paolo Bucciarelli, Giancarlo Castaman, Maria G. Mazzucconi, Massimo Morfini, Angiola Rocino, Mario Schiavoni, Flora Peyvandi, Francesco Rodeghiero and Pier Mannuccio Mannucci

**To evaluate the incidence, types and severity of spontaneous bleeding episodes requiring DDAVP and/or VWF concentrates in a large cohort of VWD patients**

**To characterize bleeding phenotype in different VWD types and to predict clinical outcome in these patients.**

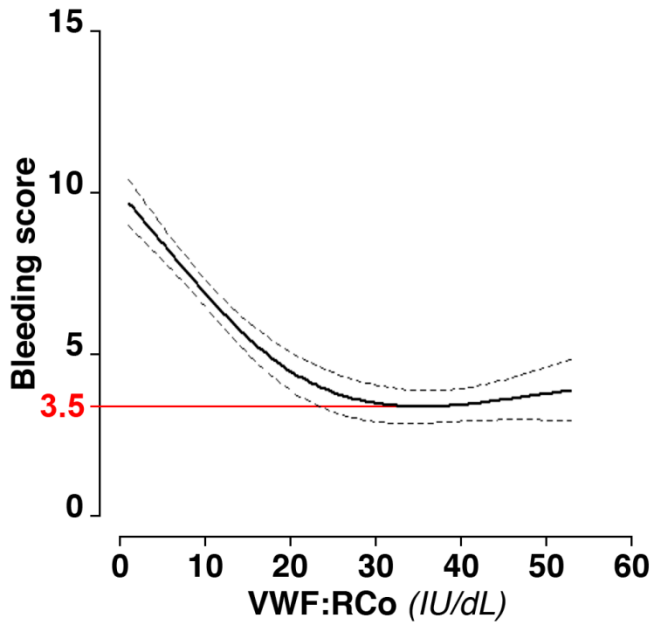


# Bleeding Phenotype in VWD

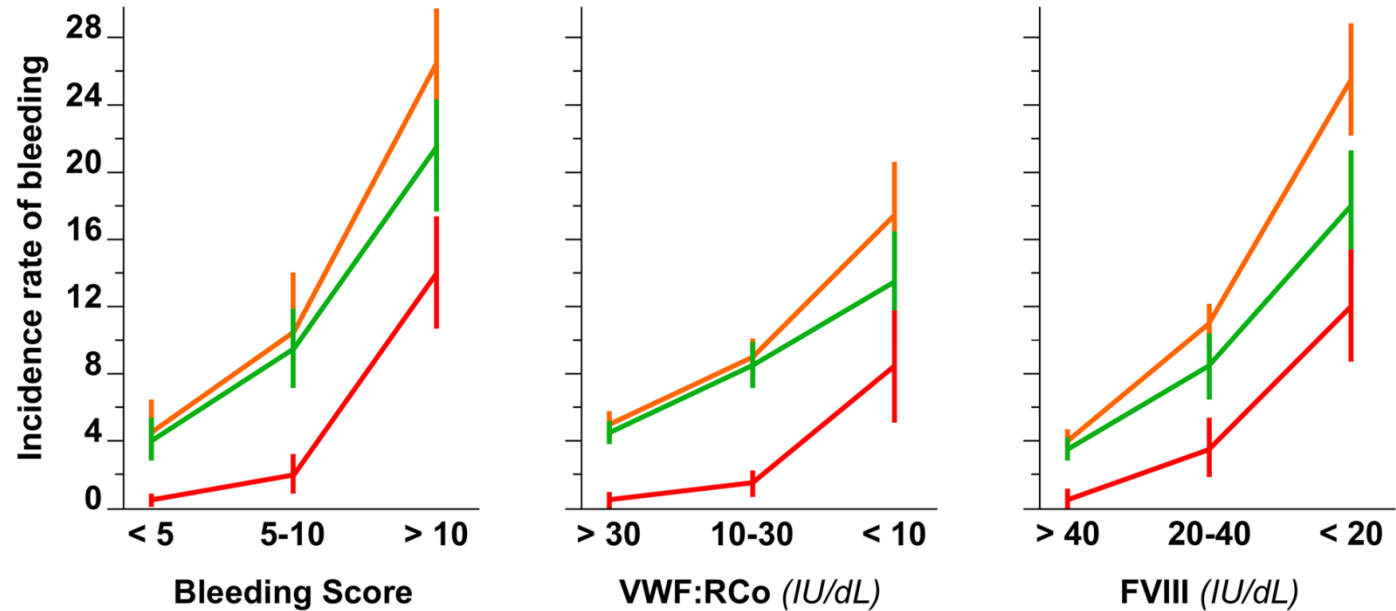
## Evidence-Based Methods



### Restricted Cubic Spline Curve



### Cox's Proportional Hazard Model



— Any bleeds — Mucosal bleeds — Non mucosal bleeds

*Federici AB et al, Blood 2014; 123: 4037-4044*





# Conclusions of RENAWI-2



blood

Prepublished online April 30, 2014;  
doi:10.1182/blood-2014-02-557264

**The bleeding score predicts clinical outcomes and replacement therapy in adults with von Willebrand disease: a prospective cohort study of 796 cases**

Augusto B. Federici, Paolo Bucciarelli, Giancarlo Castaman, Maria G. Mazzucconi, Massimo Morfini, Angiola Rocino, Mario Schiavoni, Flora Peyvandi, Francesco Rodeghiero and Pier Mannuccio Mannucci

**The bleeding score (BS) correlates with VWF levels in VWD and helps to predict clinical outcomes in adult patients with VWD.**



# Clinical and Lab Diagnosis of VWD

## *Current Perspectives in 2016*



- **BS and of specific tests for VWF activities** should be always used together in Adults to identify VWD patients with bleeding phenotype
- **More specific and automatic lab tests** should be available in most laboratory world-wide for a rapid VWD diagnosis of bleeding individuals



# Clinical and Laboratory Diagnosis of VWD

## *Current perspective 2016*



**Questions?**

**[augusto.federici@unimi.it](mailto:augusto.federici@unimi.it)**