ERN-EuroBloodNet Topic on Focus on Inherited Platelet Function Disorders (IPFD)

webinar

HEALTH PROFESSIONALS

When to suspect an inherited platelet function disorder – differential diagnoses

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CRPP Centre de Référence Pathologies Plaquettaires
MHEMO La Filière des maladies rares de l'hémostase
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Inherited platelet function disorders (IPFD): definition

- A group of rare congenital hemorrhagic disorders with altered platelet function associated or not to a reduced platelet number
- Mucocutaneous bleeding diathesis of very variable severity
- Large heterogeneity in terms of molecular/genetic defect (for some forms not yet identified)
- Diagnosis of many forms is cumbersome and sometimes requires complex assays

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Prevalence of mild-moderate bleeding disorders

- The reported prevalence of VWD varies from 0.6 to 1.3%, with 1 case per 1000 clinically relevant (Rodeghiero F, Blood 1987, 69:454; Bowman M, JTH 2010, 8:213).
- The exact prevalence of IPDs is unknown, but data from the large gnomAD database (exome sequence data of 125,748 individuals) show that 0.329% of the general population have a clinically meaningful LOF variant of a platelet function gene (Oved JH, JTH 2021,19:248).
- A study in >140,000 UK blood biobank subjects reported that up to 2.5% of individuals have a variant associated with platelet disorders (Stefanucci L, Blood 2023, 142:2055)

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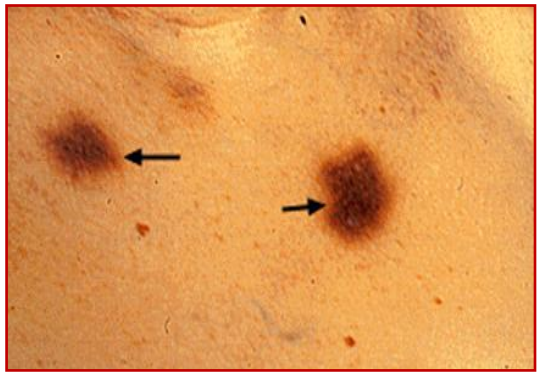
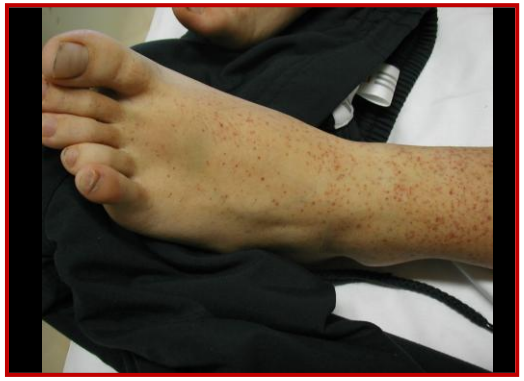
Clinical manifestations of inherited platelet disorders



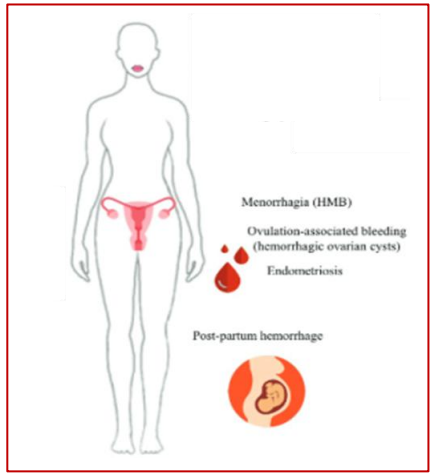
Easy bruising



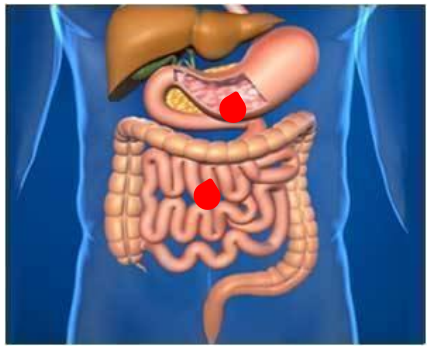
Petechiae

Epistaxis

Oral cavity bleeding



Meno/methrorragia and PPH



GI bleeding

Bleeding after invasive procedures

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Guidelines for the diagnosis of inherited platelet function disorders

When to suspect an inherited platelet function disorder

- Patients with a clinically significant history of mucocutaneous bleeding (familial or not) for whom an acquired or drug-induced cause of platelet dysfunction was excluded
- Patients for whom the following conditions have been excluded (when they fully explain the severity of the bleeding diathesis)
    - Acquired thrombocytopenia
    - Von Willebrand disease
    - Blood clotting defect
    - Afibrinogenemia

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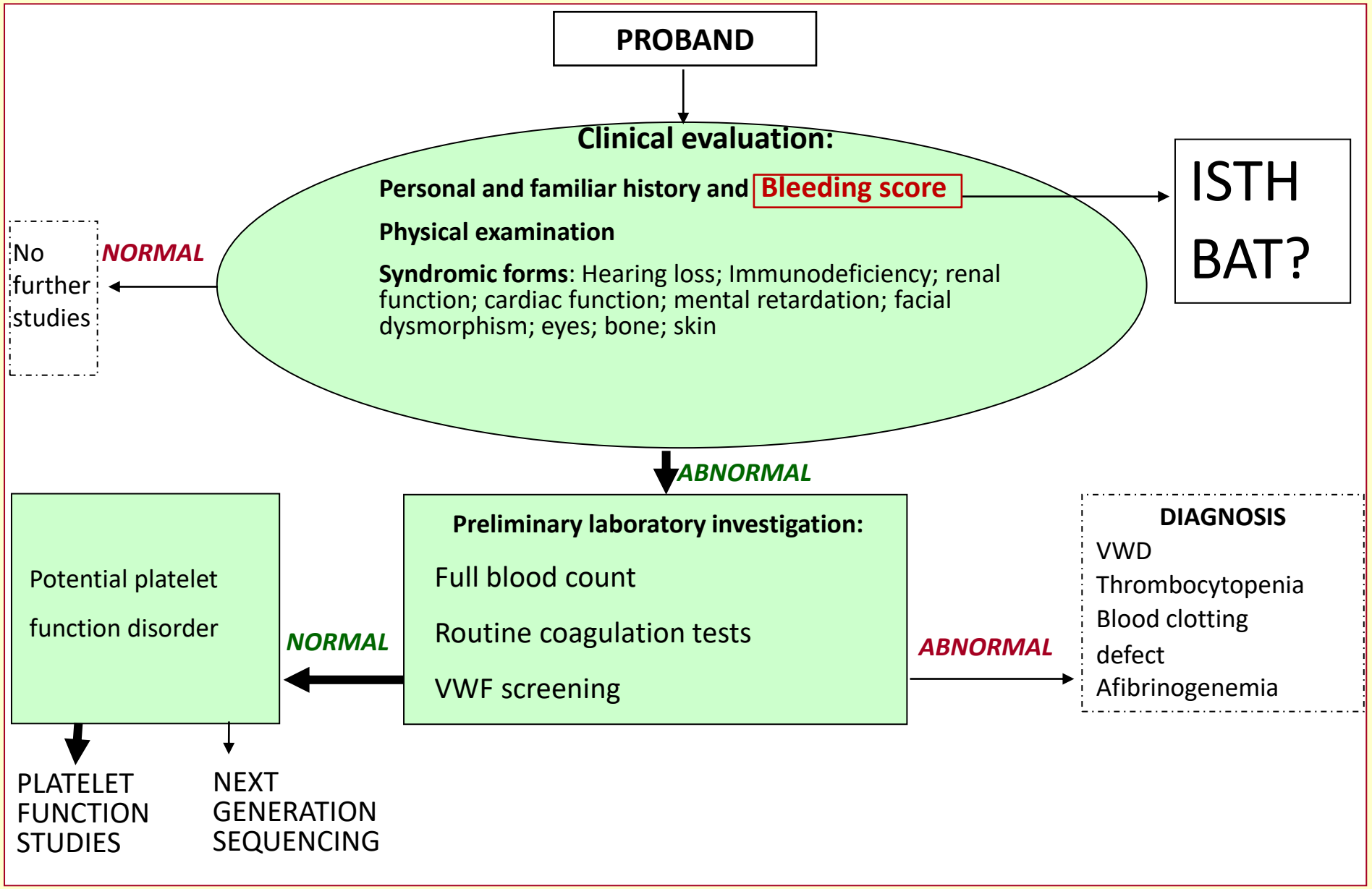
Diagnosis of inherited platelet function disorders

Guidance from the Platelet Physiology SSC of the ISTH



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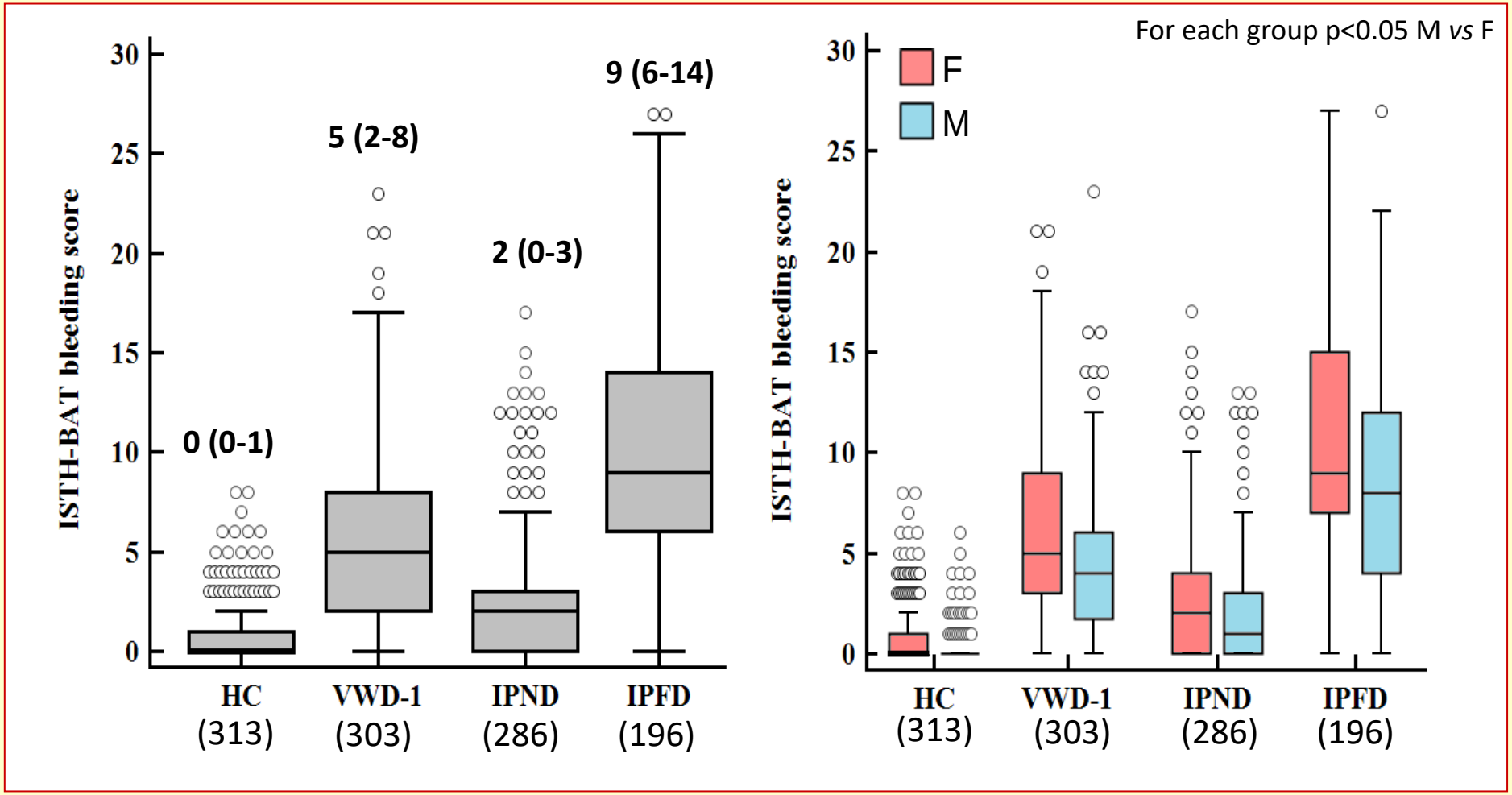
Gresele P; SSC Platelet Physiology, J Thromb Haemost 2015, 13:314

Validation of the ISTH BAT bleeding score for IPD

the BAT-VAL (ISTH-BAT in IPD evaluation) study



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Gresele P et al., J Thromb Haemost 2020, 18:732

Can the ISTH BAT bleeding score discriminate IPFD from other groups?

Sensitivity, specificity, positive and negative predictive values

	Best cut-off	AUC (p)	Sens	Spec	PPV (95% CI)	NPV (95% CI)
IPFD vs HC	>3 (F>4)	0.951 (<0.0001)	86.73	92.33	87.63	91.75
IPFD vs HC	>6	0.850 (<0.0001)	70.92	99.04	97.89	84.47
IPFD vs VWD-1	>7	0.731 (<0.0001)	72.27	67.96	69.71	63.94
IT vs HC	>1	0.684 (<0.0001)	50.70	79.87	83.85	61.73

IPFD: inherited platelet function disorder; IT: inherited thrombocytopenia , HC: healthy control; VWD-1: type 1 VWD

If a patient with a mucocutaneous bleeding diathesis has an ISTH BAT BS >6 and preliminary laboratory screening excludes VWD (Gresele P et al 2015) then it is >99% probable that he is affected by an IPFD.

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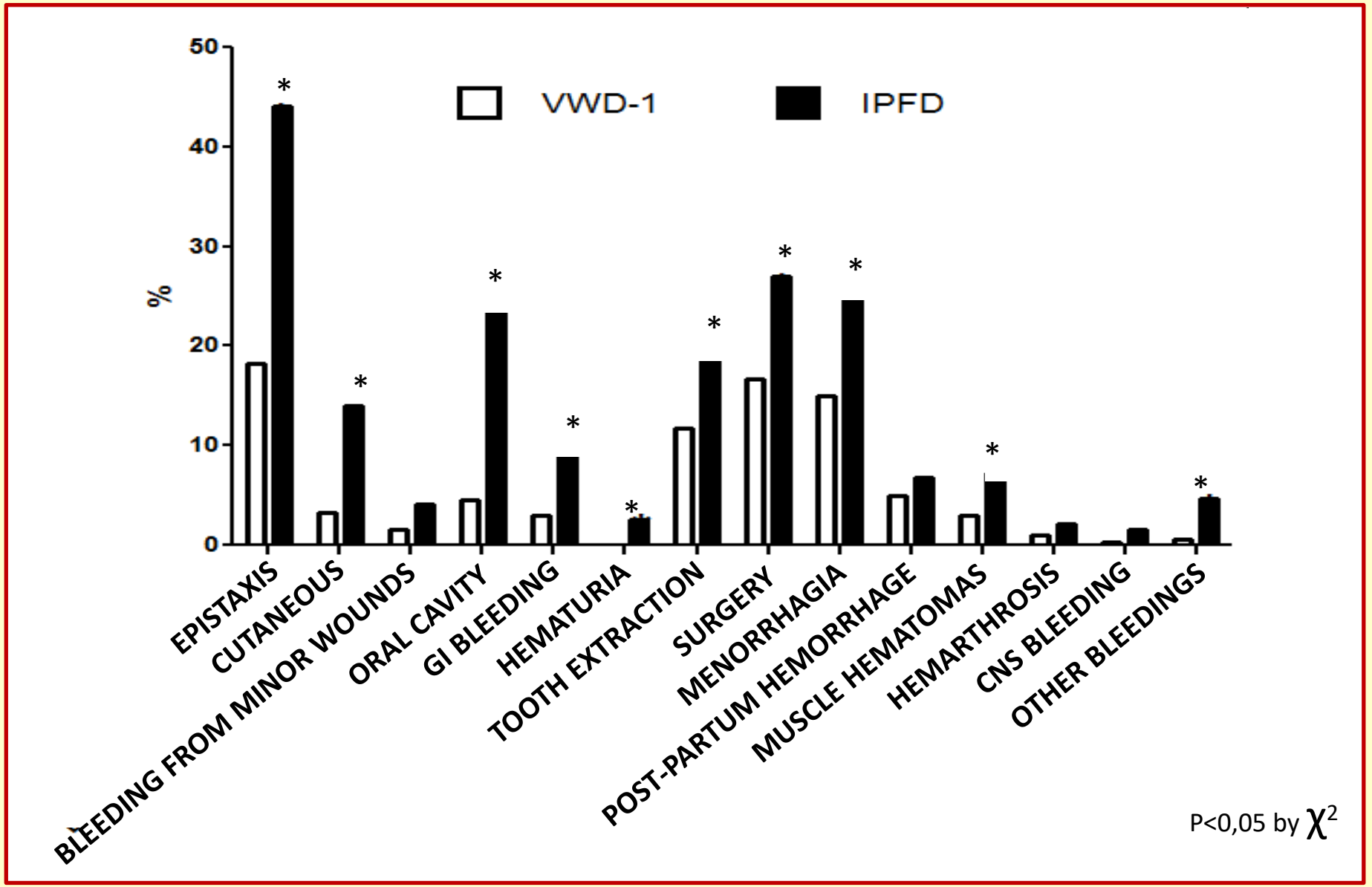
Gresele P et al., J Thromb Haemost 2020;18(3):732-739

Frequency of clinically significant bleeding symptoms (score ≥2) in IPFD and VWD-1



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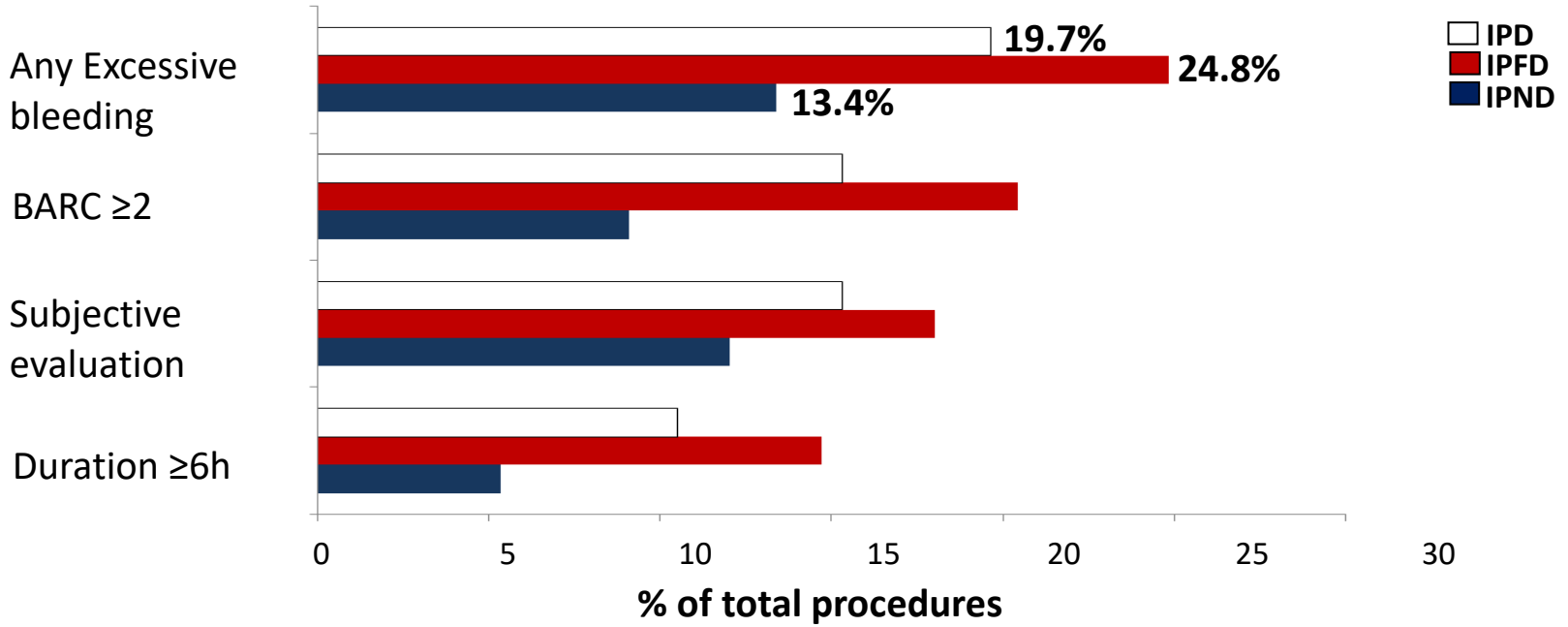
Gresele P et al., J Thromb Haemost 2020, 18:732

Frequency of excessive bleeding at surgery in IPD

The SPATA Study

49 centers, **17** countries.
829 procedures in 423 IPD patients (238 IPFD, 135 IPND)**, 16** forms of **IPFD and 9** forms of **IPND**
Median age: 40 years (IQR 23.7-54). Women: 56%



In otherwise healthy subjects:
-From literature: 1.4-6 %
-Current study: 3%

Patients with ISTH BAT > 6; OR 3.97 (2-7.8), p=0.0001

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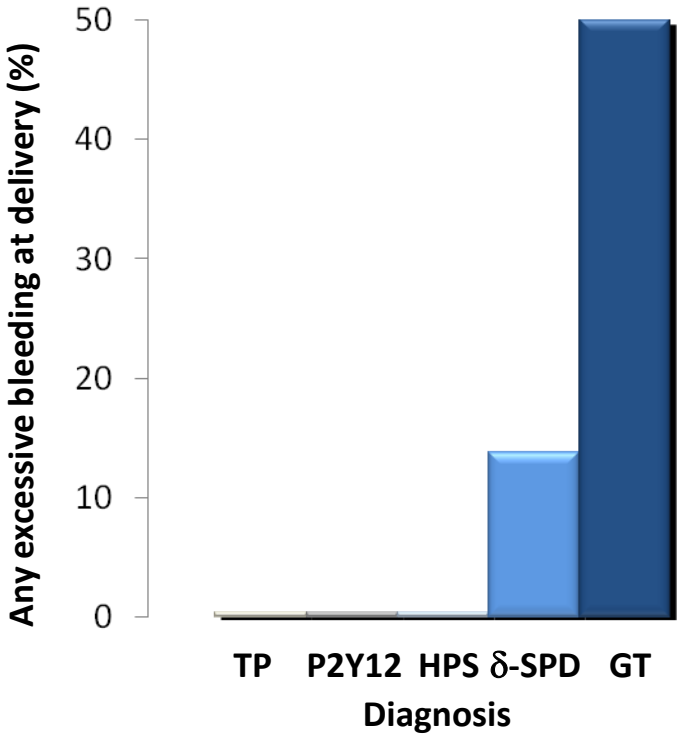
Orsini S et al., Haematologica 2017,102:119

Post partum bleeding in **IPFD**

The PIPA Study

56 deliveries in 34 women with 5 different forms of platelet function disorder
Overall frequency of AEB at delivery: 26.7%
Overall frequency of major bleeding at delivery: 12.5%
Overall frequency of hemorrhage at delivery in healthy women: 3-7%

PREDICTIVE PARAMETERS



Risk of major bleeding at delivery

	OR (95% CI)
Diagnosis of GT vs δ-SPD	41.6 (5.5-inf)
WHO grade 3-4	23.4 (2.1-256.4)

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Civaschi E et al, Br J Haematol.2015;170:559-63

Diagnosis of inherited platelet function disorders: guidance from the SSC of ISTH

- PFA-100® and Template Skin Bleeding Time: **not recommended** because of their poor diagnostic accuracy and low sensitivity

They may be used as optional test in single laboratories if a stringent cut off threshold is applied
(Gresele P for the SSC Platelet Physiology, JTH 2015;13:314-22)

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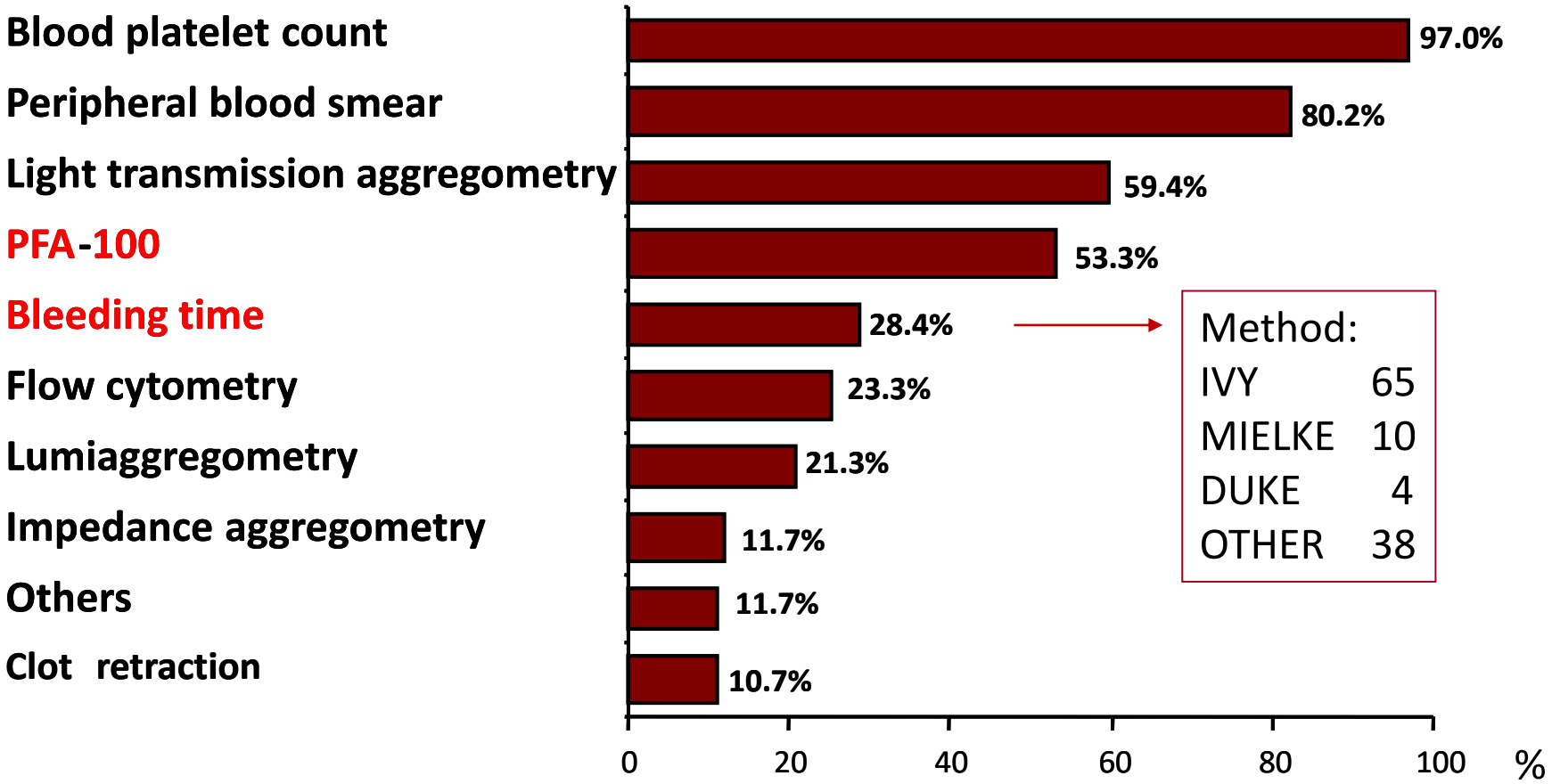
Diagnosis of suspected IPFD:
results of a worldwide survey

What kind of first step (screening) tests do you perform in patients with a suspected inherited platelet function disorder?



N. of respondents: 197/202 (97.5%)

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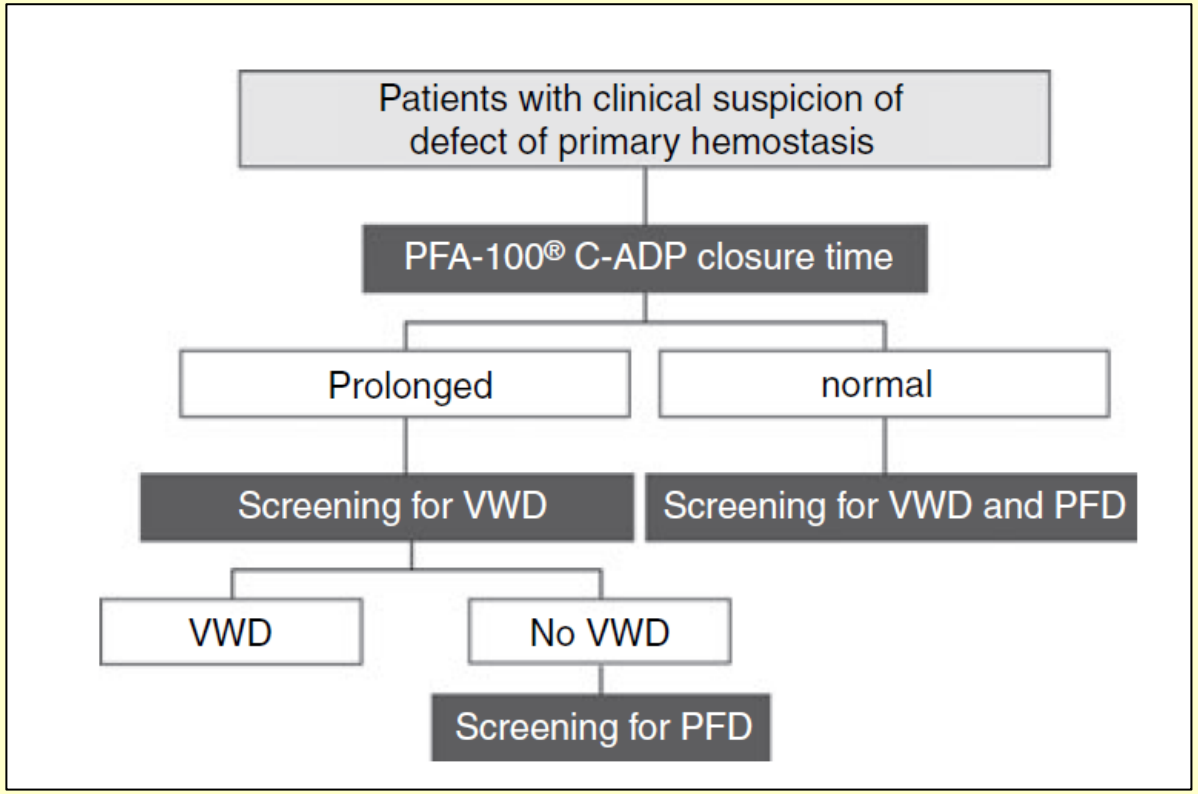
Gresele P et al., J Thromb Haemost 2014; 12:1562

USEFULNESS OF PFA-100 IN THE DIAGNOSTIC SCREENING OF SUSPECTED ABNORMALITIES OF HEMOSTASIS

Table 3 Sensitivity of collagen–epinephrine (C-EPI) closure time (CT), collagen–adenosine diphosphate (C-ADP) CT and bleeding time (BT) for abnormalities of hemostasis

		Sensitivity(%)
von Willebrand disease	C-EPI	(71)
	C-ADP	(71)
	BT	(29)
Platelet function disorders	C-EPI	(58)
	C-ADP	(8)
	BT	(33)



-7 VWD, 12 PFD; 28 clotting/fibrinolytic factor deficiencies; 18 LAC/FXII; 63 no abnormalities

«the use of the PFA-100 C-ADP cartridge might help in differentiating patients with VWD from patients with mild PFDs»

Podda GM et al. J Thromb Haemost 2007; 5: 2393–8.

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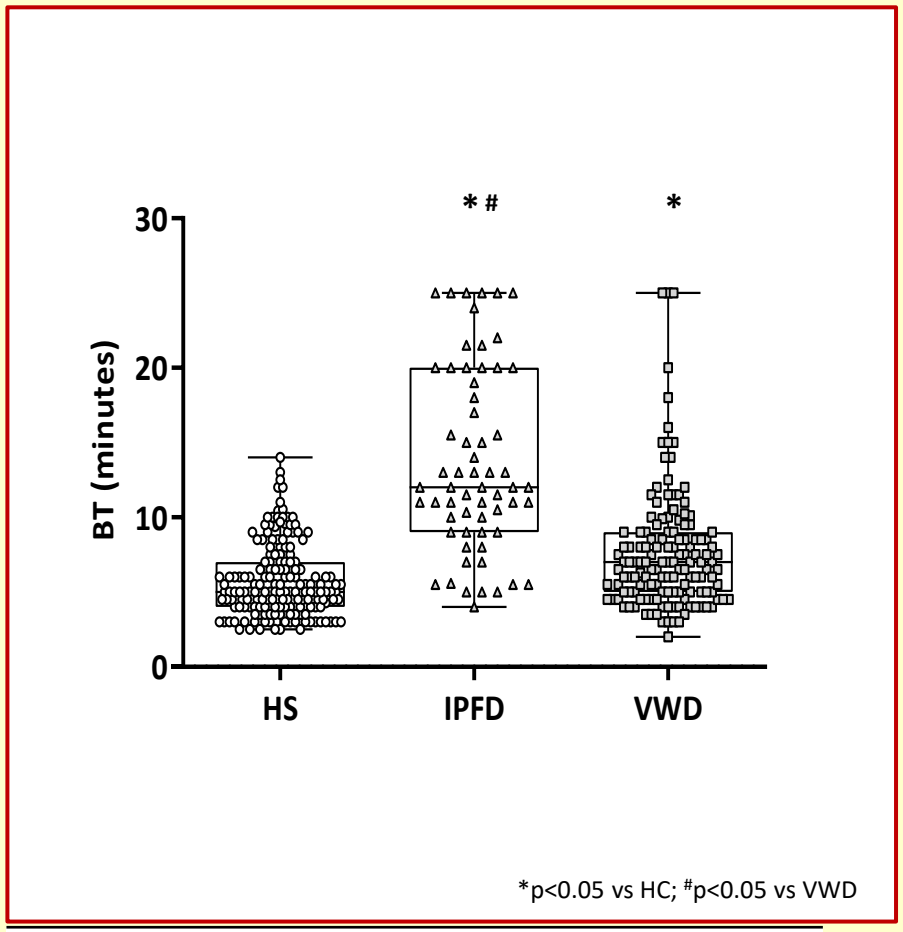
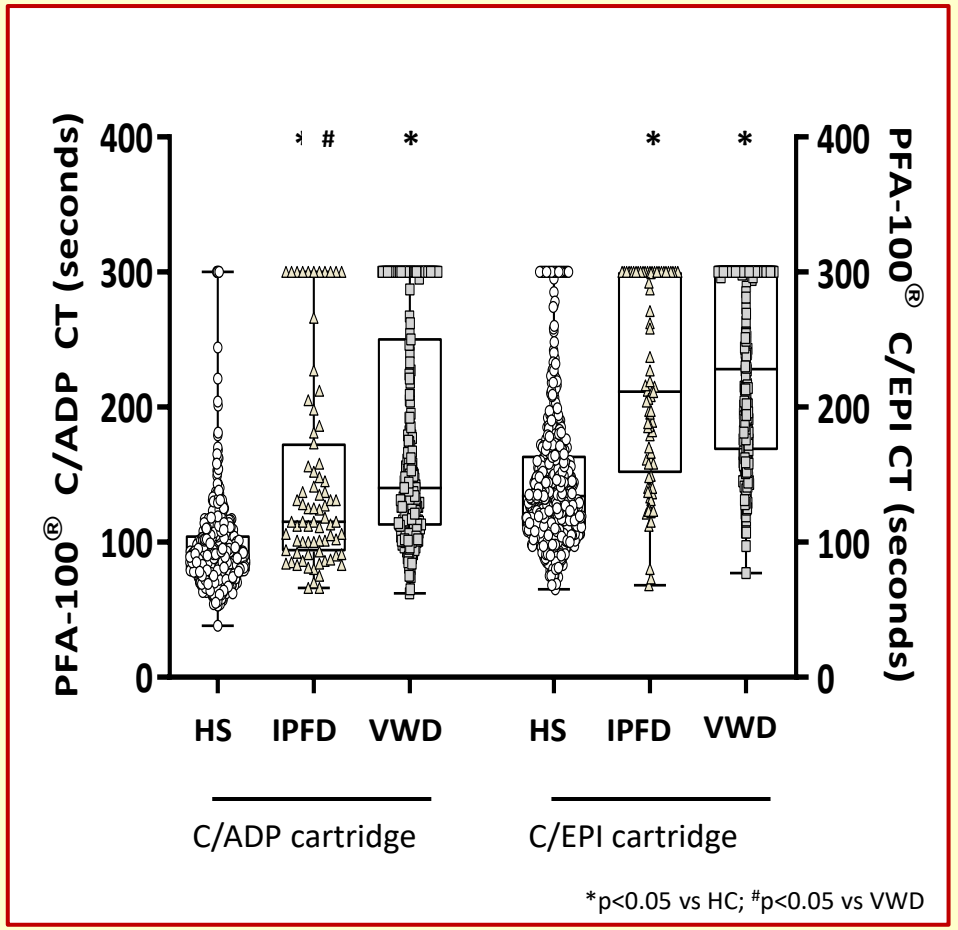
The bleeding time test, but not PFA-100, is significantly more prolonged in IPFD than in VWD





Predictors of IPFD	AUC (p)	NPV (%)
BT	**0.80** (p<0.01)	**85.3**
PFA-100® (C/ADP)	0.56 (p=ns)	11.6

n=999 subjects with suspected mild/moderate bleeding disorders

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Baccolo A, et al. Thromb Res. 2022;221:79-82

How to diagnose an IPD: a simplified approach

Confirmed clinical suspicion (ISTH-BAT, Physical examination)

Preliminary laboratory investigation:
- Full blood count
- Routine coagulation tests
- VWF screening

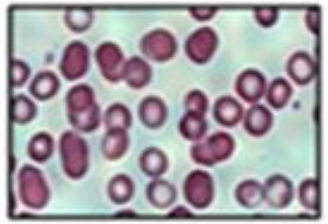
NORMAL → Potential platelet function disorder

ABNORMAL → **DIAGNOSIS**
- VWD
- Thrombocytopenia
- Clotting defect
- Afibrinogenemia

Potential platelet function disorder → PLATELET STUDIES

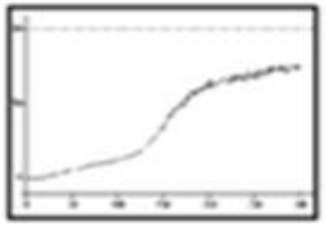
Potential platelet function disorder → NEXT GENERATION SEQUENCING

Blood smear



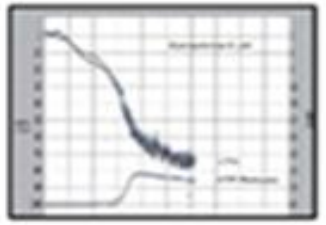
- Platelet size
- Morphologic alteration of platelets or other cells

Platelet aggregation



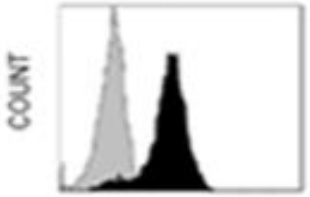
- Platelet aggregation in response to Epi, ADP, coll, AA, Ristocetin, TRAP-6, CVX, U46619

Granules assays



- α (e.g. β-TG) and δ-granules (e.g. ATP) content and release analysis

Flow cytometry (FC)



- Expression of GPIIb/IIa and GPIb/V/IX , GPVI, GPIa/IIa, GPIV
- GPIIb/IIa activation
- Procoagulant activity

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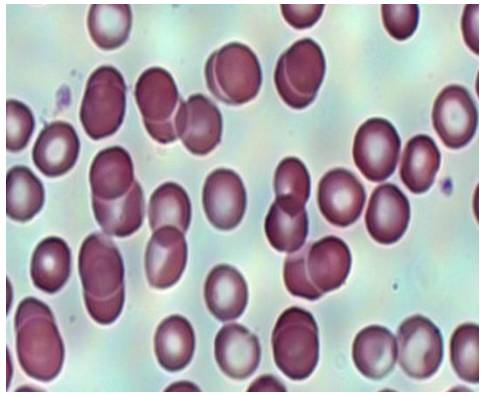
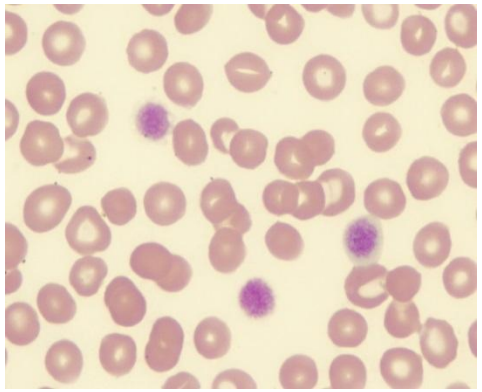
Gresele P, J Thromb Haemost 2015;13:316
Gresele P et al., Haemostaseologie 2025;45(4):335-346

Blood smear examination

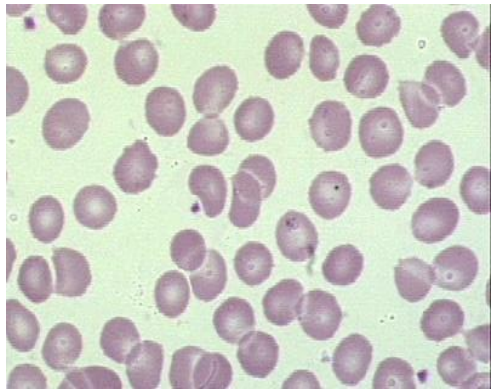
Inherited platelet disorders



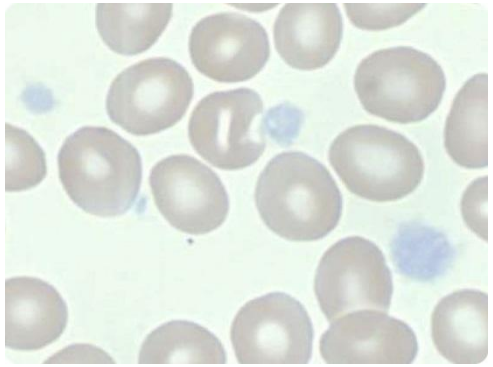
Normal



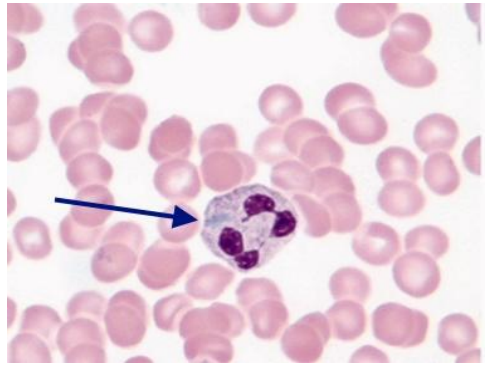
MYH9-RD
(Macrothrombocytopenia)



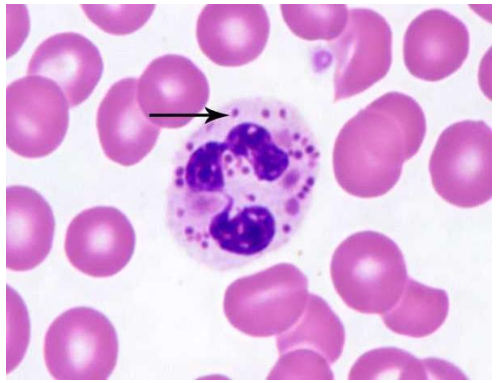
Wiskott Aldrich syndrome
(micro-thrombocytopenia)



Gray platelet syndrome
(gray platelet)



MYH9-RD
(Dohle-like bodies
in granulocytes)



Cediak-Higashi syndrome
(cytoplasmic inclusions)

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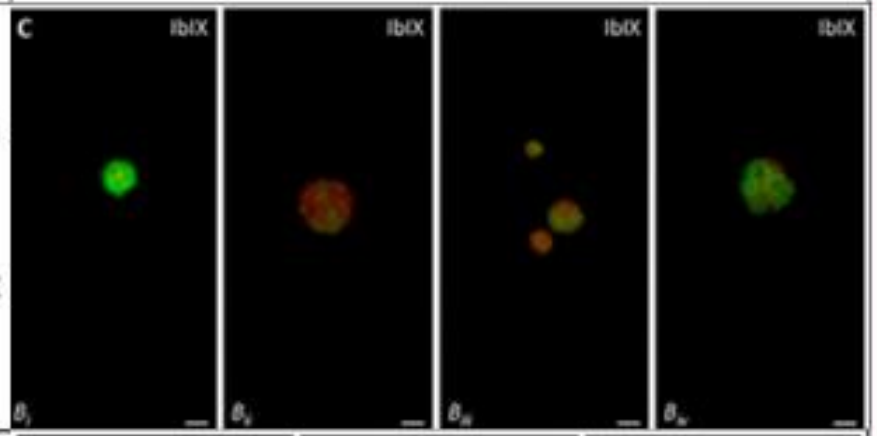
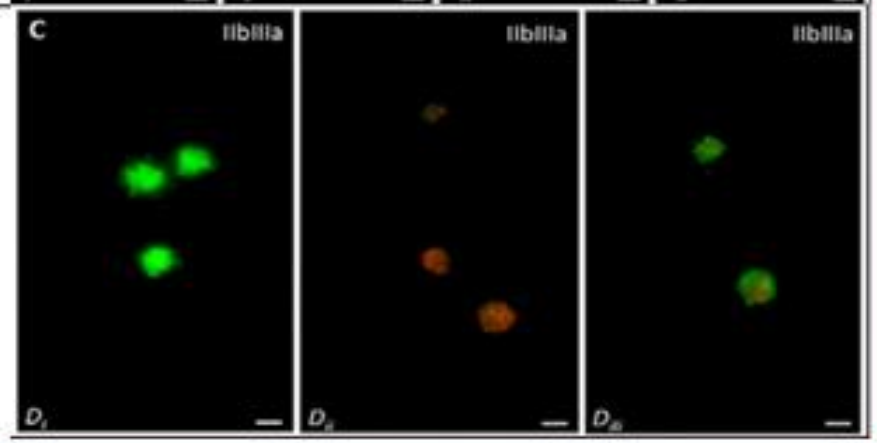
Gresele P et al., Expert Rev Hematol 2019, 12:29

Diagnosis of inherited platelet disorders on a blood smear

IPD Pathogenic mutations	Previously reported pattern	Images
BSS GP9 p.Met1 $ GP9 p.Asn61Ser $	Platelet macrocytosis with giant platelets; moderate-severe to mild reduced expression of GPIb/IX on platelet surface	
GT ITGA2B p.Arg628Ter $ ITGA2B p.Gln113* & ITGA2B p.Leu717Pro & ITGA2B p.Cys705Arg $ ITGA2B p.Trp89* $ ITGA2B p.Leu20Arg $ ITGA2B p.Glu355Lys $ ITGA2B p.Asp59Gly & ITGA2B c.848-1G>C & ITGB3 p.Met150Val $ ITGB3 p.Leu20Arg & ITGB3 p.Asn331Ser &	Reduced or absent expression of GPIIb/IIIa on platelet surface	

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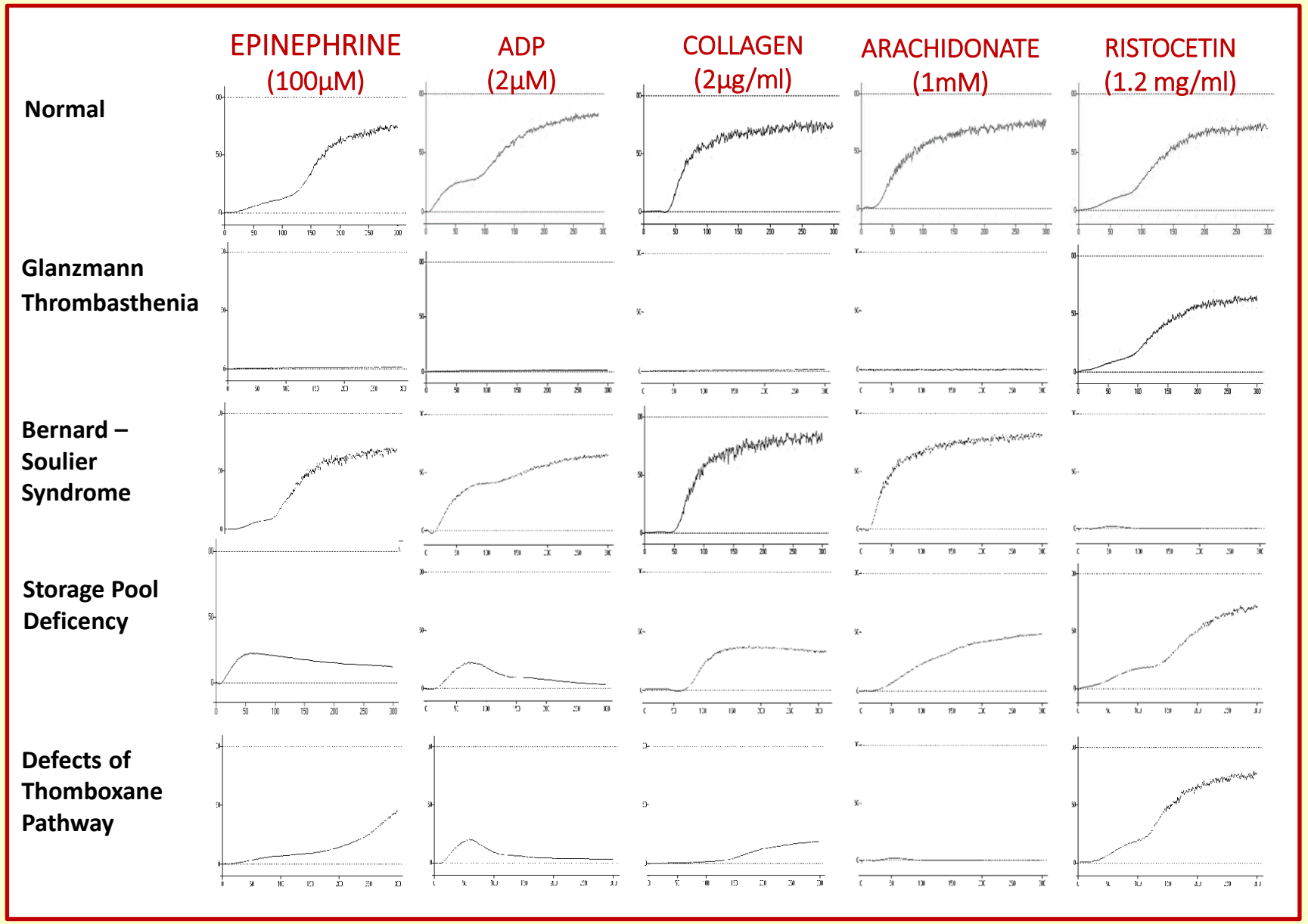
Zaninetti C et al., J Thromb Haemost. 2023;21:1010-1019

Diagnosis of inherited platelet function disorders by LTA



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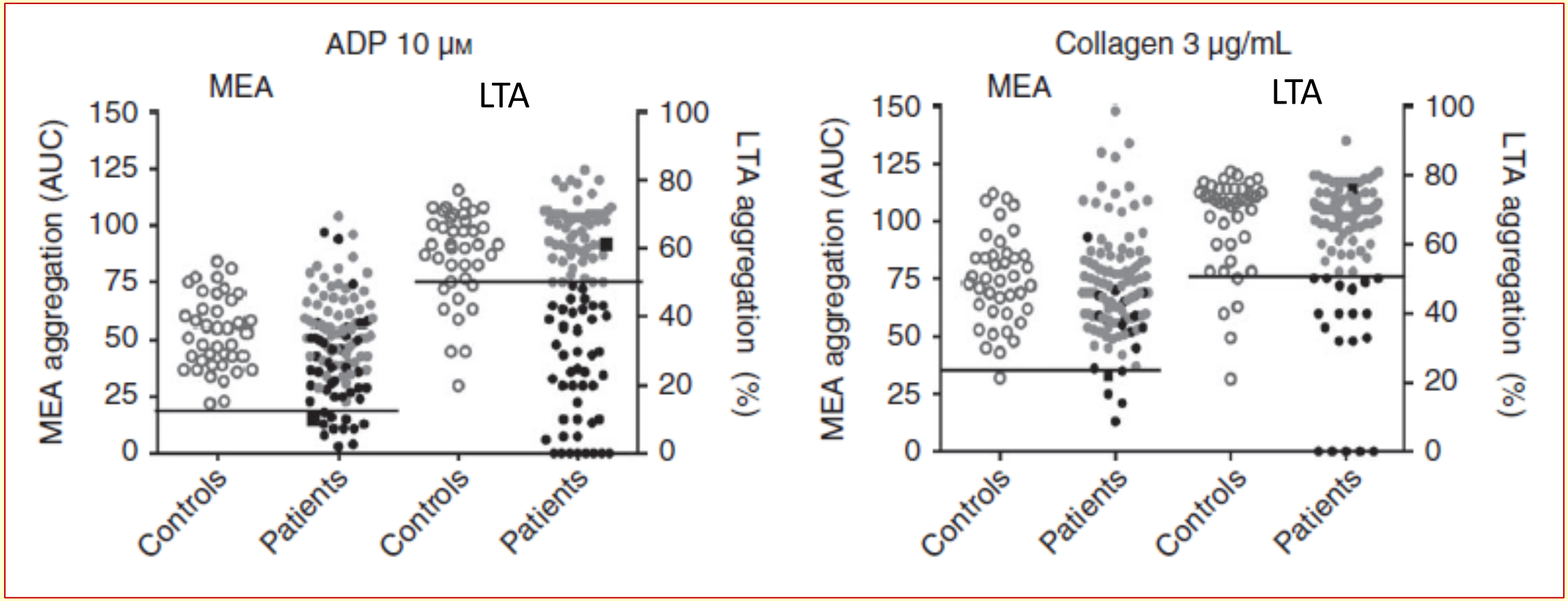
Gresele P et al., Expert Rev Haematol 2019, 12:29-46

Light transmission aggregometry (LTA) vs multiple electronic aggregometry (MEA) for the diagnosis of platelet function disorders



- 40 healthy subjects, 109 IPFD patients

«…MEA is less sensitive in identifying patients with abnormal platelet function than LTA»

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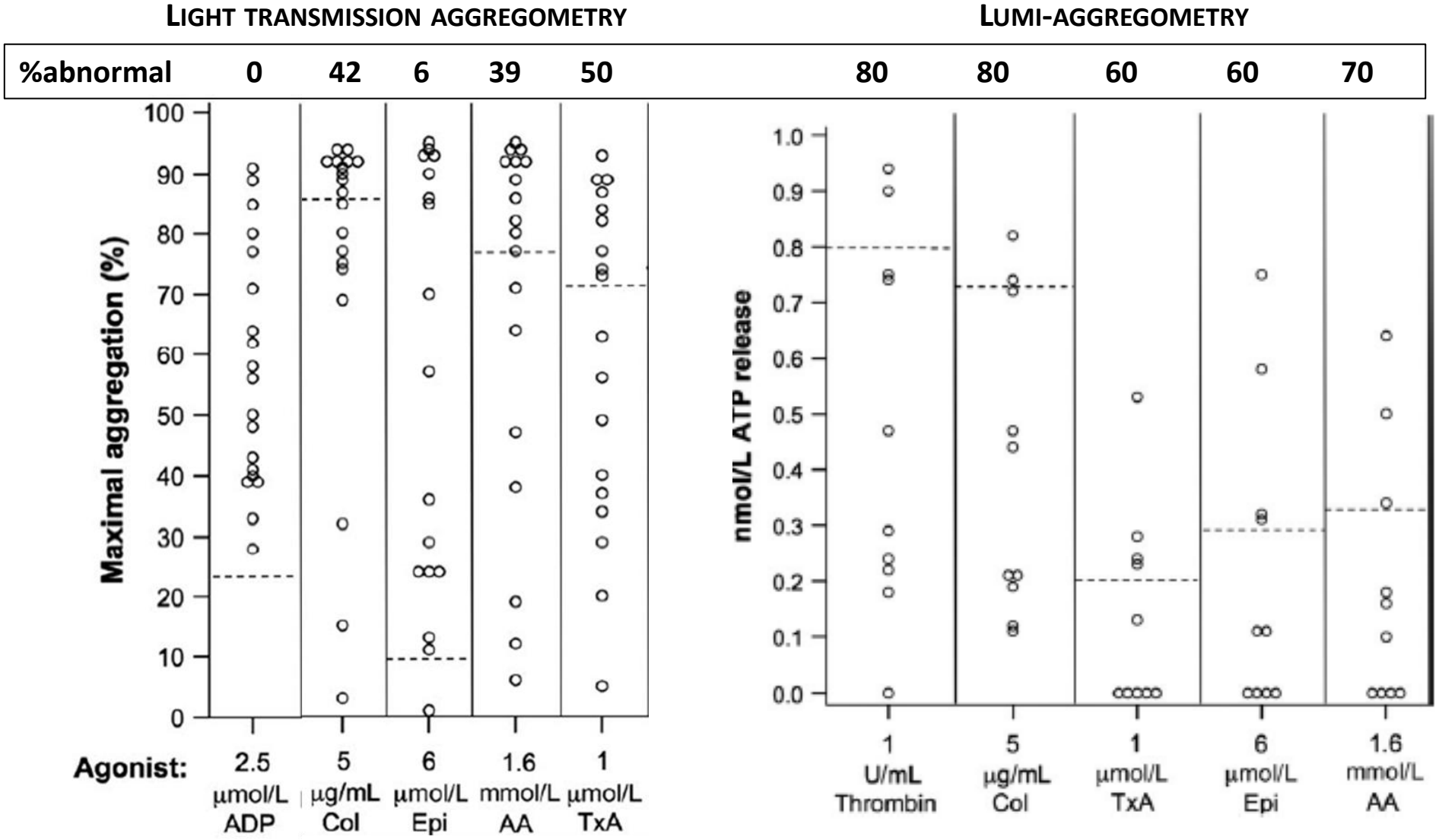
Al Ghaithi R et al., J Thromb Haemost 2017; 15: 2045–2052

LTA may be normal in some patients with dense granule deficiency



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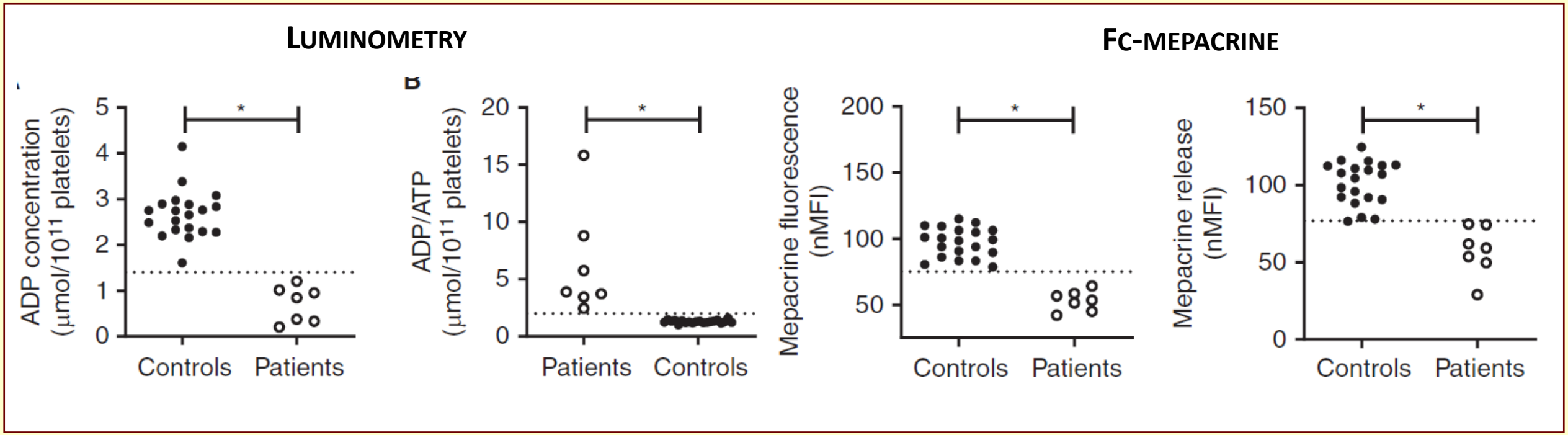
Brunet JG et al., Int J Lab Hem 2018;40:400

Comparison of FC mepacrine fluorescence assay and ATP/ADP luminometry in patients with δ-SPD



«…FC mepacrine fluorescence assay has high NPV and can be used for exclusion of δ-SPD»

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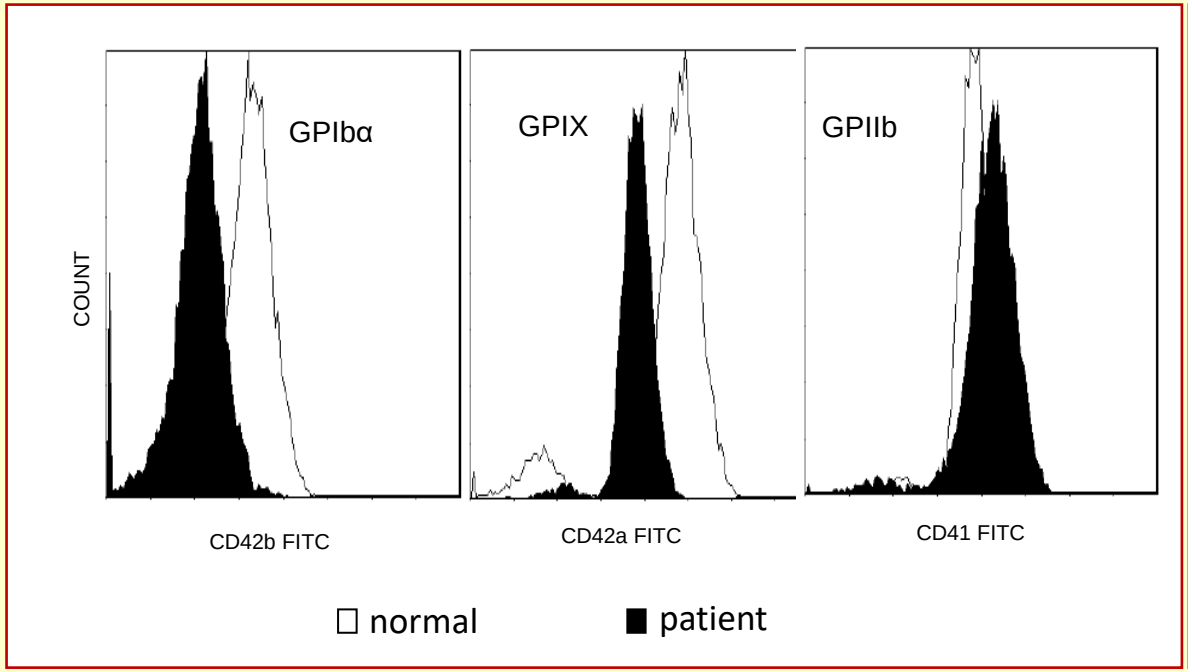
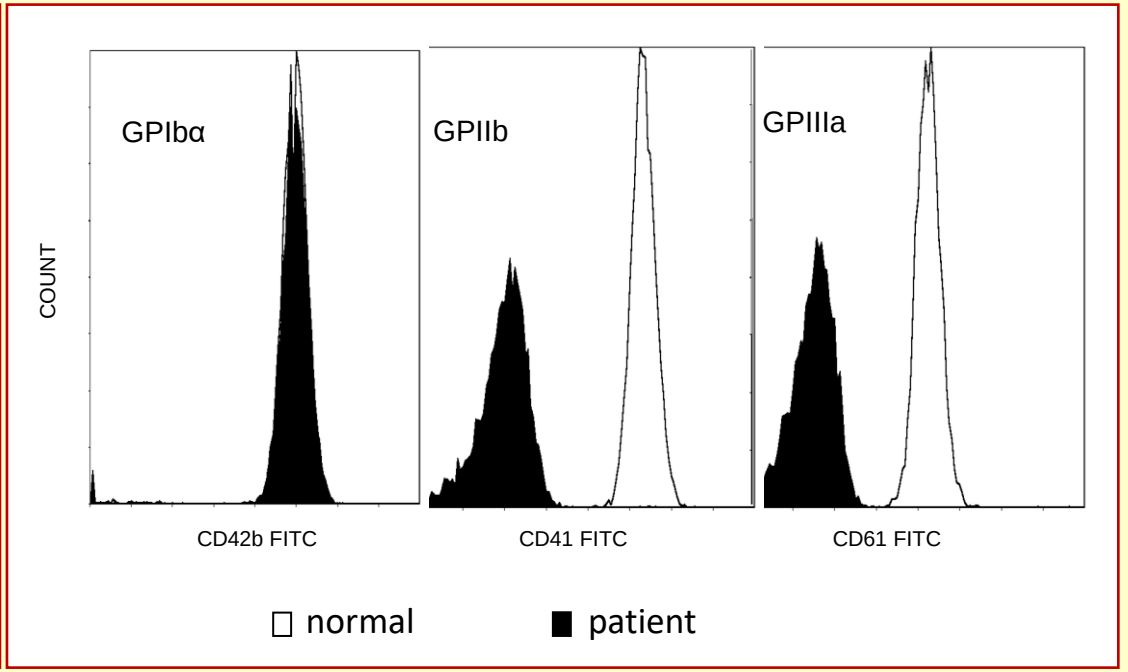
Van Asten I et al., J Thromb Haemost 2020;18(3):706-713.

Diagnosis of IPFD by Flow Cytometry: Platelet surface glycoprotein defects

biallelic Bernard Soulier syndrome (BBS)



Glanzmann thrombasthenia (GT)



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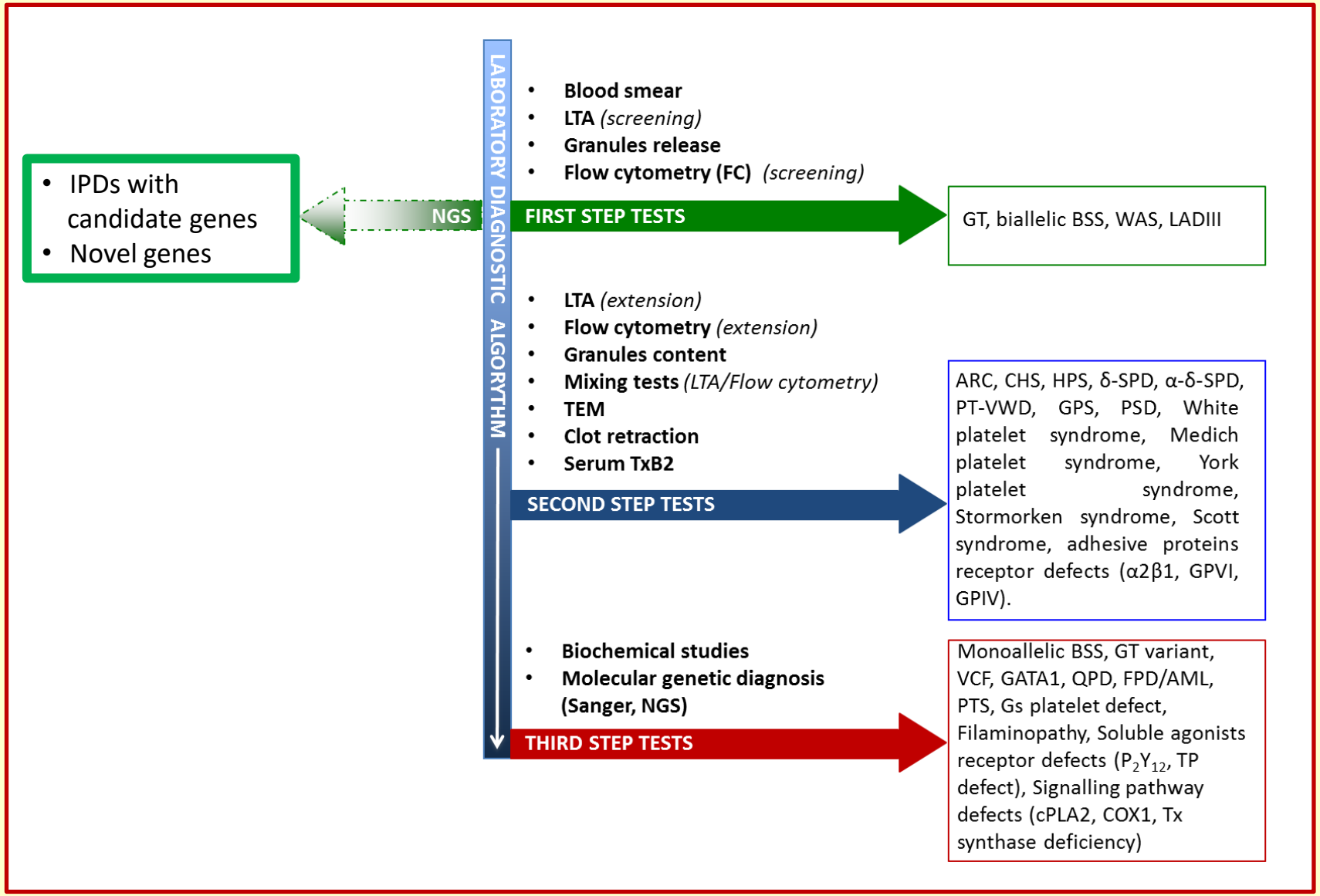
Gresele P, et al. Expert Rev Hematol. 2019; 12:29-46

Diagnoses of IPFDs made by the application of a standardized diagnostic algorithm



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Gresele P et al., Semin Thromb Haemost 2016, 42:292

Indications to genetic diagnosis of IPDs

- **Not necessarily required:** when clinical phenotype or first/second step tests are sufficient for a conclusive diagnosis (e.g. GT, BSS)
- **Advisable:** when the platelet phenotype may not be indisputably attributed to a specific disorder (e.g. Stormorken syndrome, PT/VWD, FDP/AML) or when genotype/phenotype prognostic correlations exists (e.g. MYH9-RD, HPS)
- **Recommended:** when the clinical and laboratory picture is disorienting, functional alterations are heterogeneous, or characterization is uncertain for too few cases described (e.g. GT variants, $cPLA_2$ deficiency, etc.)

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Gresele P et al., Semin Thromb Haemost 2016, 42:292

A puzzling MMBD clinical case

A 30-year old female with a mucocutaneous bleeding diathesis. She had a lifelong history of mild thrombocytopenia and easy bruising and post-partum hemorrhage, and an ISTH-BAT BS of 11 (normal ≤ 5). Bone marrow aspirate was normal with a mild increase of megakaryocytes.

Preliminary screening assays

TEST	Result	Normal values
Skin bleeding time	**>20 min**	2-10 min
PFA-100 Coll/Epi	**>300sec**	85-165 sec
PFA-100 Coll/ADP	**>300sec**	71-118 sec
PT, aPTT, fibrinogen	normal	10-13s, 27-38s, 200-400 mg/dL
Factor VIII	94 %	50-150 %
Ristocetin cofactor	**55 %**	58-97 %
VWF Ag	63 %	60-150 %
CBA %	**45.7 %**	50-400 %

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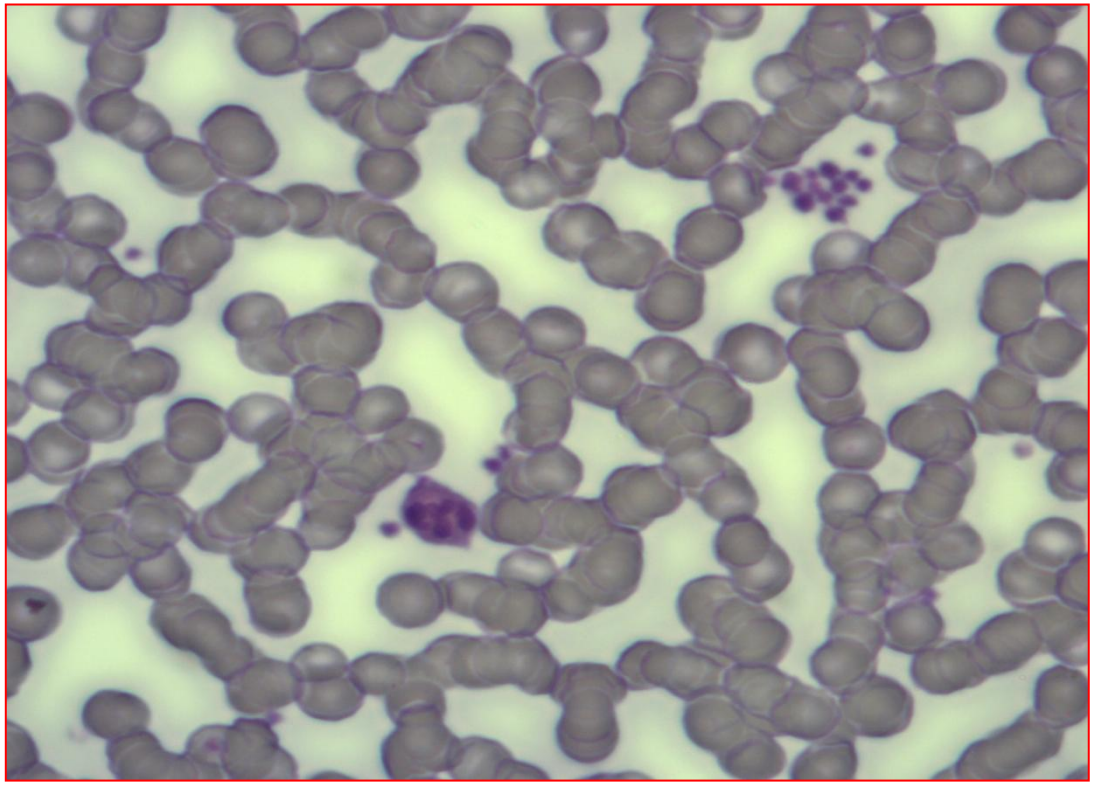
Giannini S et al, Haematologica 2010, 95::2021

Peripheral blood count and blood smear

TEST	Result	Normal values
WBC	5.3 K/µL	4-11 K/µL
RBC	4.29 K/µL	3.8-6.5 K/µL
Hb	12 g/dL	11.5-18 g/dL
Platelets (automatic)	**130 K/µL**	150-400 K/µL
Platelets (microscopic)	**120 K/µL**	150-400 K/µL
MPV	**12.7 fL**	8-12 fL



- Mild macrothrombocytopenia
- Presence of platelet clumps

Giannini S et al, Haematologica 2010, 95: 2021

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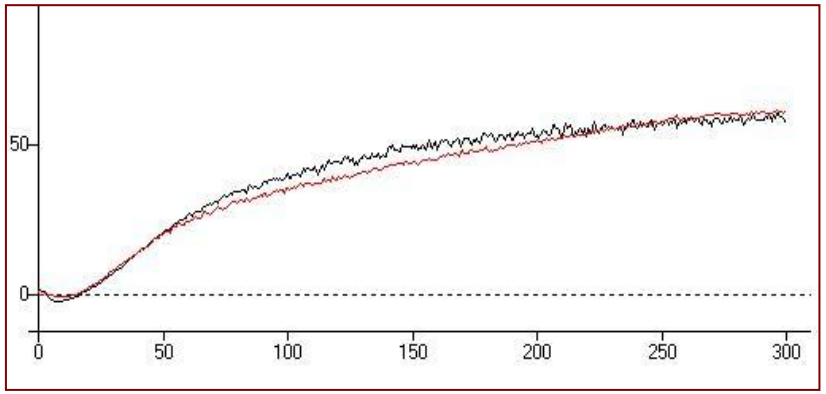
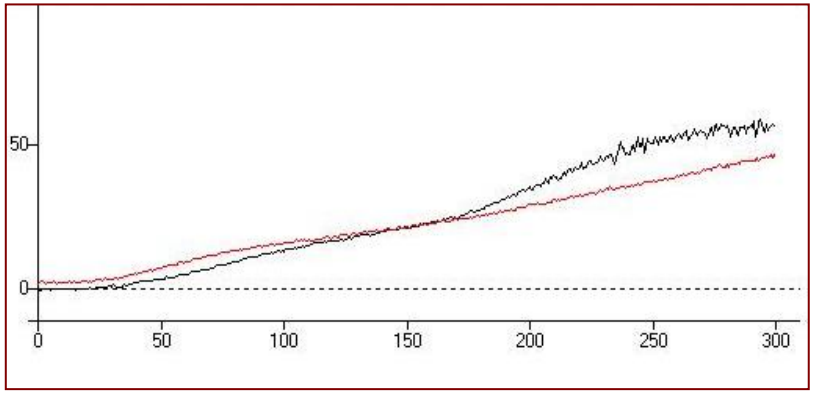
Light Trasmission Aggregometry

control
patient

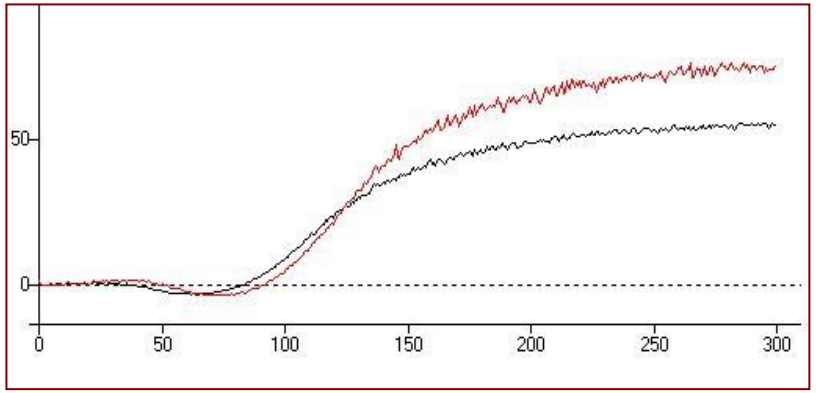
ADP (2µM)



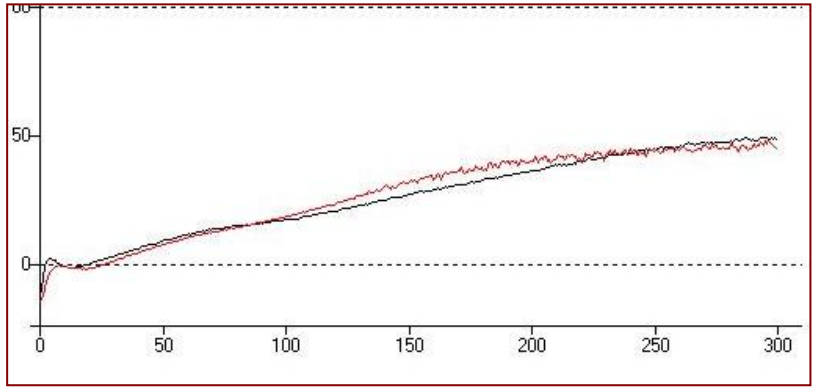
Epinephrine (5µM)



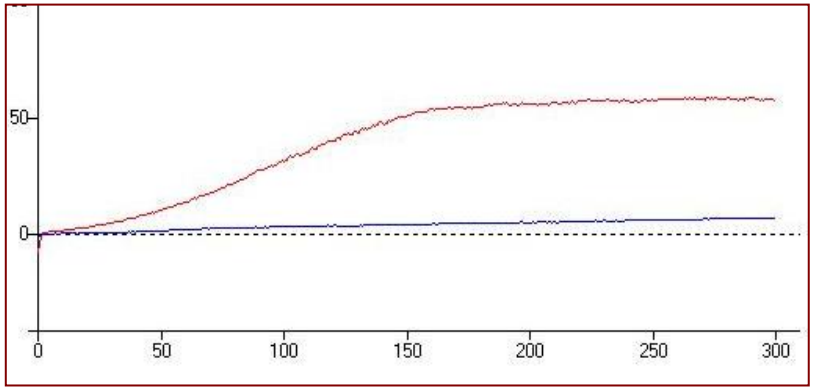
Collagen (2µM)



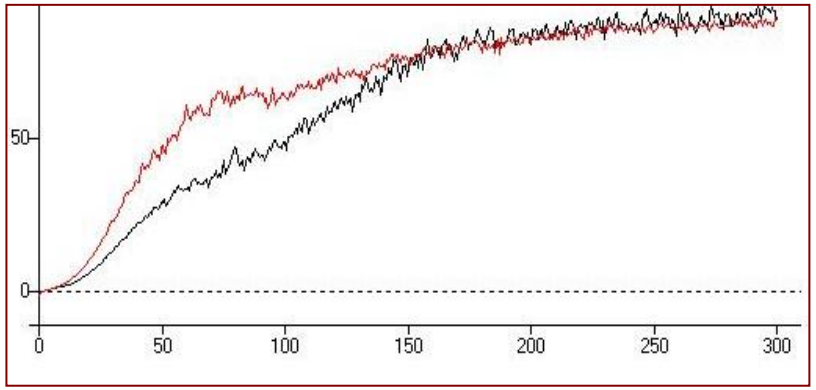
Arachidonic Acid (1mM)



Ristocetin (0.3 mg/ml)



Ristocetin (0.9 mg/ml)



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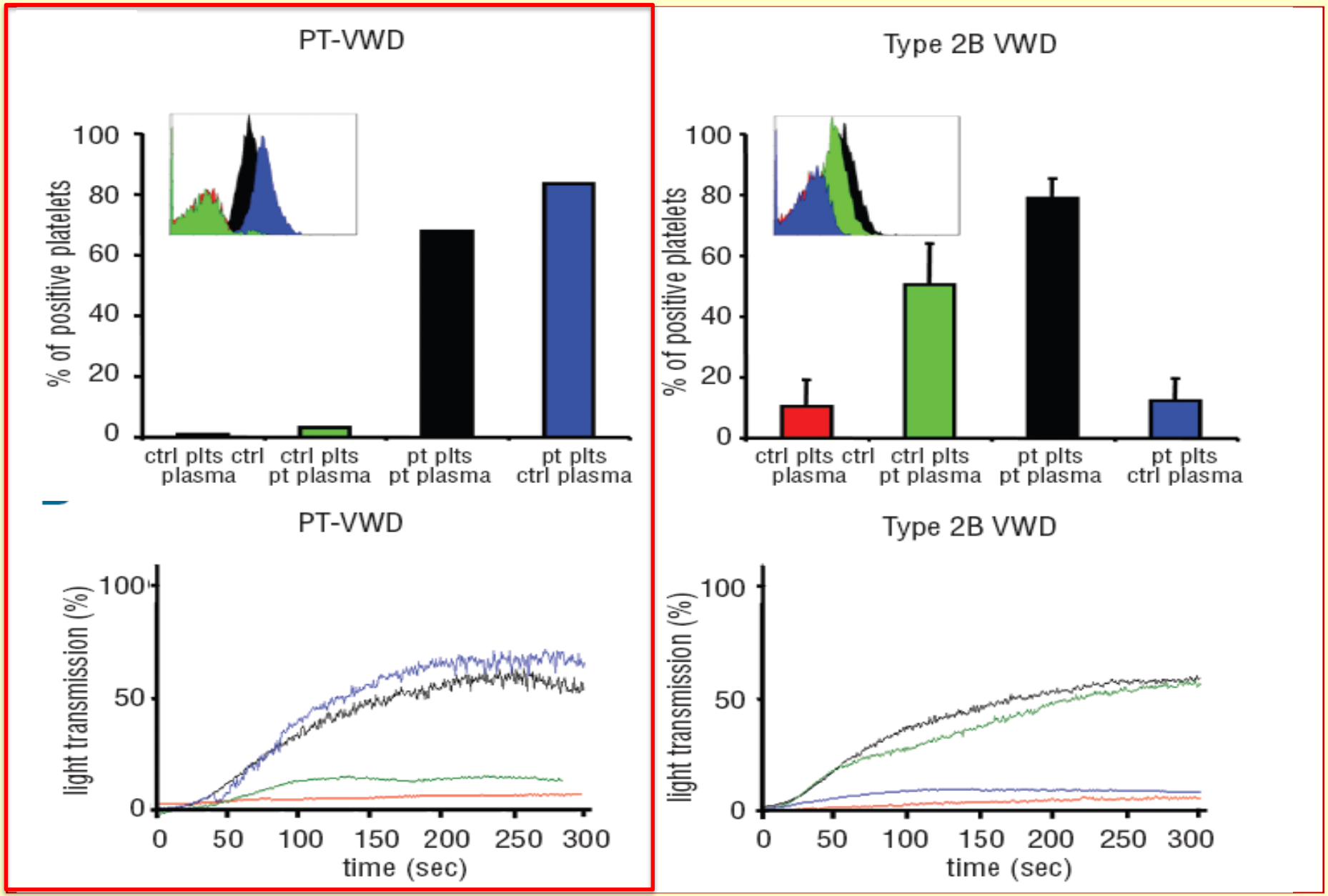
Mixing test: differential diagnosis PT-VWD vs Type 2B-VWD



Giannini S et al., Haematologica 2010, 95:1021

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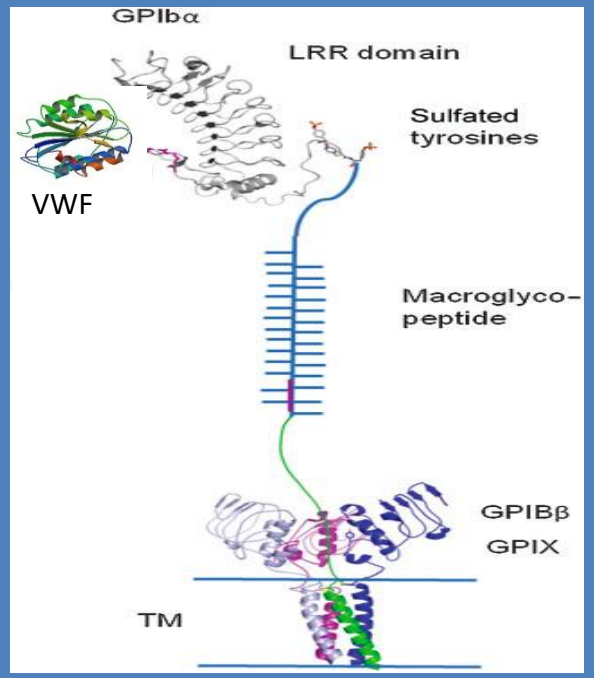
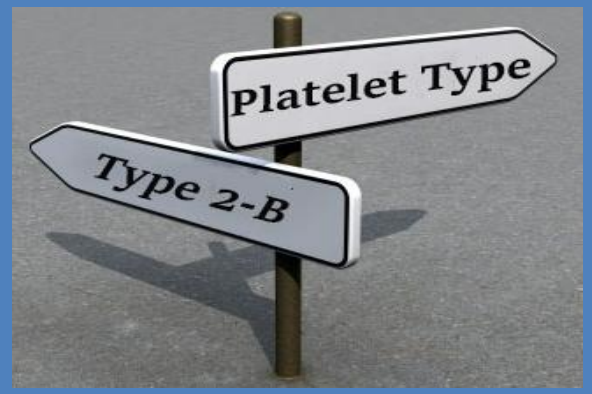
Phenotype may not be indisputably attributed to a specific disease





PT-VWD	2B-VWD
Hemorrhagic diathesis	Hemorrhagic diathesis
Intermittent thrombocytopenia	Intermittent thrombocytopenia
Loss of HMWM-VWF	Loss of HMWM VWF
Increased VWF/Platelet interaction	Increased VWF/Platelet interaction
Platelet GPIbα defect	**Plasmatic VWF defect**
Mutations in GP1BA	**Mutations in VWF**
Platelet trasfusions	**Administration of VWF/FVIII**

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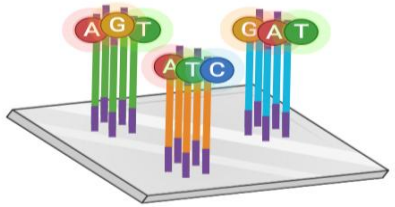
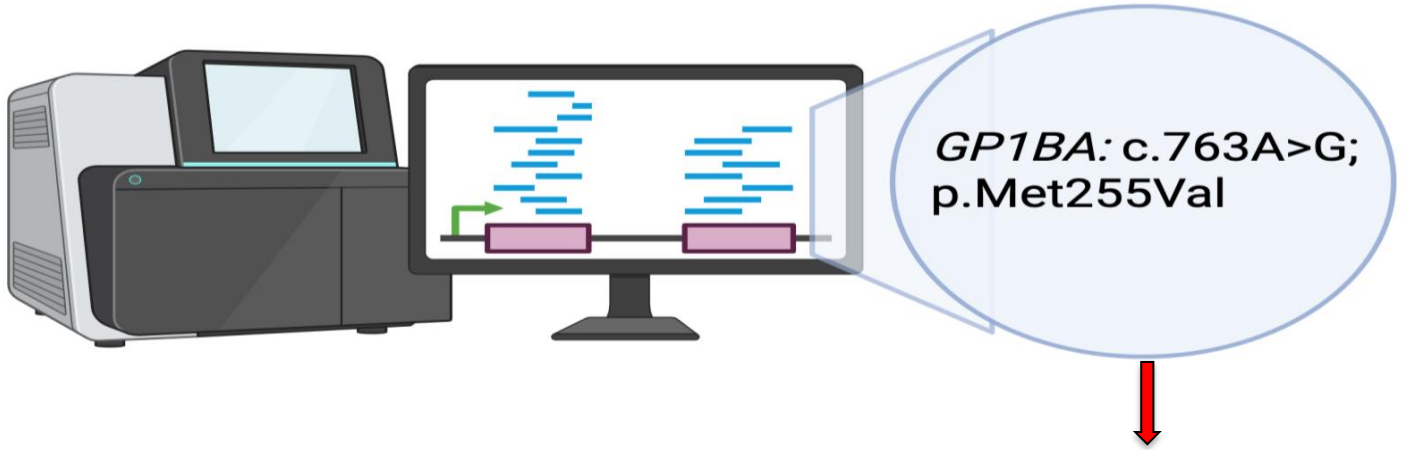
Giannini S et al. Haematologica 2010; 95: 1021

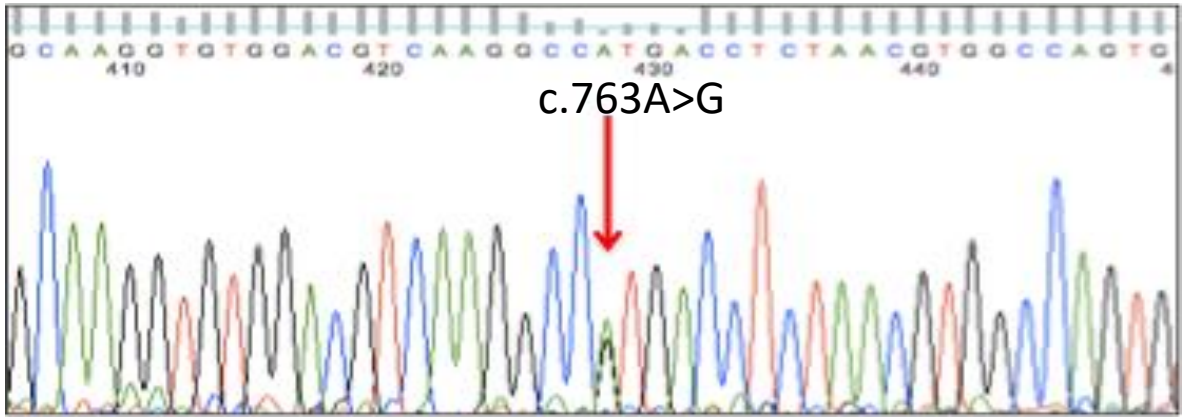
Genetic analysis

NGS (gene panel) identified a pathogenic *GP1BA* variant typical of PT-VWD





Confirmed by Sanger sequencing



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Conclusions

- Inherited platelet disorders represent a significant fraction of all bleeding diatheses
- A careful clinical evaluation, possibly based on the ISTH BAT bleeding score, and a screening for VWD exclusion, are the mainstay for the decision to test platelet function
- A simplified streamlined panel of tests allows to identify most of the known inherited platelet disorders
- Genetic diagnosis may complement platelet function testing to decipher complex phenotypes, to formulate prognostic predictions (genotype/phenotype correlation) and to identify new forms

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EHA-SWG Scientific Meeting on Bleeding and Platelet Disorders: Advances in Pathology, Diagnosis, and Management

Dates: April 9-11, 2026
Location: Florence, Italy

Chairs: Giancarlo Castaman and Paolo Gresele

Collaborating Specialized Working Groups (SWGs):

- SWG on Thrombocytopenias and Platelet Function Disorders (TPFD)
- SWG on Bleeding and Thrombosis

Registration for the meeting is open. For full details, visit our registration and accommodation page.

https://ehaweb.org/connect-network/meetings/eha-swg-scientific-meeting-on-bleeding-and-platelet-disorders

Assessment of the bleeding severity of hemorrhagic disorders

- Measurement of history of spontaneous or provoked hemorrhage by bleeding assessment tools
- Systematic evaluation of the prevalence of excessive bleeding during invasive procedures

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HemaSphere
Powered by EHA

Fundamentals for a Systematic Approach to Mild and Moderate Inherited Bleeding Disorders: An EHA Consensus Report

Francesco Rodeghiero[1], Ingrid Pabinger[2], Margaret Ragni[3], Rezan Abdul-Kadir[4], Erik Berntorp[5], Victor Blanchette[6], Imre Bodó[7], Alessandro Casini[8], Paolo Gresele[9], Riitta Lassila[10], Frank Leebeek[11], David Lillicrap[12], Diego Mezzano[13], Patrizia Noris[14], Alok Srivastava[15], Alberto Tosetto[16], Jerzy Windyga[17], Barbara Zieger[18], Mike Makris[19], Nigel Key[20]

Definition of bleeding disorders

Bleeding disorders (BD)	Any distinct disease entity associated with an excessive bleeding tendency qualified by a unique set of hemostatic and/or genetic abnormalities
Mild-moderate bleeding disorder (MMBD)	Any BD associated with a mild to moderate bleeding phenotype

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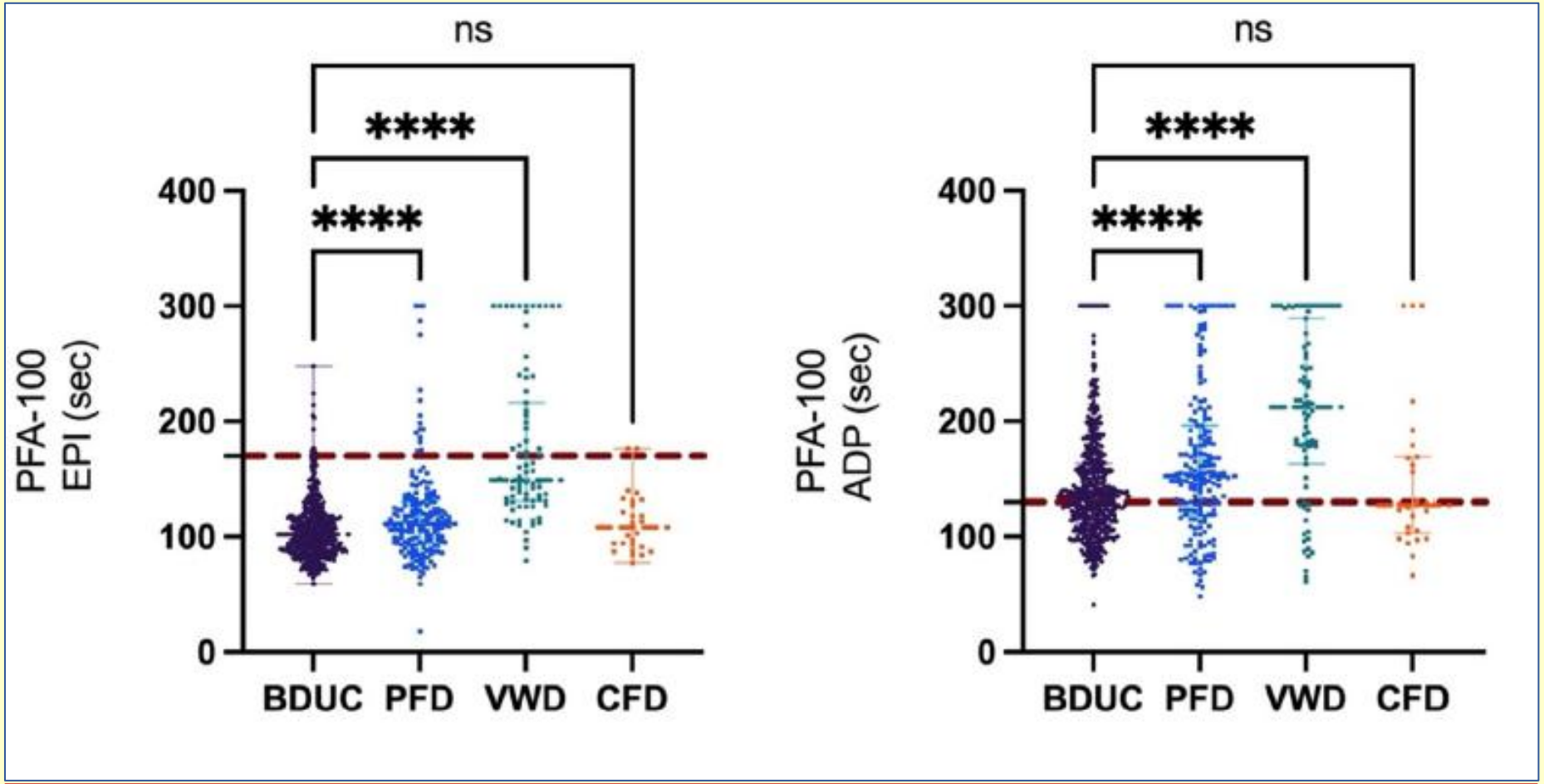
Rodeghiero F et al., HemaSphere 2019; 17 (3):e286

PFA-100 values in patients with mild-to-moderate bleeding disorders



BDUC: bleeding disorder of unknown cause; PFD: platelet function defect; VWD: von Willebrand disease; CFD: clotting factor deficiency

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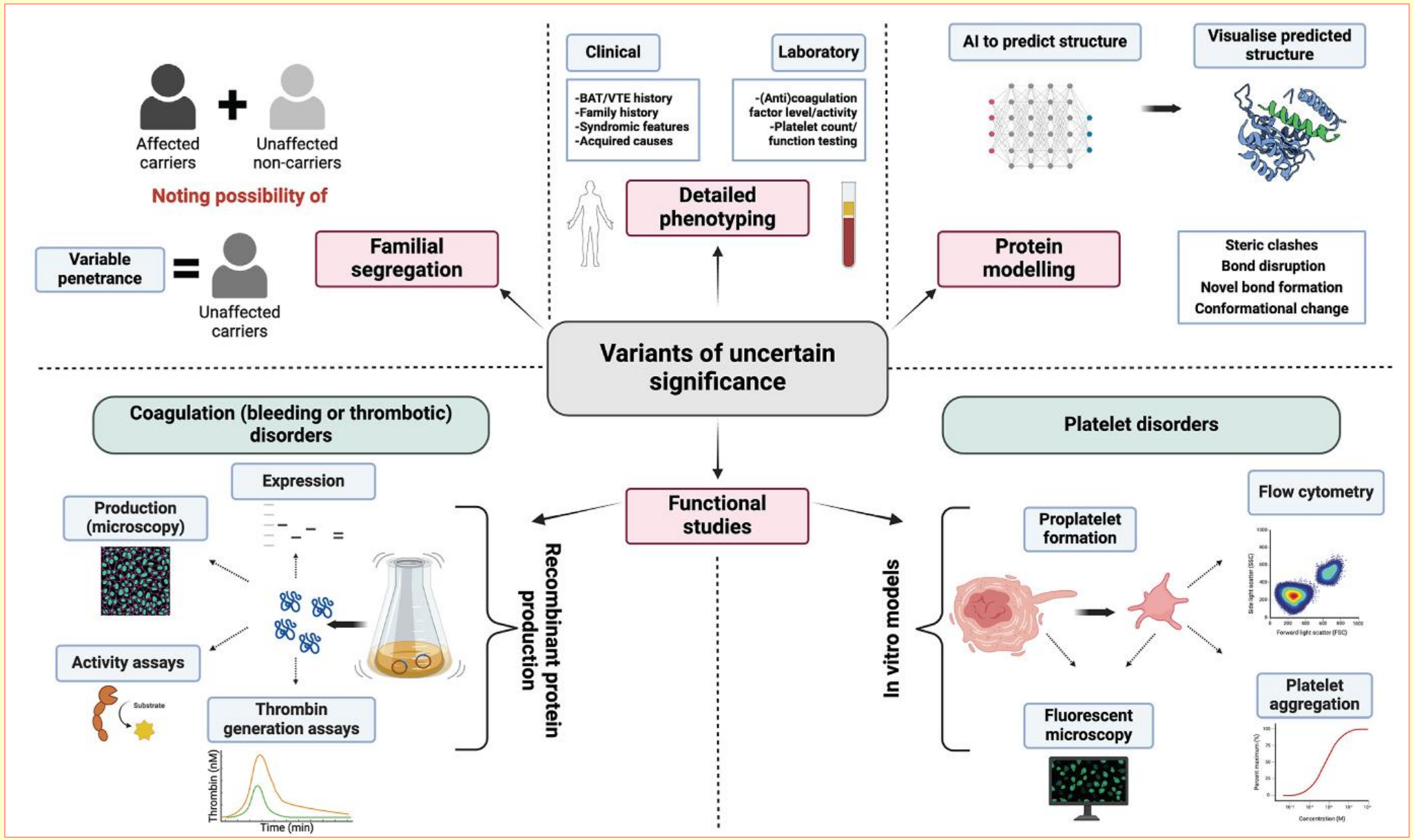
Mehic D et al., J Thromb Haemost. 2025;23(9):2982-298

Approach to the interpretation and reclassification of VUS in bleeding disorders



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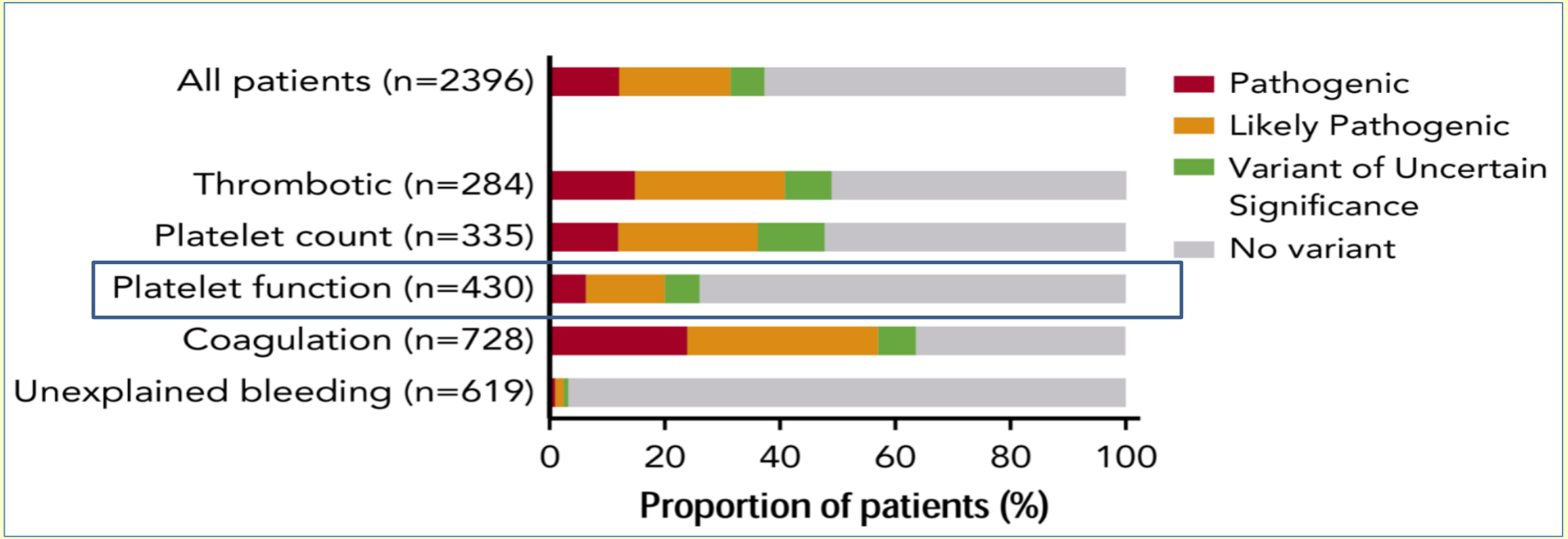
Khourssaji M et al., Thromb Haemost 2025; 125:618-6

Diagnostic rates of the Thrombogenomics



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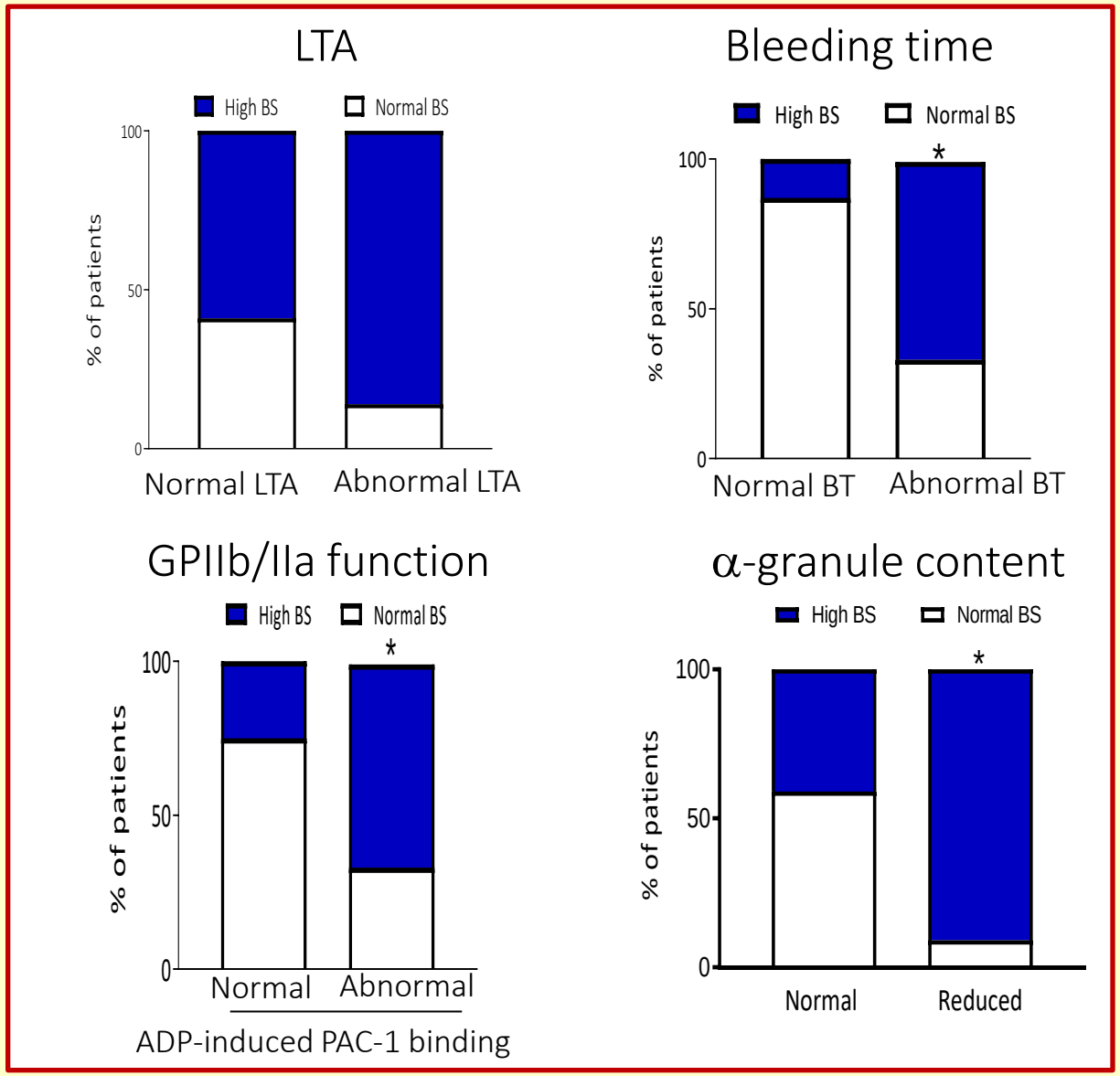
Downes K et al, Blood. 2019;134(23):2082-2091

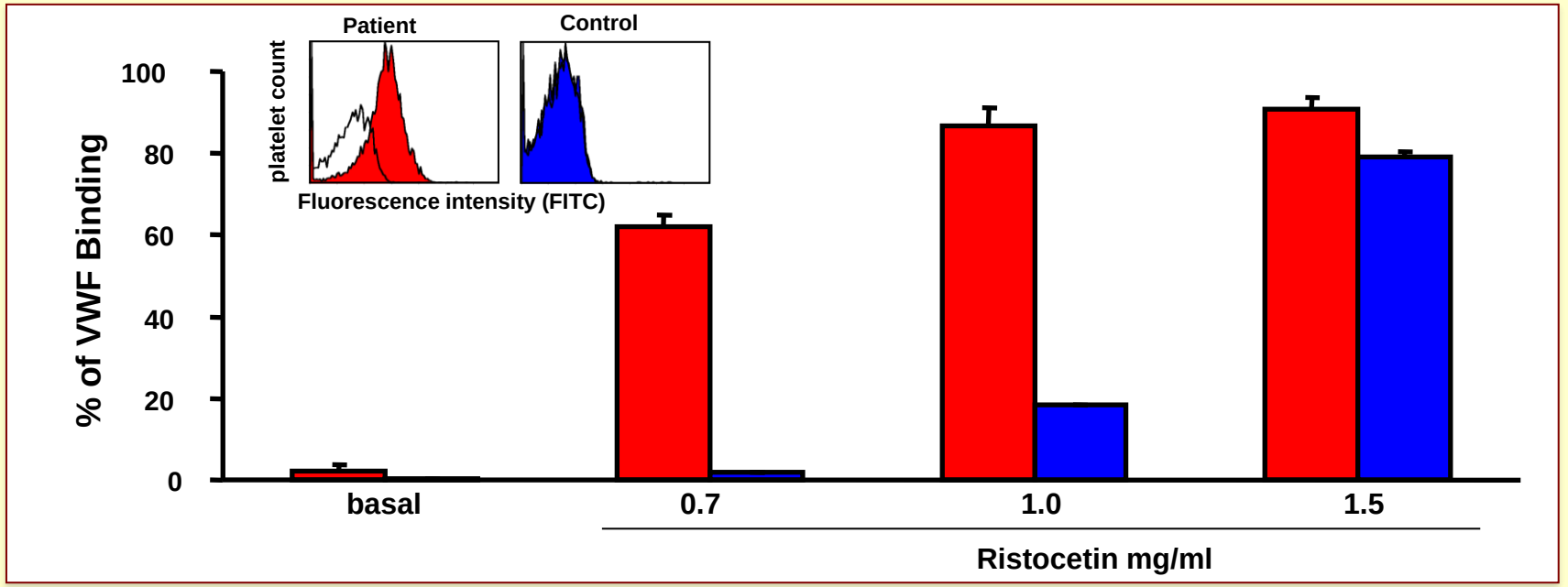
Association of platelet function assays with the ISTH BAT BS

68 patients included from 11 centers worldwide: Median baseline BS was 8 (IQR 2.2-12).
15 different IPFD forms were represented.



Gresele P, et al 2023. Submitted

Binding of VWF to platelets by flow cytometry



Flow cytometry of platelet glycoproteins

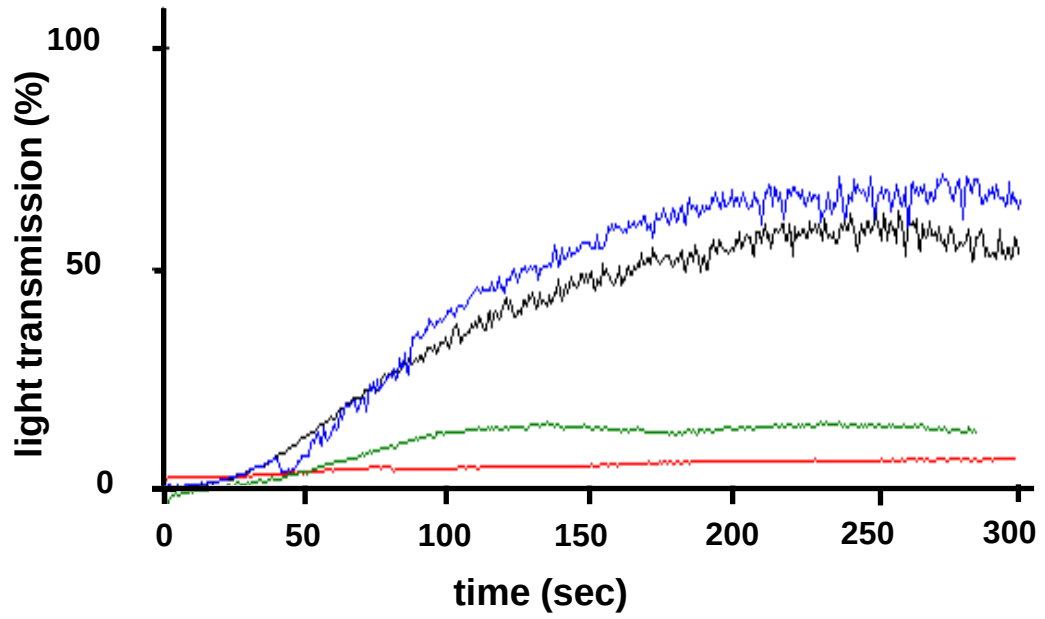
GLYCOPROTEIN	Result (Mean Fluorescence Intensity)	Normal values (Mean Fluorescence Intensity)
GPIIb/IIIa (CD41/61)	21.5	22.1±3.2
GPIIb (CD41)	4	4.5±1.06
GPIIIa (CD61)	15.6	16.3±5.2
GPIb/IX (CD42a)	6.8	7.5±1.2
GPIbα (CD42b)	20	19.7±1.8

Giannini S et al. Haematologica. 2007;92:1647-54

Aggregometric mixing test

RIPA (Ristocetin mg/ml)

	CONTROL PLASMA	PATIENT PLASMA
CONTROL PLATELETS	1.1	1.2
PATIENT PLATELETS	**<0.3**	**0.3**



Patient plts+ctrl plasma

Patient plts+patient plasma

Ristocetin
0.3 mg/ml

Ctrl plts+patient plasma

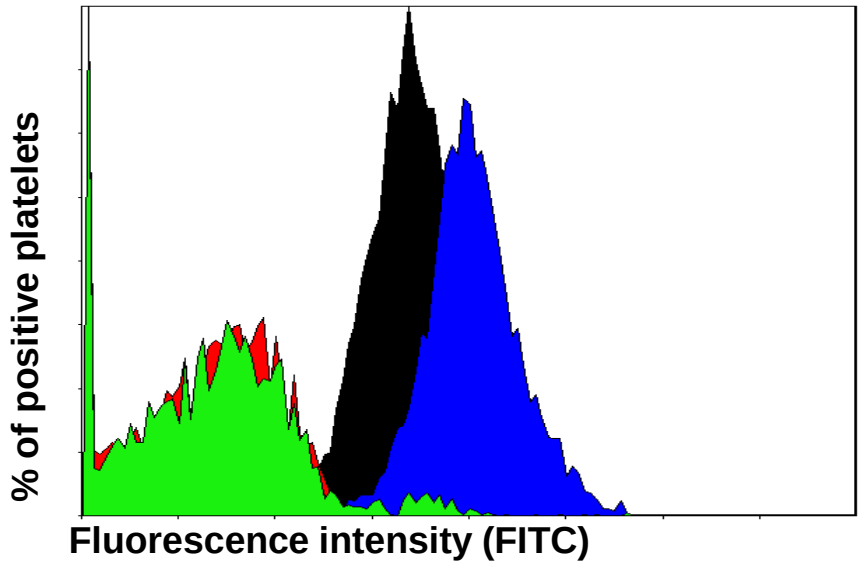
Ctrl plts+ctrl plasma

Ristocetin
0.1 mg/ml

Flow cytometric mixing test

VWF binding (% positive platelets)
Ristocetin 0.75 mg/ml

	CONTROL PLASMA	PATIENT PLASMA
CONTROL PLATELETS	1.7 %	4.1 %
PATIENT PLATELETS	**68.6 %**	**70.8 %**



Ctrl plts
Ctrl plasma

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Pt plasma

Pt plts
Pt plasma

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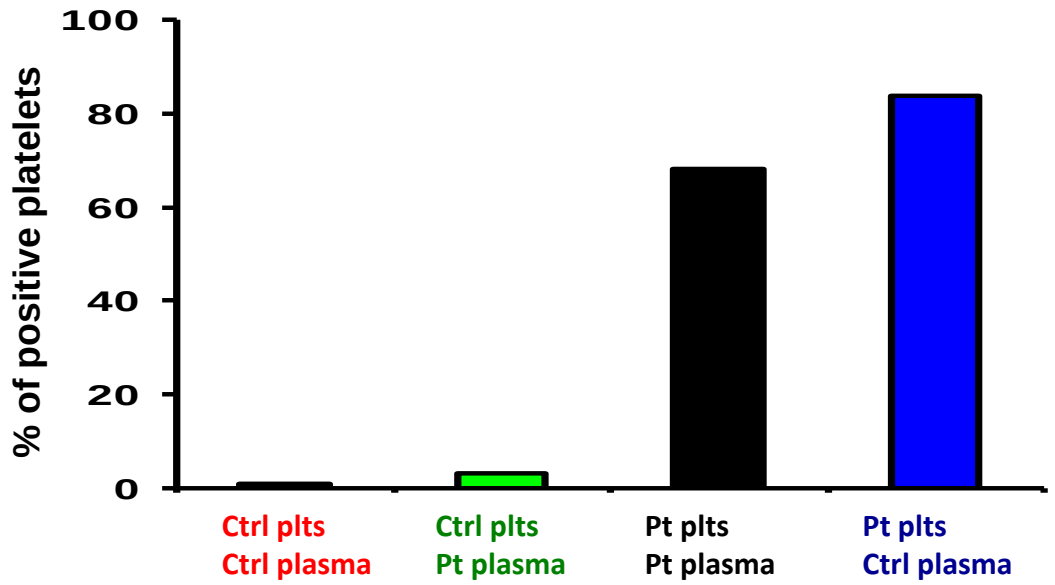
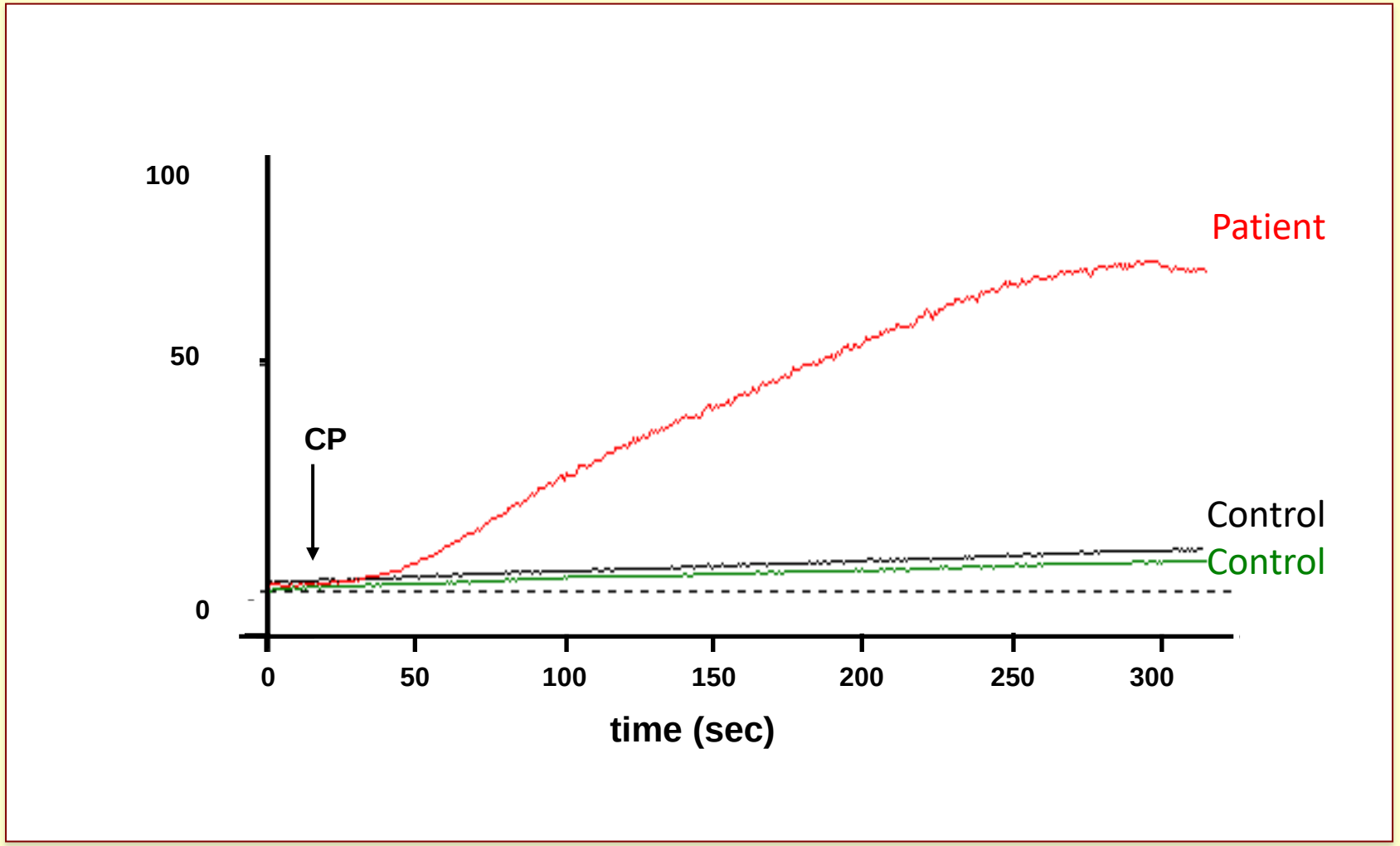


Cryoprecipitate assay

50 µl of cryoprecipitate dissolved in TrisHCl 0.01M, NaCl 0.15M, 10.6 mM trisodium citrate were added to 250 µl of PR and aggregation was followed for 300 sec.



Possible future developments in the diagnostic approach to platelet function disorders

- **MICROFLUIDIC DEVICES**
  - multi-microspot microfluidic test (very informative; only research)
  - total thrombus formation assay system (T-Tas)(only preliminarily tested in δ-SPD)
- **MULTICOLOR FLOW CYTOMETRY**
  - enables the study of various intracellular signalling events (only preliminarily tested in δ-SPD)
- **MASS CYTOMETRY**
  - enables the simultaneous analysis of numerous platelet surface markers and functional proteins (only preliminarily tested in GT patients)
- **ARTIFICIAL INTELLIGENCE (ML)-ASSISTED DIAGNOSIS**
  - differential diagnosis of IPD vs ITP/acquired PFD.

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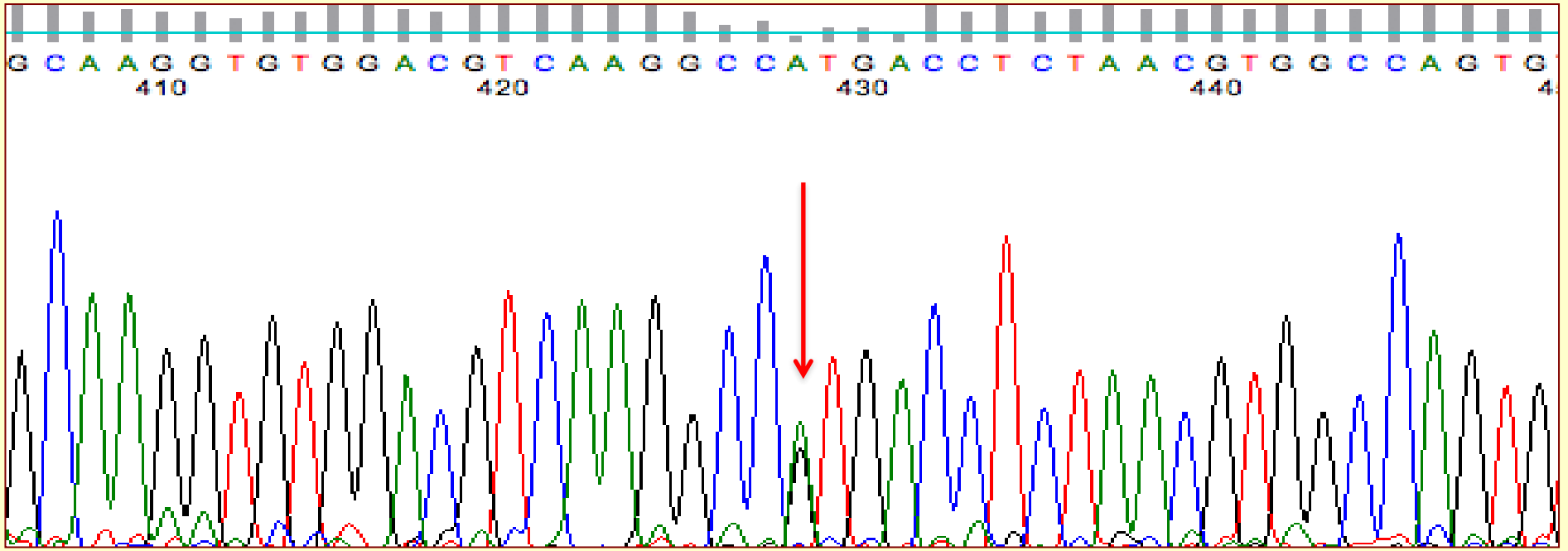
Gresele P et al., Haemostaseologie 2025, in pres

DNA sequencing



Heterozygous A/G

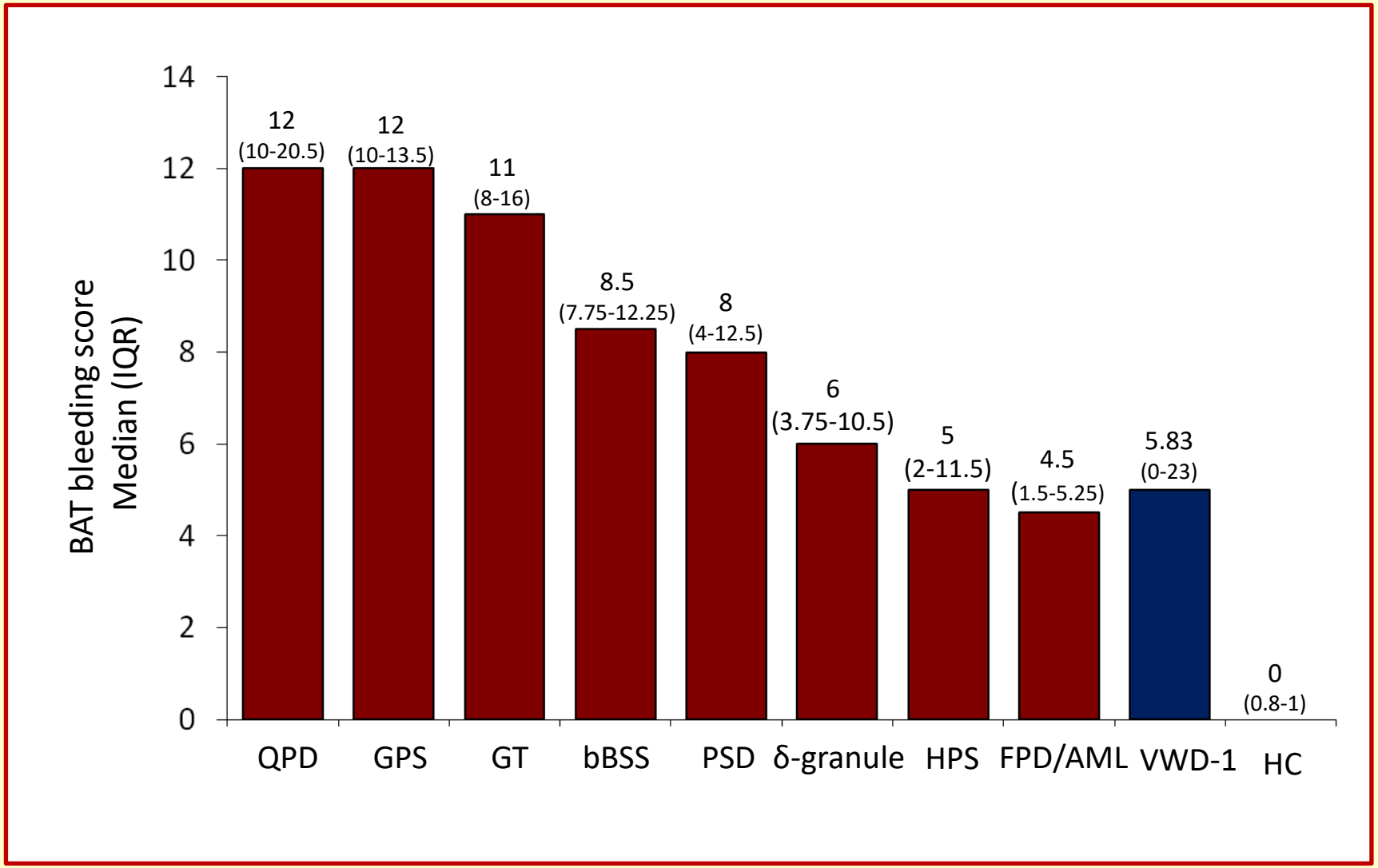
Met239Val of glycoprotein Ibα (GPIbα)

Typical of platelet-type VWD

Diagnosis confirmed

Giannini S et al, Haematologica 2010, 952021:

BAT bleeding score in IPFD by principal diagnoses



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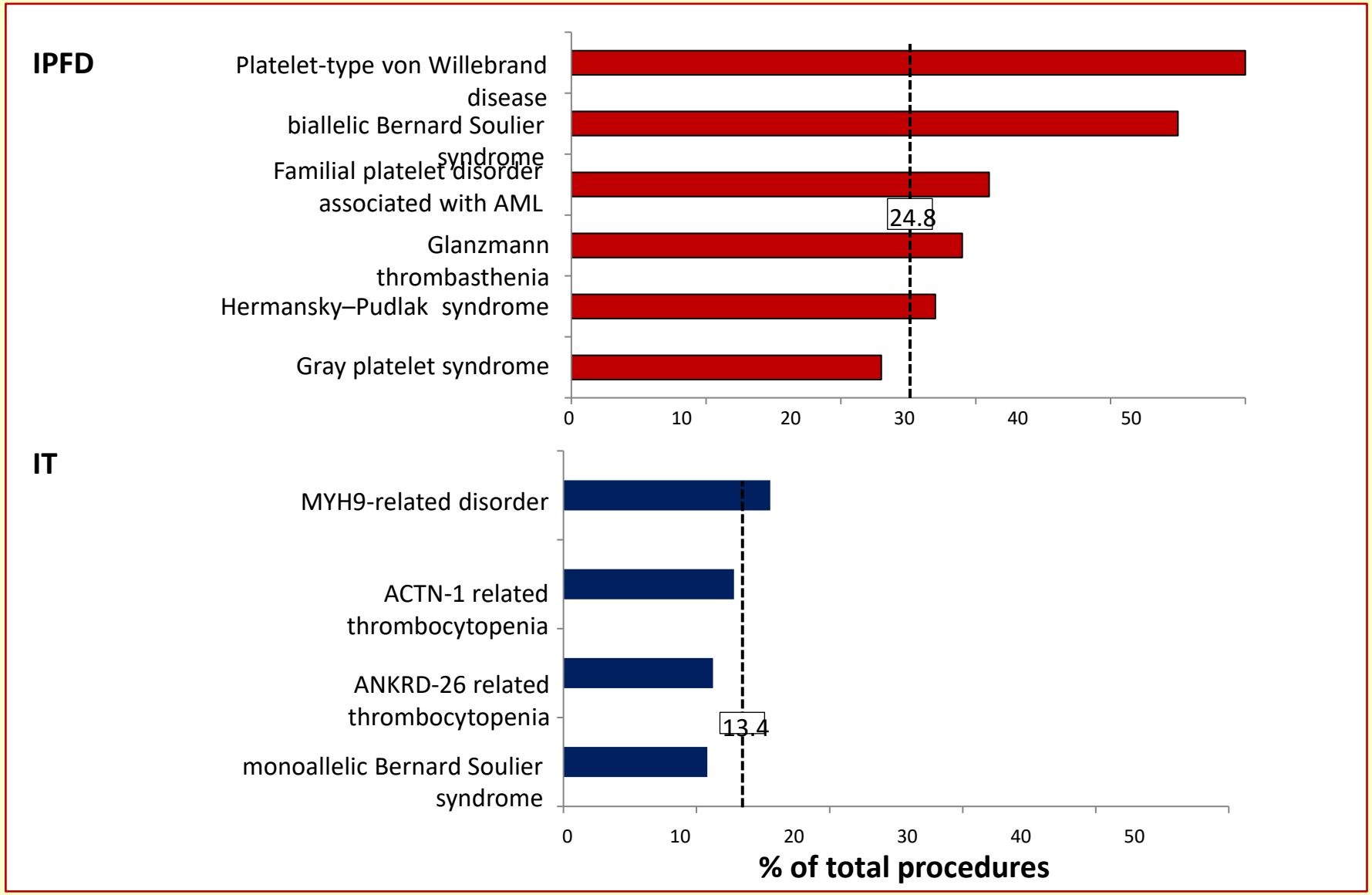
Gresele P et al., J Thromb Haemost 2020, 18:732

Frequency of AEB at surgery according to diagnosis



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Orsini S et al., Haematologica 2017, 102:119

How to diagnose an IPFD: a simplified approach

Confirmed clinical suspicion (ISTH-BAT, Physical examination)

Preliminary laboratory investigation:
- Full blood count
- Routine coagulation tests
- VWF screening

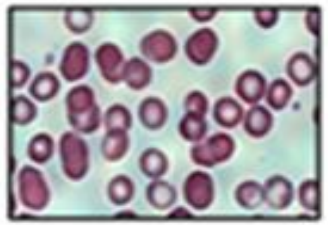
NORMAL → Potential platelet function disorder

ABNORMAL → **DIAGNOSIS**
- VWD
- Thrombocytopenia
- Clotting defect
- Afibrinogenemia

Potential platelet function disorder → PLATELET STUDIES

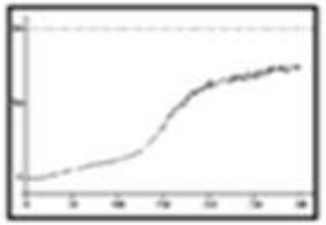
Potential platelet function disorder → NEXT GENERATION SEQUENCING

Blood smear



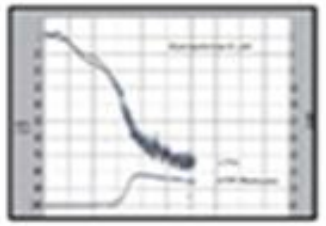
- Platelet size
- Morphologic alteration of platelets or other cells

Platelet aggregation



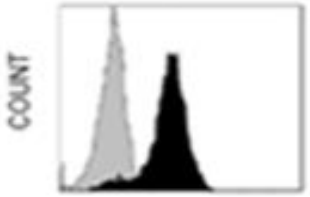
- Platelet aggregation in response to Epi, ADP, coll, AA, Ristocetin, TRAP-6, CVX, U46619

Granules assays



- α (e.g. β-TG) and δ-granules (e.g. ATP) content and release analysis

Flow cytometry (FC)



- Expression of GPIIb/IIa and GPIb/V/IX , GPVI, GPIa/IIa, GPIV
- GPIIb/IIa activation
- Procoagulant activity

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Gresele P, J Thromb Haemost 2015;13:316
Gresele P et al., Haemostaseologie 2025;45(4):335-34