# Inquadramento clinico delle piastrinopatie congenite

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# INHERITED PLATELET FUNCTION DISORDERS

- A heterogeneous group of rare congenital hemorrhagic disorders with normal (or reduced) platelet number and an altered platelet function
- Mucocutaneous bleeding diathesis of variable severity
- Large heterogeneity in terms of molecular/genetic defect (for several forms not yet identified)

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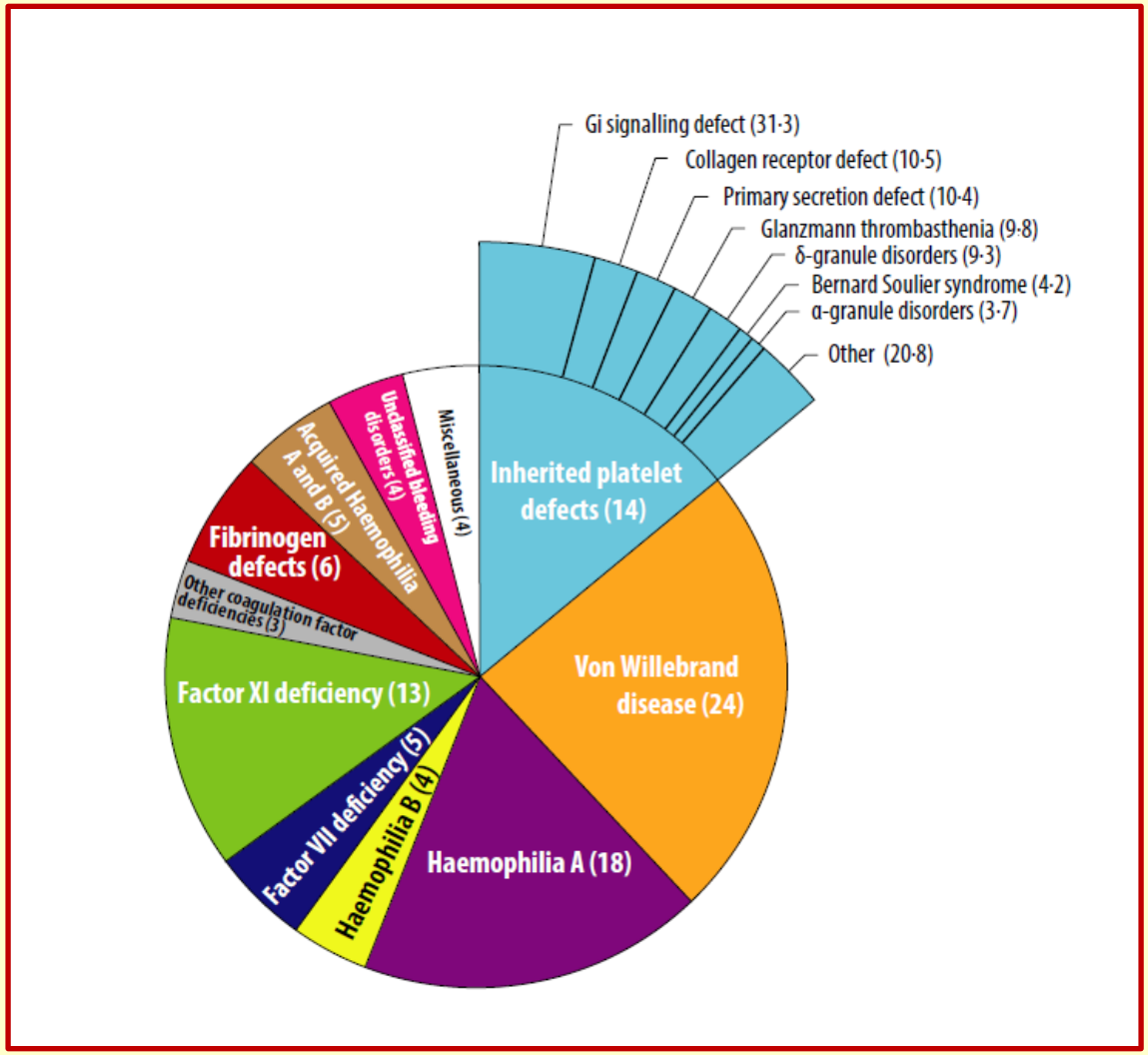
# Prevalence of IPD

- The prevalence of IPNDs in Italy is estimated to be at least 2.7/100,000 (Balduini C et al. Hamostaseologie 2012;32:259-270).
- The exact prevalence of IPFDs is unknown, but estimates go from 2/1,000,000 (Israels SJ et al. *Pediatr Blood Cancer* 2011; 56:975-83) to more than 1/100, a prevalence higher than that of von Willebrand disease (Quiroga T et al. *Haematologica* 2007;92:357).

# Recorded incidence of bleeding disorders from the 2015 annual registration data from the UK-HCDO




Bariana TK et al., BJH 2016

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# Diagnosis of suspected IPFD: **results of a worldwide survey**

- Many laboratories worldwide are involved in the diagnosis of IPFD
- Tests for IPFD represent a major committment these diagnostic laboratories (>14.000 patients studied each year)
- High variability in the diagnostic approaches
- In 40-60% of studied patients with confirmed platelet dysfunction, a diagnosis is not achieved despite demanding laboratory investigations

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

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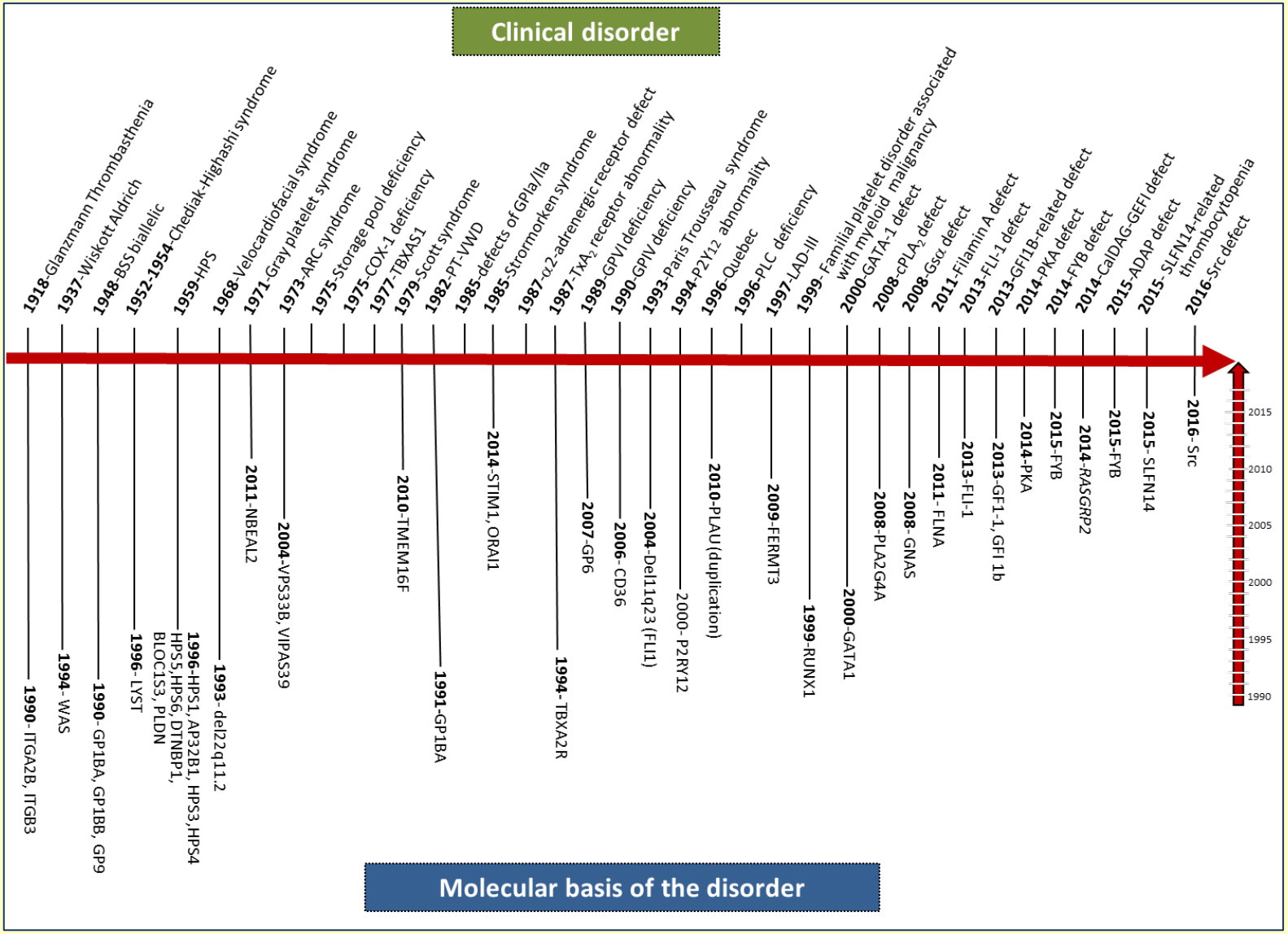
# History of clinical and molecular discovery of inherited platelet function disorders

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Bury L, Falcinelli E, Gresele P, Wintrobe's Clinical Hematology 14° Edition, in press

# INHERITED PLATELET FUNCTION DISORDERS
## A revised classification

**1-ADHESIVE PROTEIN RECEPTOR DEFECTS**

BSS, GT, PT-VWD, VCF, $\alpha_2\beta_1$, GPVI, GPIV

**2-ABNORMALITIES OF G-PROTEIN COUPLED RECEPTORS**

$P_2$ purinergic receptors, TP-receptor, $\alpha_2$ adrenergic receptor, PAR-1

**3-DEFECT OF PLATELET GRANULES (isolated/syndromic)**

α-granules, δ-granules, α+δ granules

**4-SIGNAL TRANSDUCTION PROTEINS DEFECTS**

Gs, Tx-synthase, $cPLA_2$, PKA, LADIII, CalDAG-GEFI, COX-1, SRC

**5-DEFECTS OF MEMBRANE PHOSPHOLIPIDS**

Scott syndrome, Stormorken syndrome

**6- DEFECTS OF TRANSCRIPTION FACTORS**

FPD/AML/MDS, FLI1-related dense granule defect,

Paris-Trousseau syndrome, GATA1

**7- DEFECTS OF CYTOSKELETAL PROTEINS**

Filaminopathy, WAS/XLT, Cytosolic adaptor protein (ADAP)

**8- ENHANCED PLATELET FIBRYNOLITIC ACTIVITY**

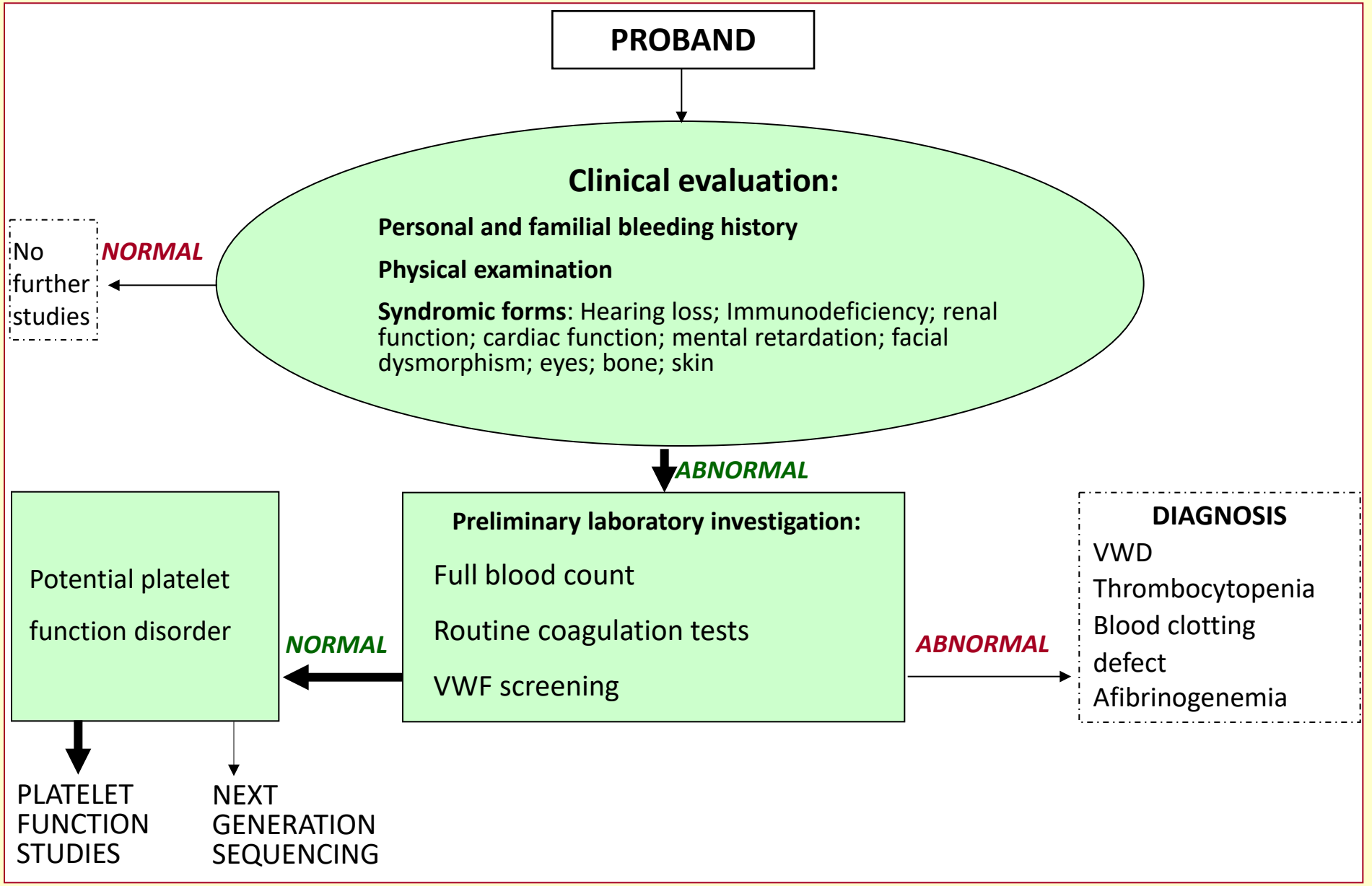
Quebec platelet disorder

**9- UNCLASSIFIED**

SLFN14-related thrombocytopenia

Bury L, Falcinelli E, Gresele P, Wintrobe's Clinical Hematology 14° Edition, in press

# DIAGNOSTIC ALGORITHM - Flowchart




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

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# Clinical evaluation of the patient with a suspected inherited platelet function disorder

- Personal and family bleeding history
- Drug and food history
- Sites of bleeding (easy bruising, epistaxis, gum bleeding, menorrhagia)
- Severity (objective assessment?), recurrence
- Concomitant systemic alterations

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# 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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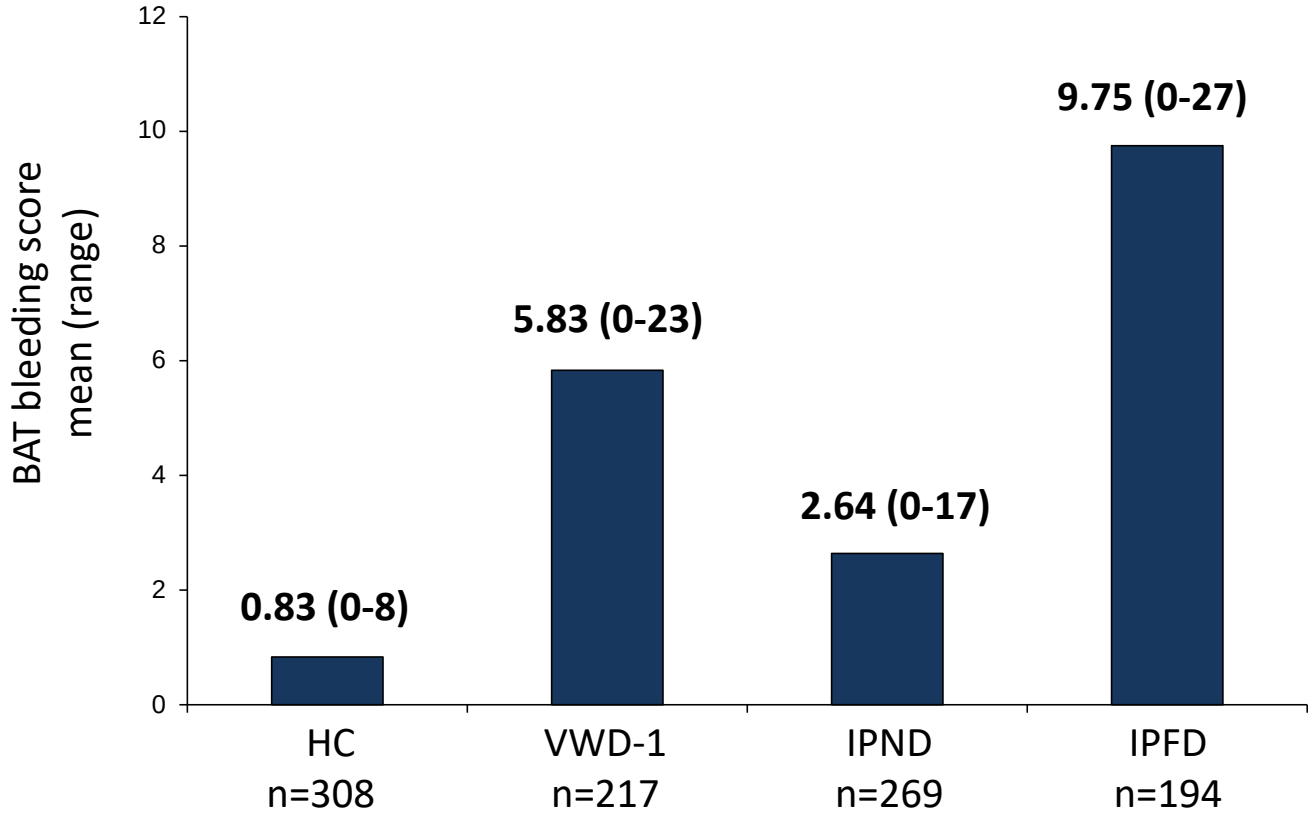
# BAT bleeding score according to disease group

1042 subjects enrolled from 42 centers




Each group p<0.05 *vs* all other groups

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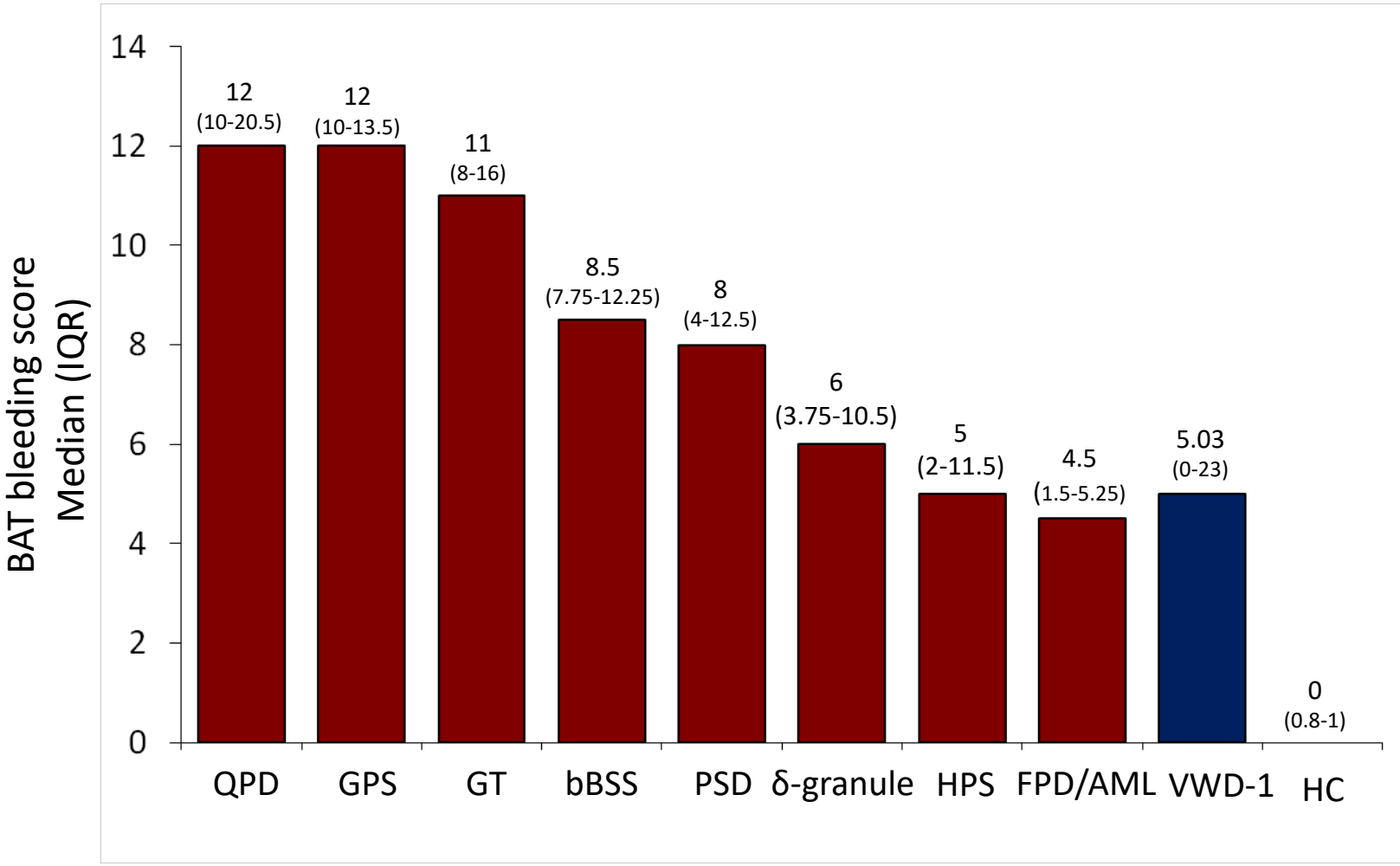
# BAT bleeding score in IPFD by principal diagnoses

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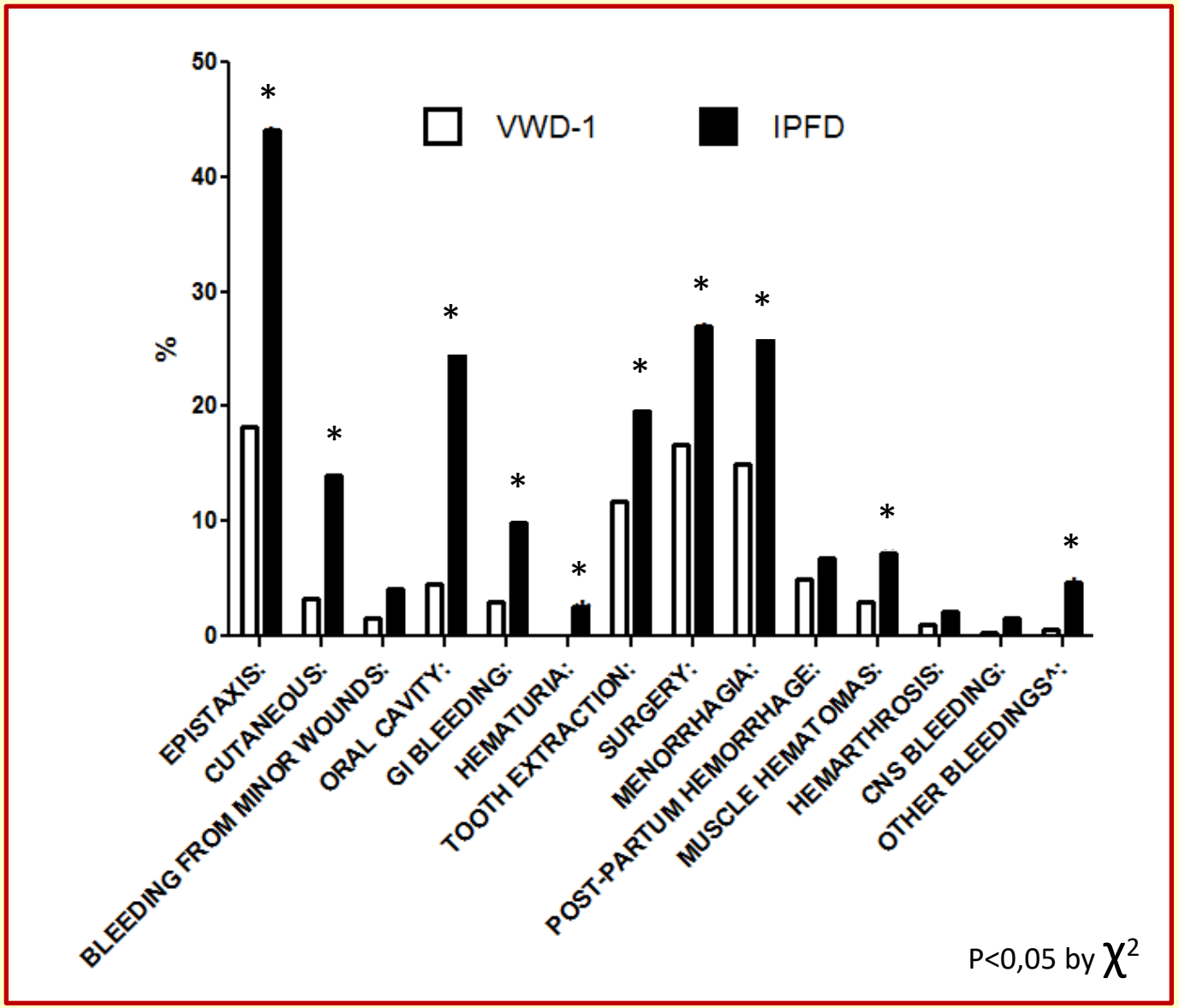
# Frequency of clinically significant bleeding symptoms (score ≥2) in IPFD and VWD-1

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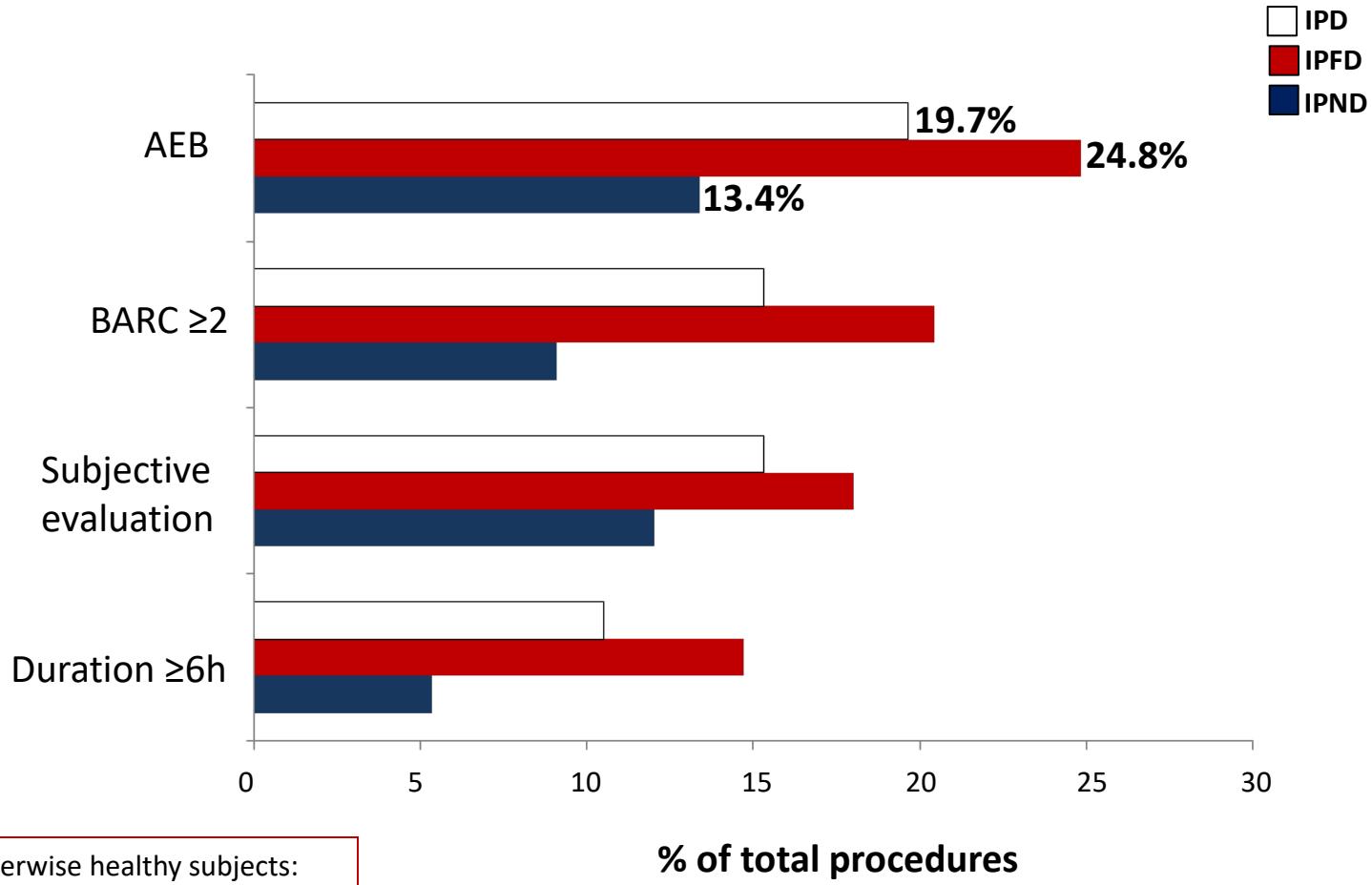
# The SPATA Study

**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%

## Frequency of any excessive bleeding (AEB) at surgery




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

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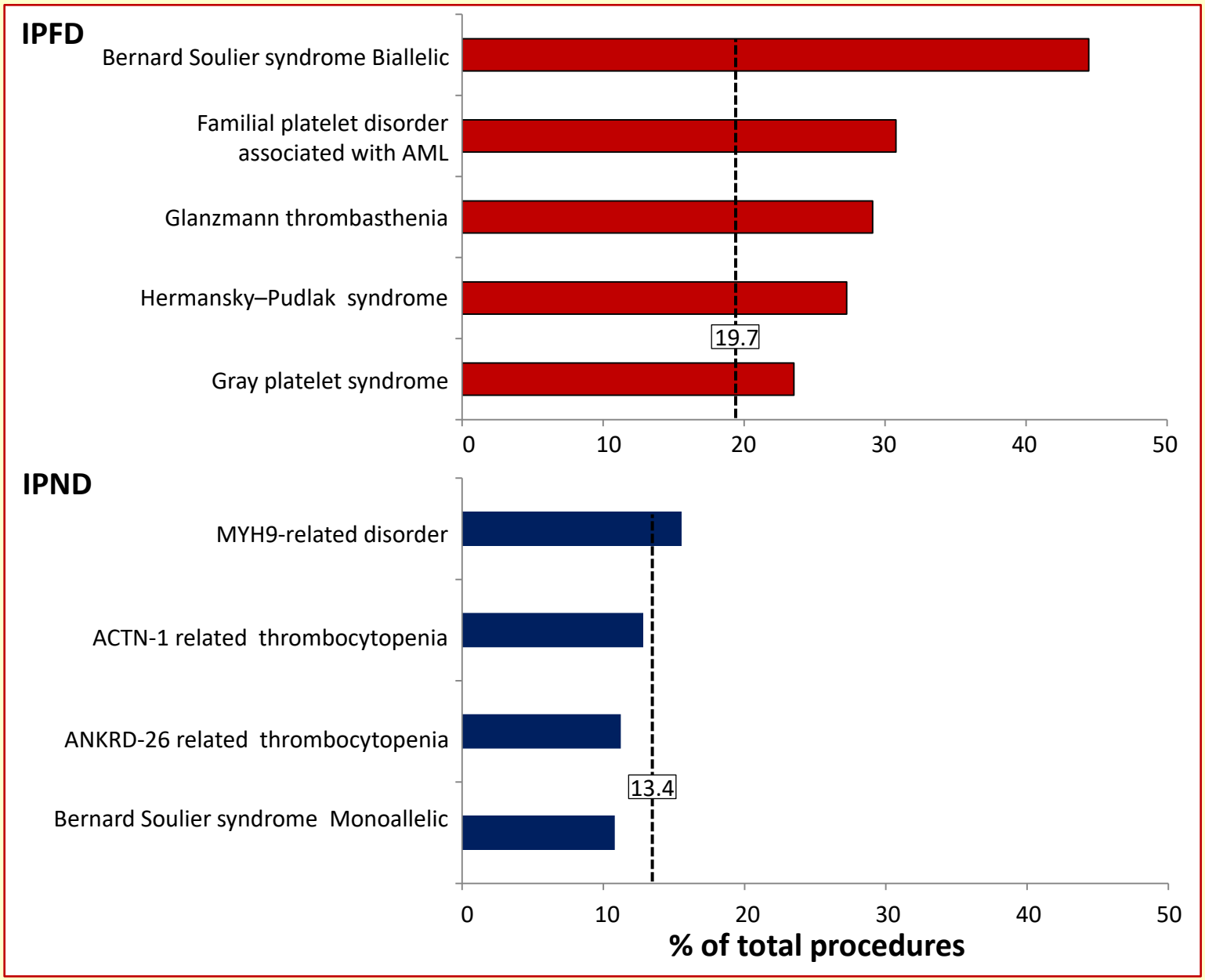
Orsini S et al., Haematologica 2017, 102:1192.

# Frequency of AEB at surgery according to diagnosis

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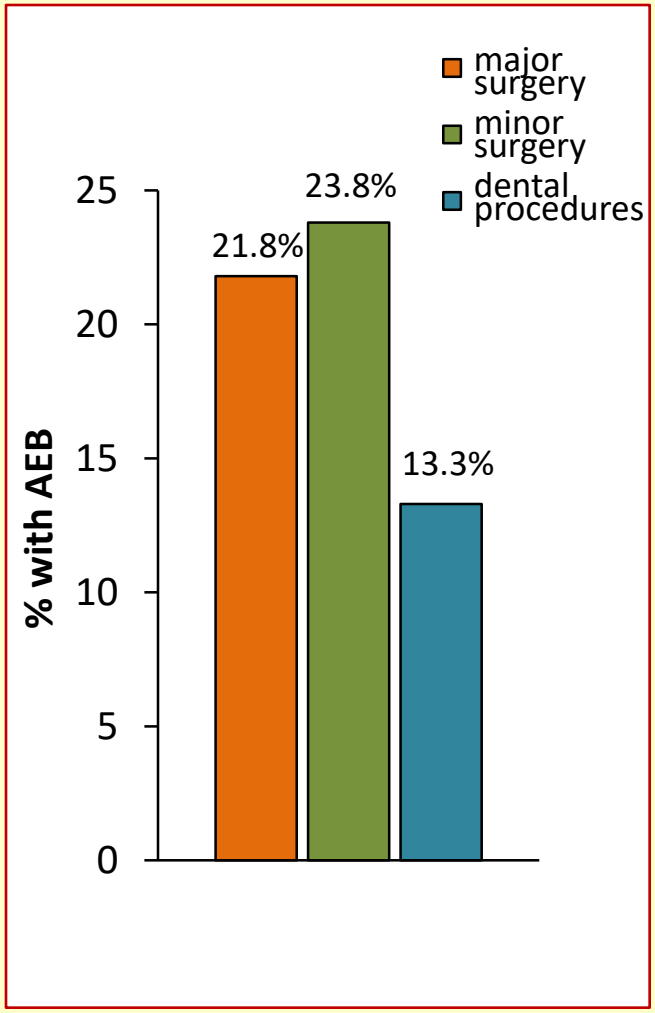
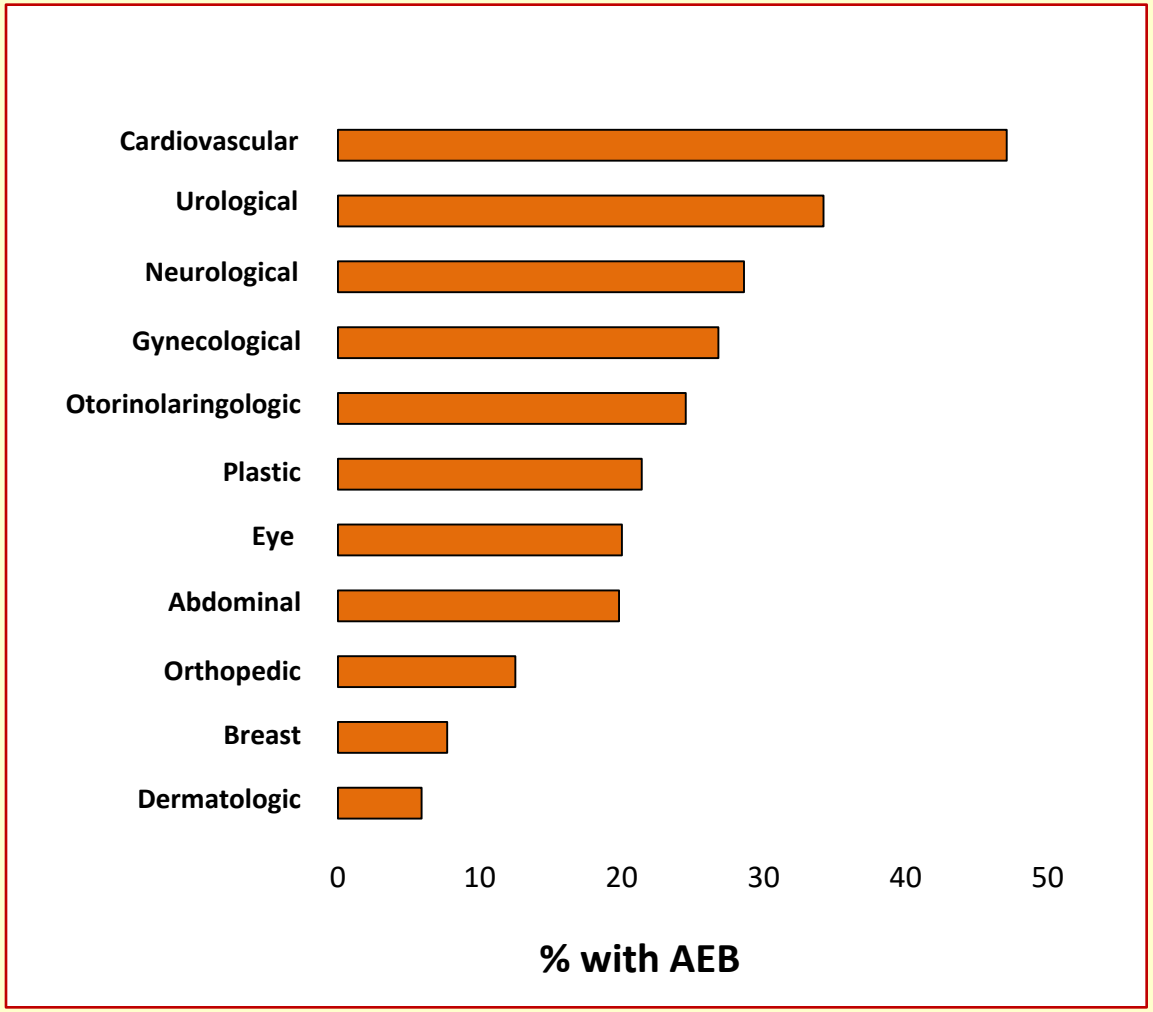
Orsini S et al., Haematologica 2017, 102:1192.

# Frequency of AEB at surgery according to type of surgery

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Orsini S et al., Haematologica 2017, 102:1192.

# Syndromic IPFDs

| Disorder | Associated abnormalities |
|---|---|
| ***Arthrogryposis renal dysfunction and cholestasis syndrome*** | Arthrogryposis, renal dysfunction, cholestasis, cerebral malformations, dysmorphic features |
| ***Filaminopathy related macrothrombocytopenia*** | Skeletal dysplasia, mental retardation, cardiac valvular dystrophy, congenital intestinal pseudo-obstruction, terminal osseous dysplasia |
| ***Gsα platelet defect*** | Short stature, mental disability, brachydactyly. Pseudohypoparathyroidism Ib (PHPIb) |
| ***Hermansky-Pudlak syndrome, Chediak-Higashi syndrome*** | Skin, ocular and hair hypopigmentation, nystagmus Immunodeficiency |
| ***Leukocyte adhesion deficiency III*** | Leukocytosis, recurrent bacterial infections |
| ***Paris-Trousseau syndrome*** | Psychomotor retardation, facial and cardiac abnormalities |
| ***Stormorken syndrome*** | Miosis, muscle weakness, dyslexia, ichthyosis, asplenia |
| ***Velocardiofacial syndrome*** | Cardiac abnormalities, learning disabilities, velopharyngeal insufficiency, immunodeficiency, facial dysmorphisms and thymic hypoplasia |
| ***Wiskott-Aldrich syndrome*** | Eczema, immunodeficiency |

Gresele P et al., Semin Thromb Haemost 2016, in press

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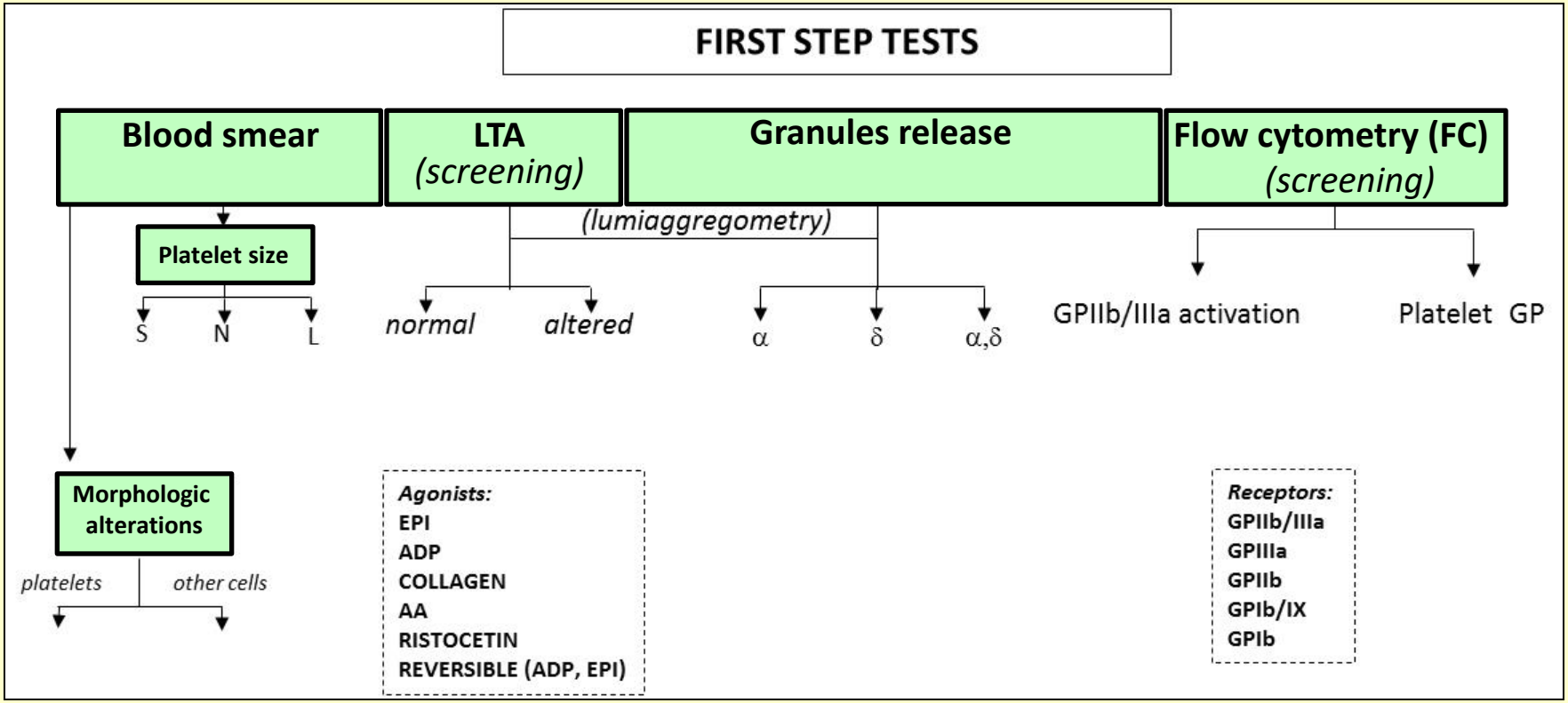
# Diagnosis of IPFD: guidance from the SSC of ISTH




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

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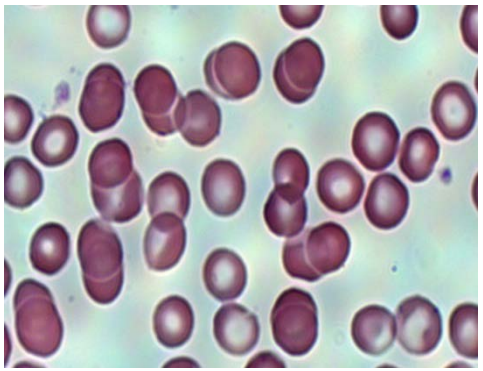
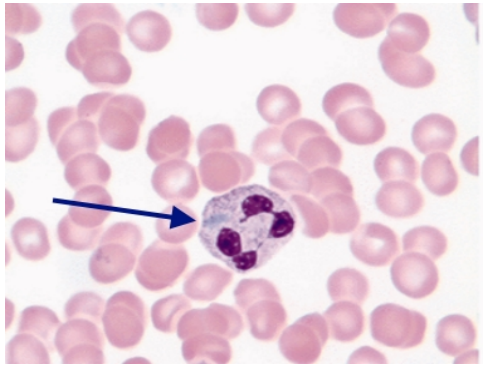
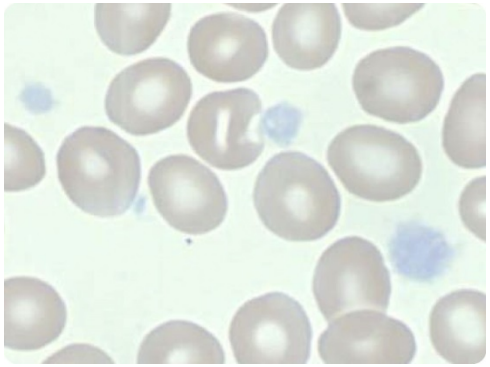
# Blood smear examination



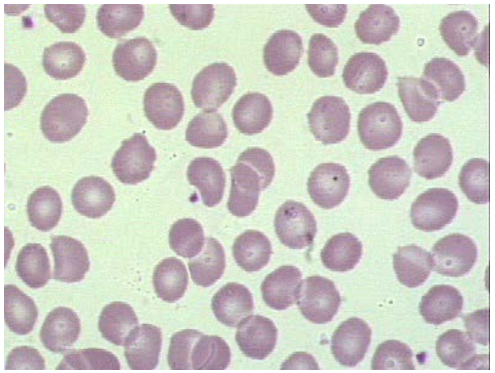
Normal



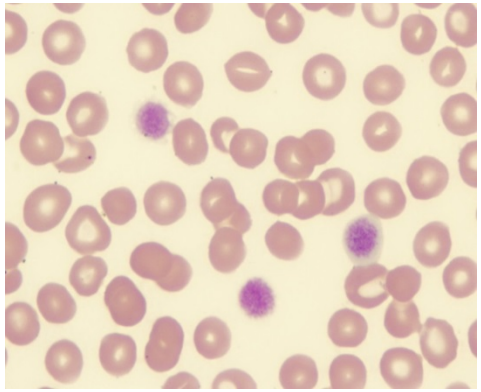
MYH9-RD
(Dohle-like bodies
in granulocytes)



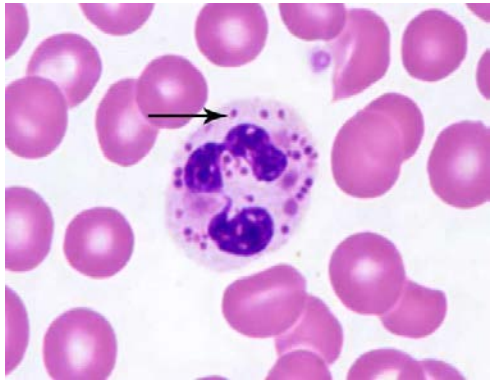
Gray platelet syndrome
(gray platelet)



Wiskott Aldrich syndrome
(micro-thrombocytopenia)



MYH9-RD
(Macrothrombocytopenia)



Cediak-Higashi syndrome
(cytoplasmic inclusions)

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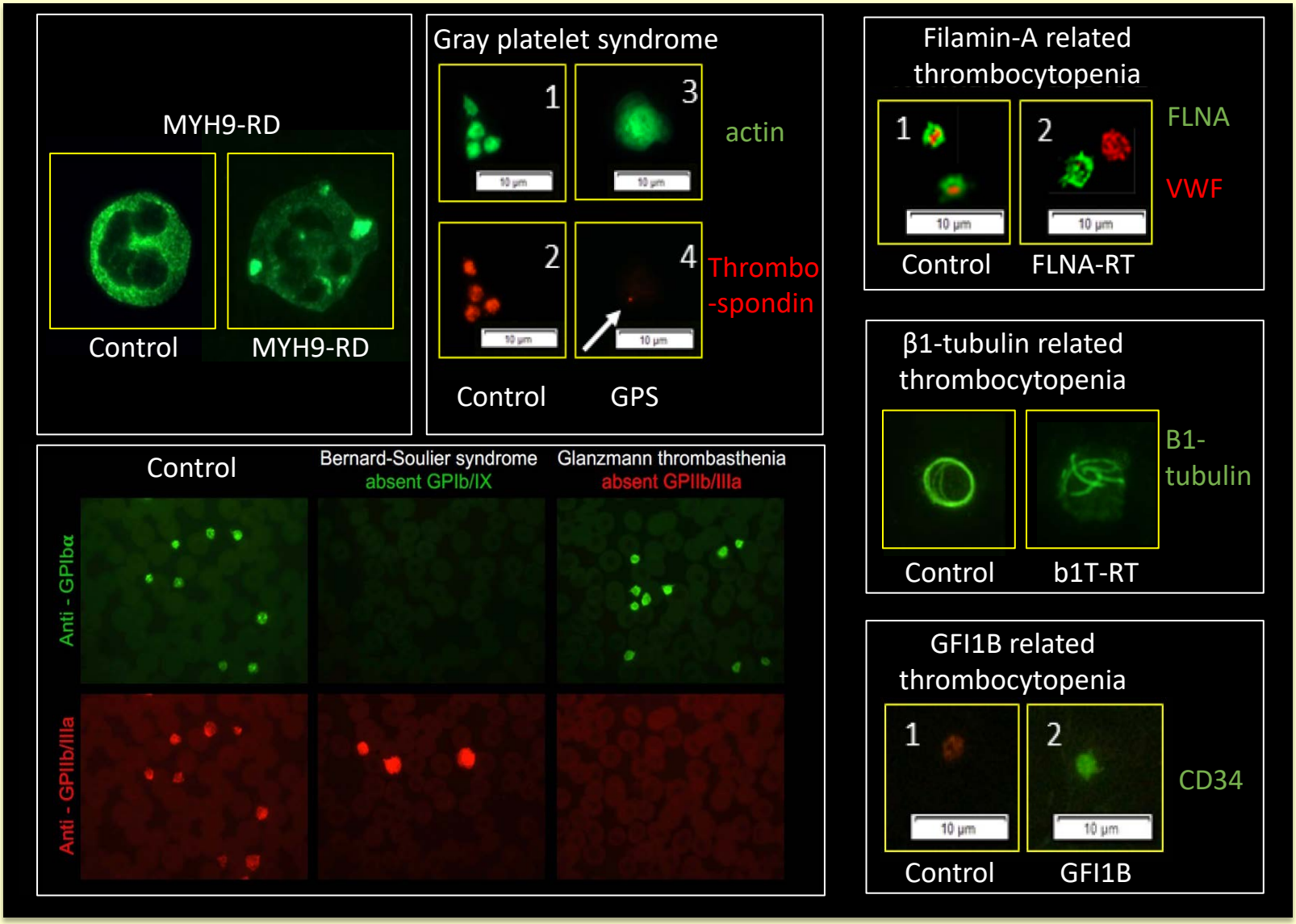
# Diagnosis of inherited platelet disorders on a blood smear

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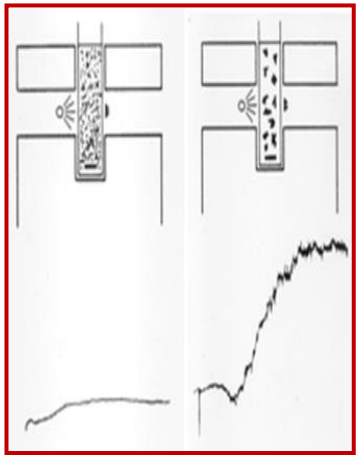
Greinacher A et al. J Thromb Haemost 2017; 15: 1511–21

# Light transmission aggregometry (LTA)

**Basic principle**: it measures the increase of light transmission through a suspension of platelets during platelet clump formation



**ALTERED IN**

- Inherited platelet function disorder
- Acquired platelet function disorder
- Drugs
- Afibrinogenemia

**ADVANTAGES**

- Historical gold standard
- Composite readout
- Wide range of use

**DISADVANTAGES**

- High sample volume
- Time consuming
- Separation of platelets from other cells
- Poorly standardized
- Lack of reference standard materials

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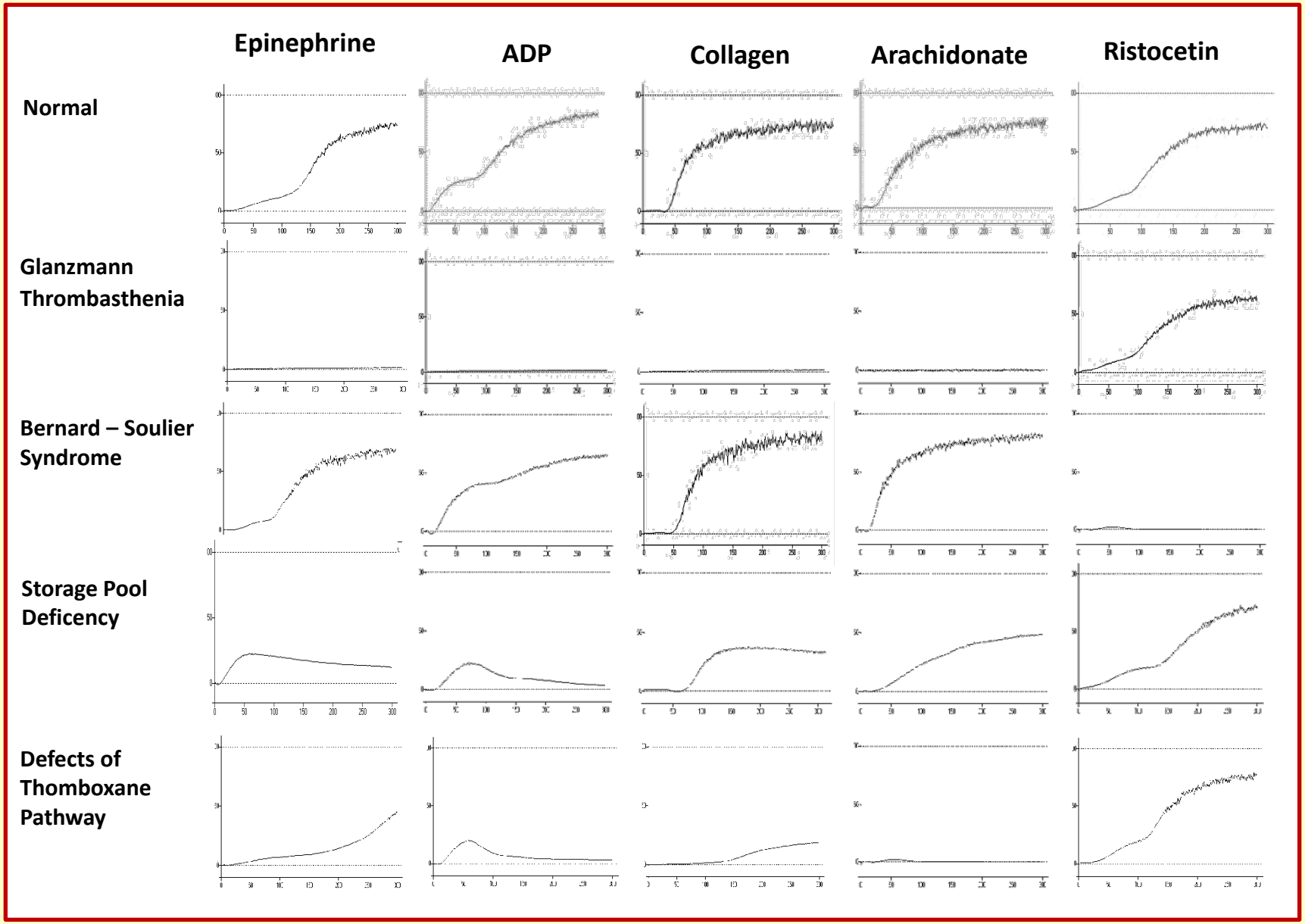
# Diagnosis of inherited platelet function disorders by LTA

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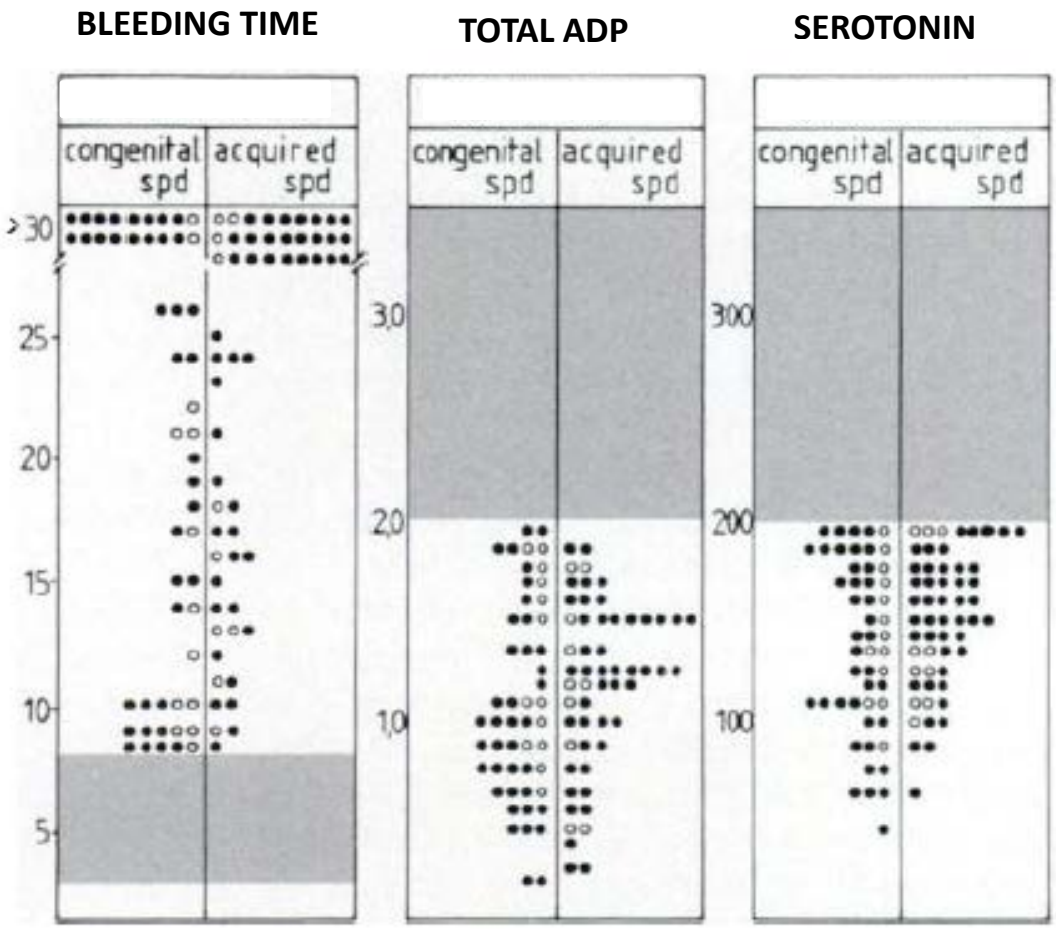
# Defective platelet secretion with normal aggregation in δ-SPD




106 patients with δ-SPD

● Patients with abnormal platelet aggregation
○ Patients with normal platelet aggregation

Nieuwenhius HK et al., Blood 1987;70:620

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# Flow cytometry in hemostasis and thrombosis

| ADVANTAGES | DISADVANTAGES |
| --- | --- |
| • Small sample volume<br>• Short time of analysis<br>• Possibility to study platelets in whole blood<br>• Possibility to study platelet characteristics/function in thrombocytopenic patients | • Expensive<br>• Technically complex<br>• Lack of standardization<br>• Need to process the sample immediately after blood collection |

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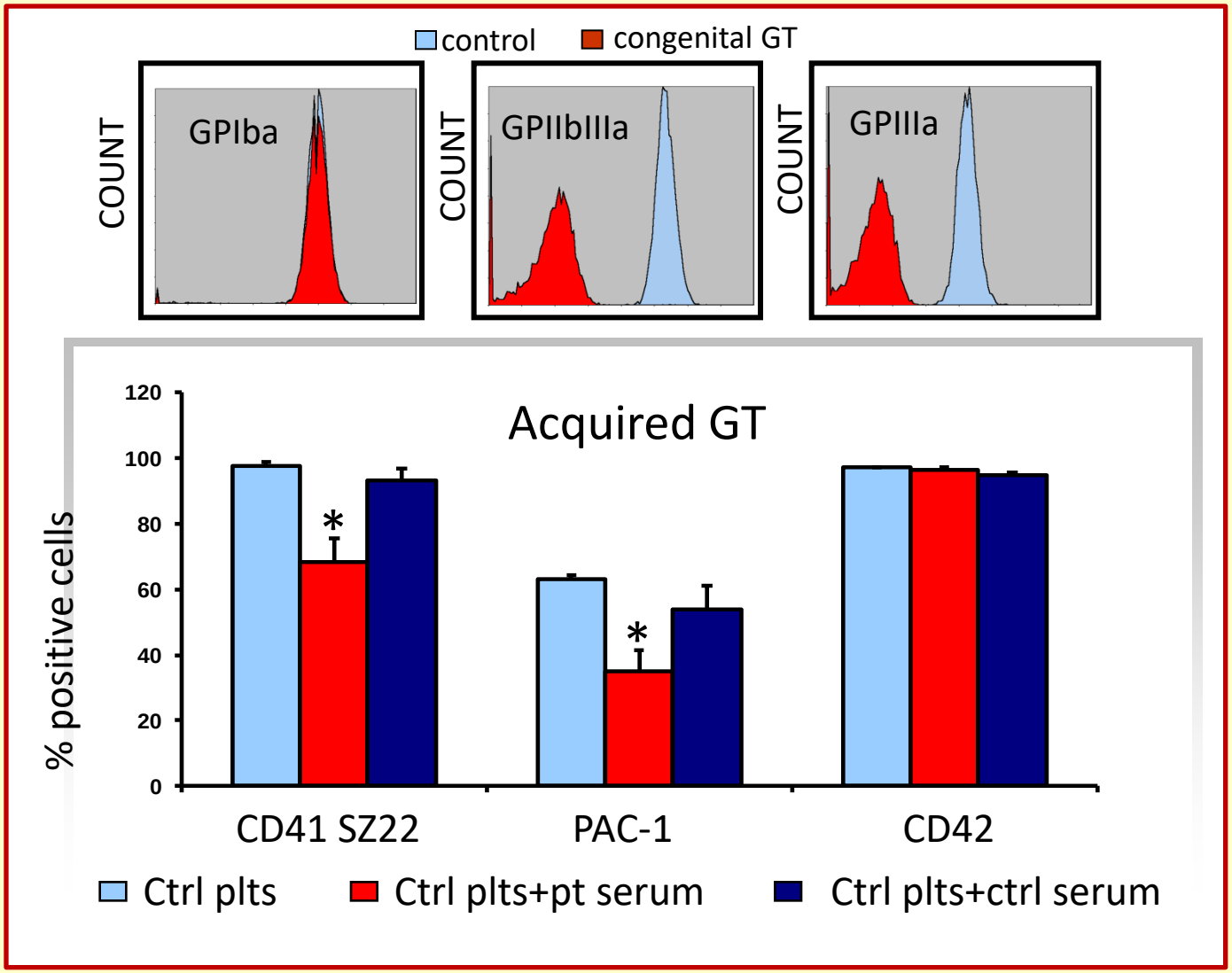
# Flow cytometry in the diagnosis of IPFD

## Glanzmann Thrombasthenia




Giannini S et al., Cytometry B Clin Cytom 2008, 74:194

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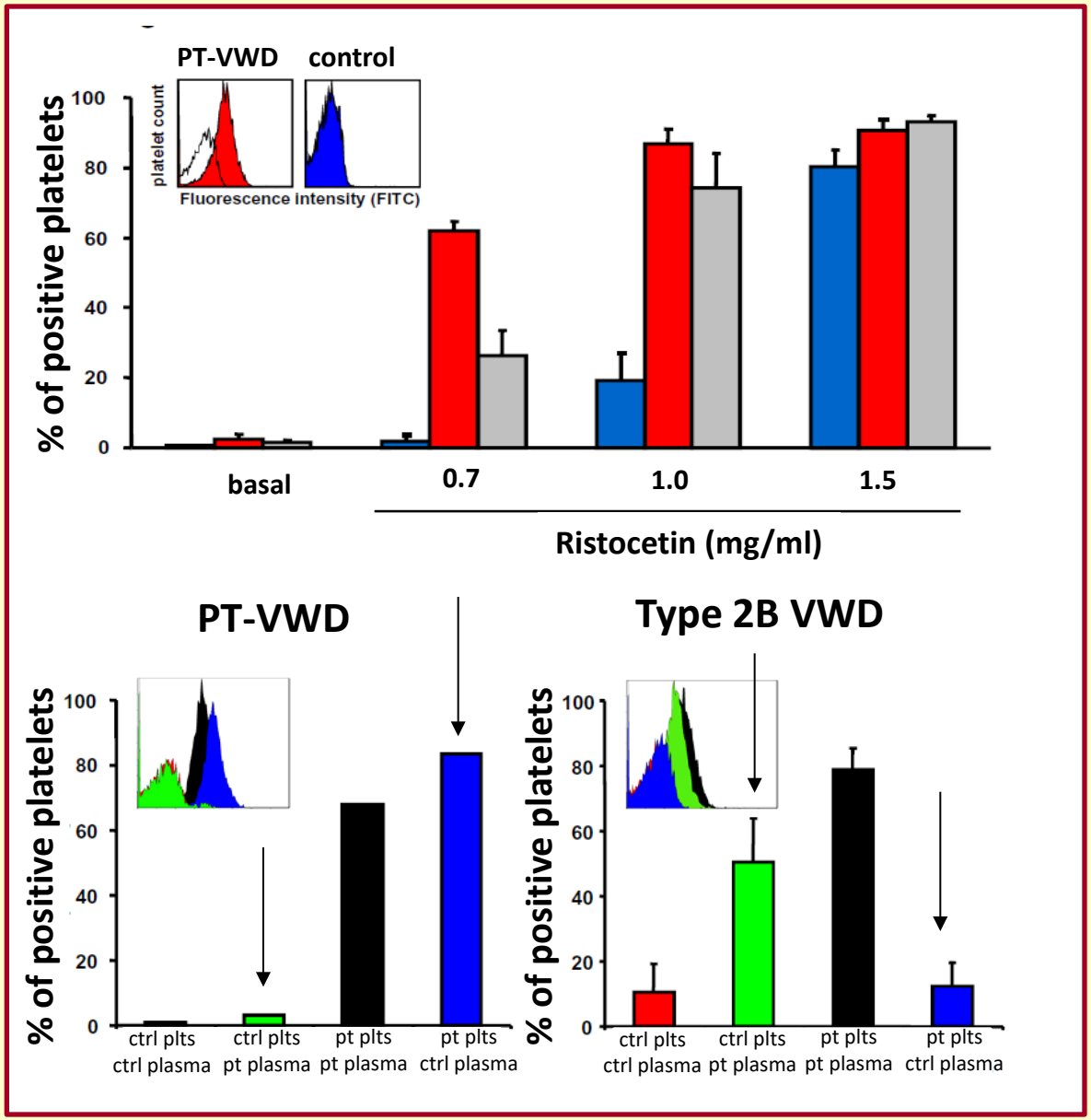
# Differential diagnosis of Platelet Type-Von Willebrand Disease and Type 2B VWD by flow cytometry

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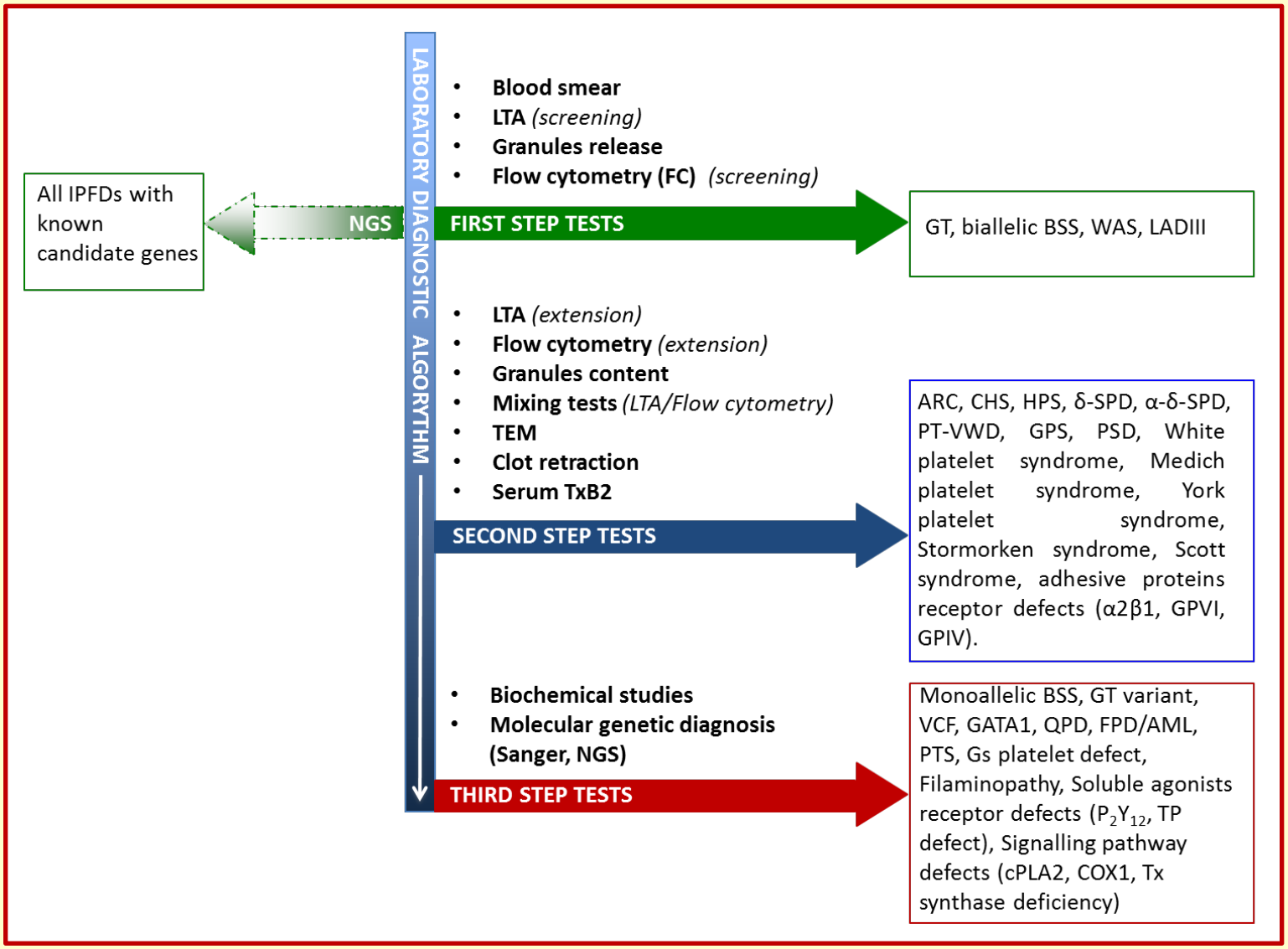
Giannini S, et al., Haematologica 2010; 95:1021

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

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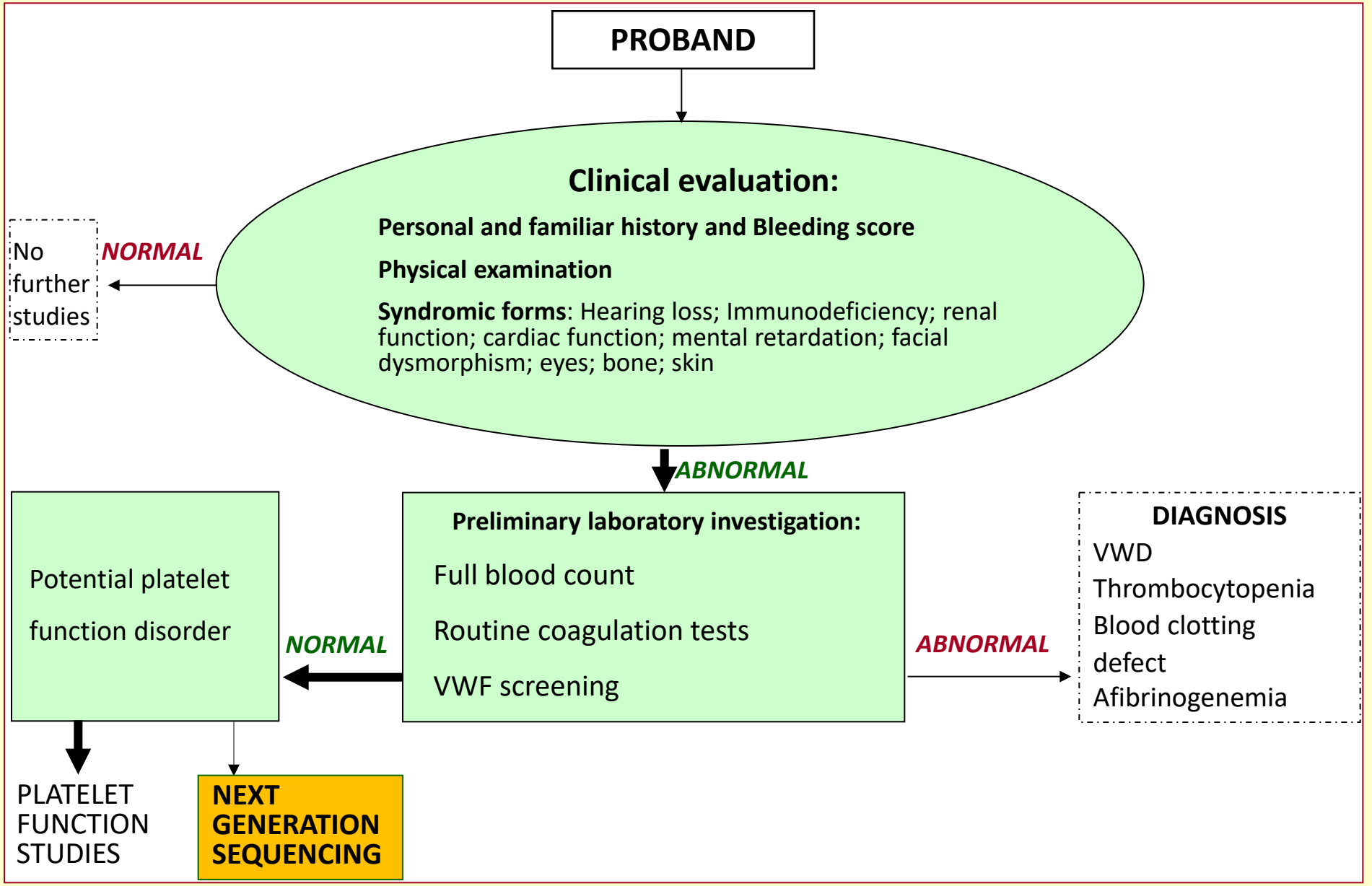
Gresele P et al., Semin Thromb Haemost 2016, 42:292

# DIAGNOSTIC ALGORITHM - Flowchart

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

# Genetic Diagnosis: pros and cons

- Next Generation Sequencing enables the simultaneous analysis of large groups of candidate genes, allowing the rapid identification of a mutation in a known gene.
- Diagnosis of some IPFD can be reached only after genetic analysis (e.g. GT Variants).
- For some disorders (e.g. MYH9-RD) a phenotype/genotype correlation exists.

*however*

- We need to consider that several centers still do not have access to molecular testing.
- Genetic testing (especially WES) is a potentially valuable investigation for gene discovery only if backed up by good phenotyping.
- Ethics of predictive testing should be considered (e.g. diagnosis of FPD/AML)
- It can not be considered yet as an initial diagnostic test, but rather as complementary and/or confirmatory.

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# Indications to genetic diagnosis of IPFDs

- **Not required:** when clinical phenotype or first/second step tests are sufficient for a conclusive diagnosis (e.g. GT, BSS, PT-VWD)
- **Advisable:** when the platelet phenotype may not be undisputably attributed to a specific disorder (e.g. Stormorken syndrome) 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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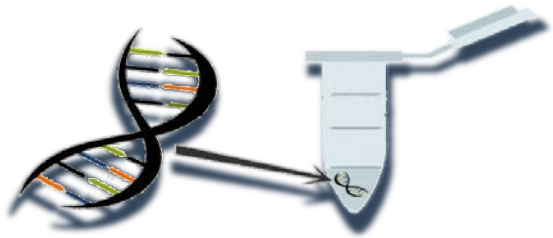
Gresele P et al., Semin Thromb Haemost 2016, 42:292

# ThromboGenomics - Methods

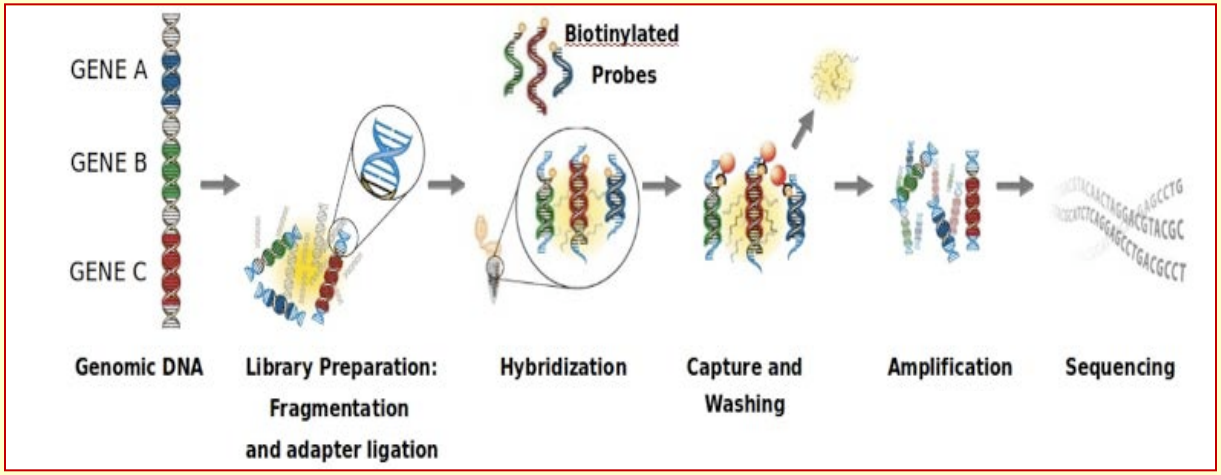
## 1. Patient phenotyping (HPO terms)

- Mucocutaneous bleeding
- Impaired aggregation ADP
- Impaired aggregation collagen
- Impaired aggregation epinephrine
- Impaired aggregation arachidonic acid
- Normal aggregation ristocetin
- Defective CD41/CD61 expression
- Absent clot retraction
- Normal granule content

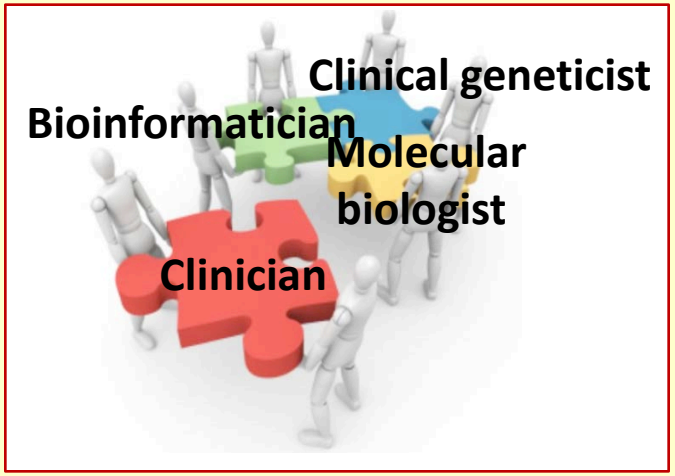
## 2. DNA extraction



## 3. Next Generation Sequencing: targeted sequencing of 63 genes from 24 samples simultaneously




## 4. Results analysis
(Multi-disciplinary team)

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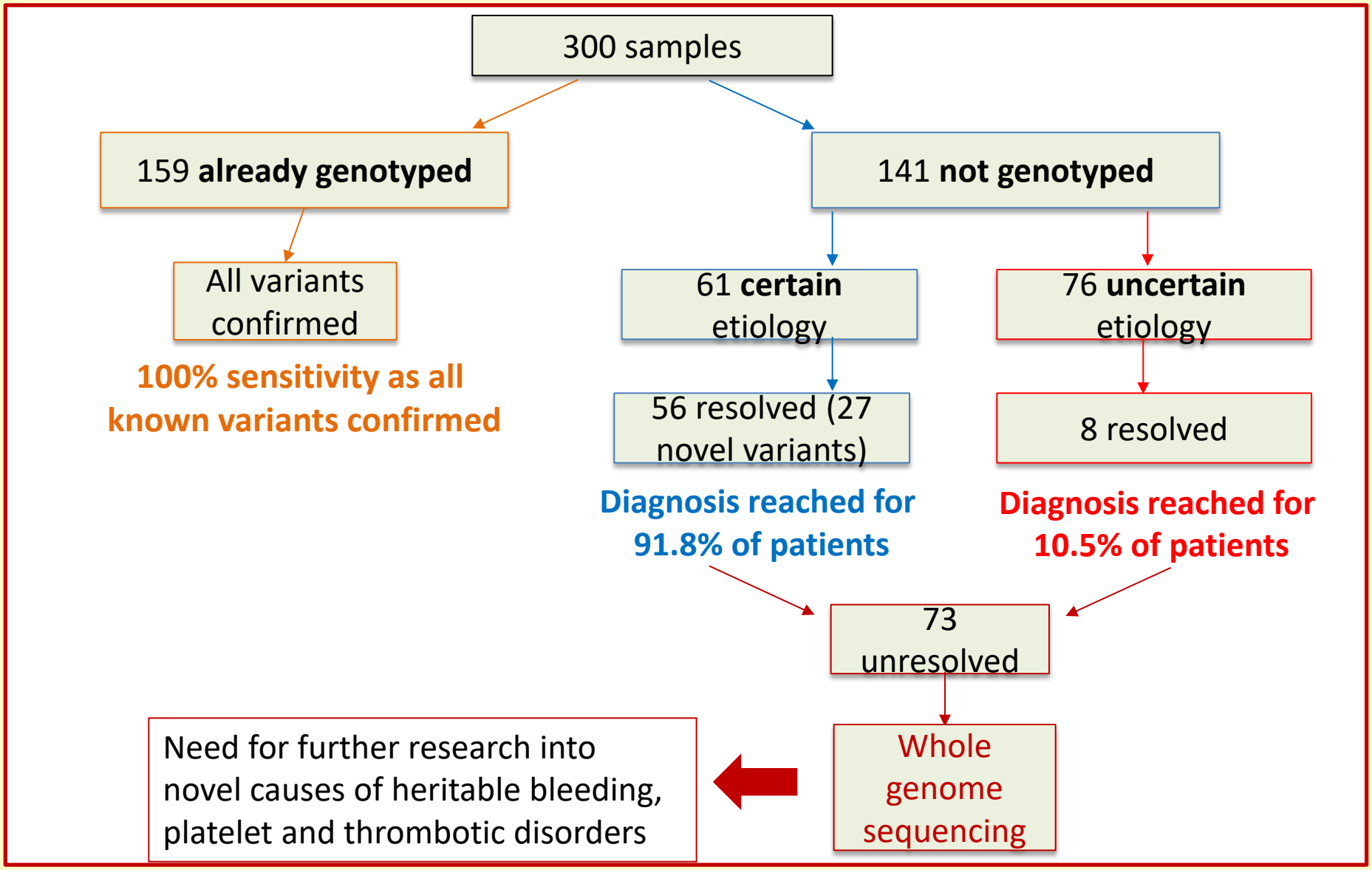
Simeoni I et al. Blood 2016;127:2791

# ThromboGenomics - Results

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Simeoni I et al. Blood 2016;127:2791

# Treatment options for IPFDs

- **Antifibrinolytic agents**: local or systemic; arrest/prevent epistaxis, gingival bleeding or menorrhagia; used for the prevention of bleeding for minor surgery
- **DDAVP:** i.v., s.c., nasal spray; efficacious in preparation to invasive procedures or surgery (contraindicated in PT-VWD; uncertain efficacy in some IPFDs)
- **Platelet transfusion:** treatment of acute bleeding or prior to surgery; should be used only when other agents have failed (alloimmunization and infectious risks)
- **rFVIIa:** approved for treatment of acute bleeding and for perioperative management of GT refractory to platelet transfusions; little or no informations for other IPFDs

# Conclusions

- IPFDs are a heterogeneous group of bleeding diseases which represent a significant fraction of all the bleeding diatheses
- A careful clinical evaluation and a rational diagnostic algorithm based on a streamlined panel of tests allows diagnosis in a large part of the cases.
- Genetic diagnosis is becoming a conceivable alternative to extensive platelet function testing for many IPFDs
- IPFDs are associated with a significant bleeding risk
- Correct diagnosis and the use of prompt and appropriate treatment may minimize bleeding risk

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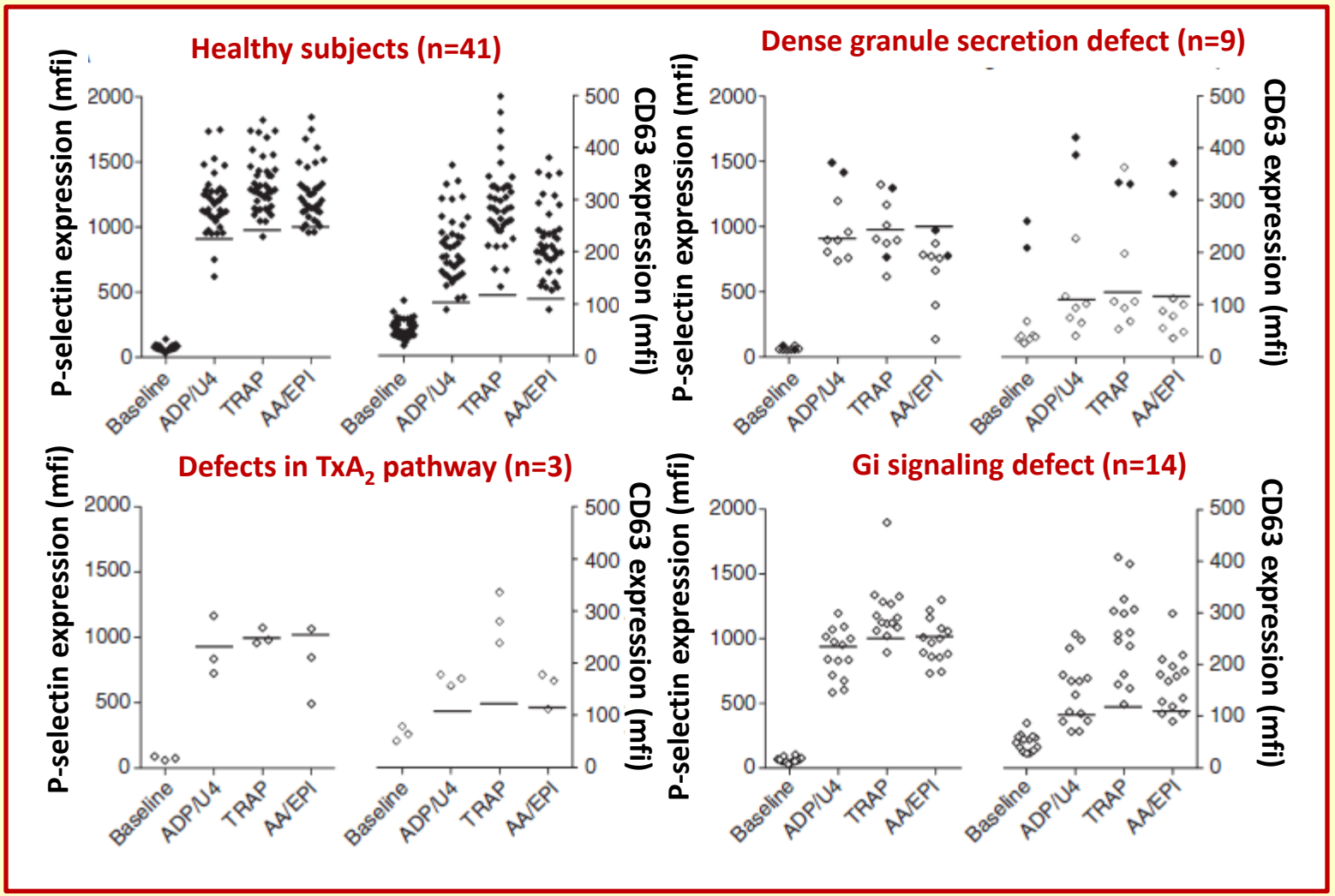
# Use of a whole blood remote flow cytometry platelet function test for the diagnosis of mild bleeding disorders




-61 patients; samples fixed and analyzed within 3 days
-Lumiaggregometry vs remote flow cytometry: concordance 84% ($p<0.0001$)

Dovlatova N et al, J Thromb Haemost 2014;12:660

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# The ThromboGenomics platform

- Currently, 90% of patients with an heritable bleeding disorder with the exception of hemophilia and VWD never receive a conclusive molecular diagnosis.
- Targeted sequencing platform covering 63 genes linked to heritable bleeding, thrombotic and platelet disorders.
- The Thrombogenomics platform provides a sensitive genetic test to obtain molecular diagnoses in patients with a suspected etiology.


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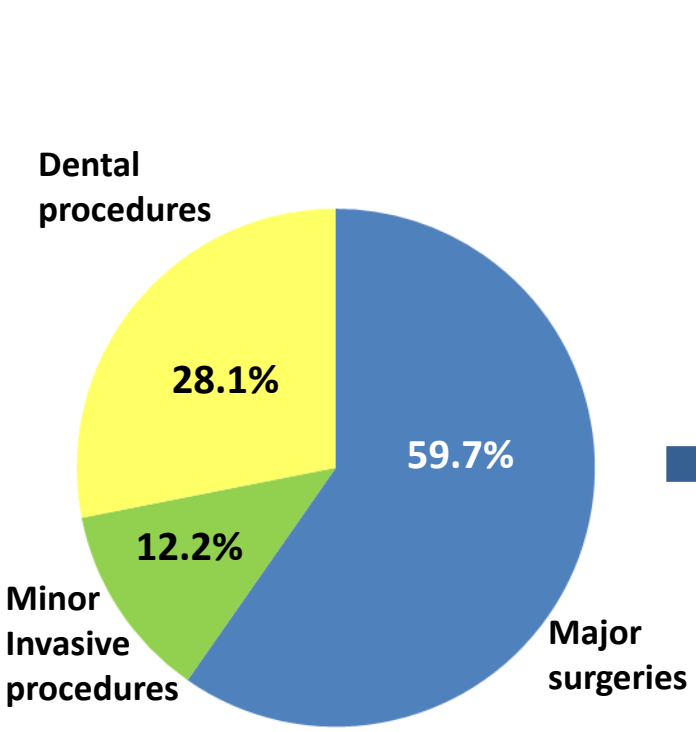
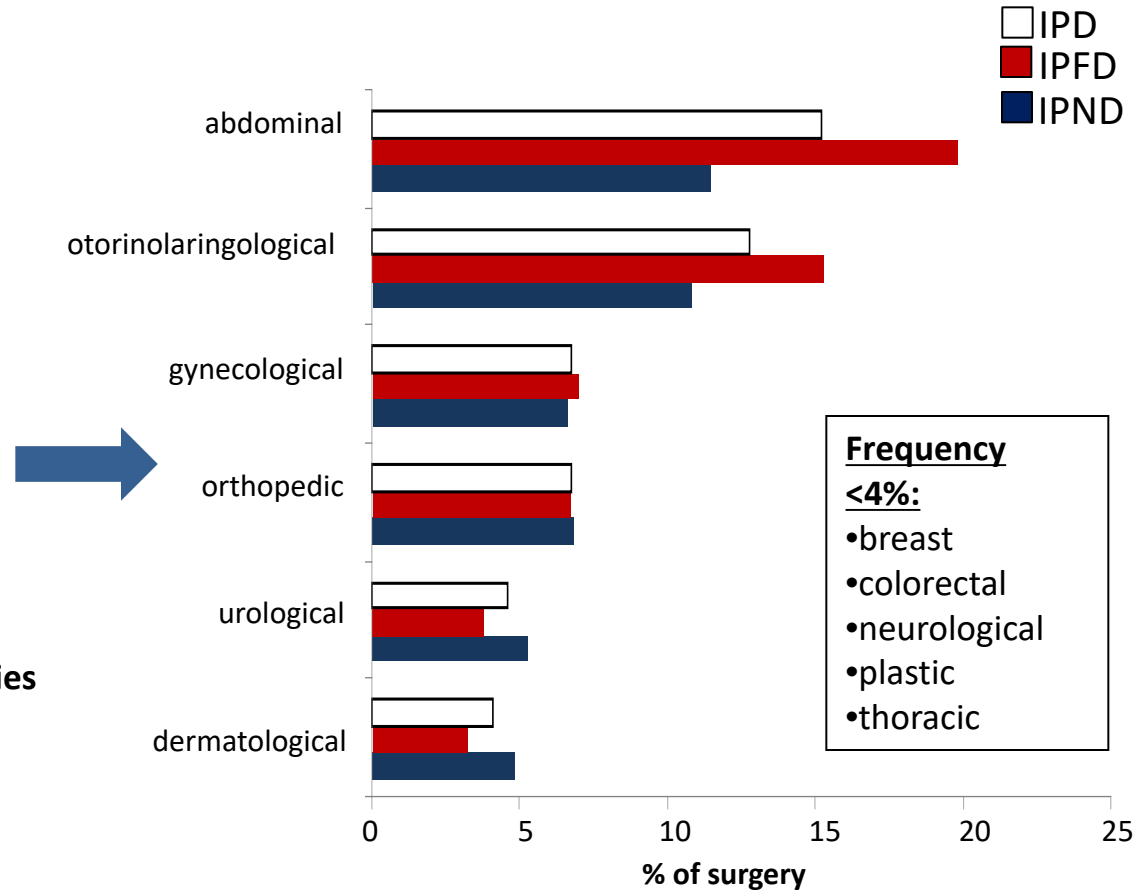
Simeoni I et al. Blood 2016;127:2791

# Invasive procedures

**829** procedures (355 in IPFD, 374 in IPND)**.** Median age at surgery: 31 years (IQR 15-52)




**Major**: any procedure in which a body cavity was entered, a mesenchymal barrier was crossed, a facial plane was opened, an organ was removed or normal anatomy was altered;
**Minor invasive**: any operative procedure in which only skin, mucous membranes or superficial connective tissue were manipulated, gastroscopy, colonoscopy and similar;
**Dental**: extraction, abscess removal, apicectomy and similar

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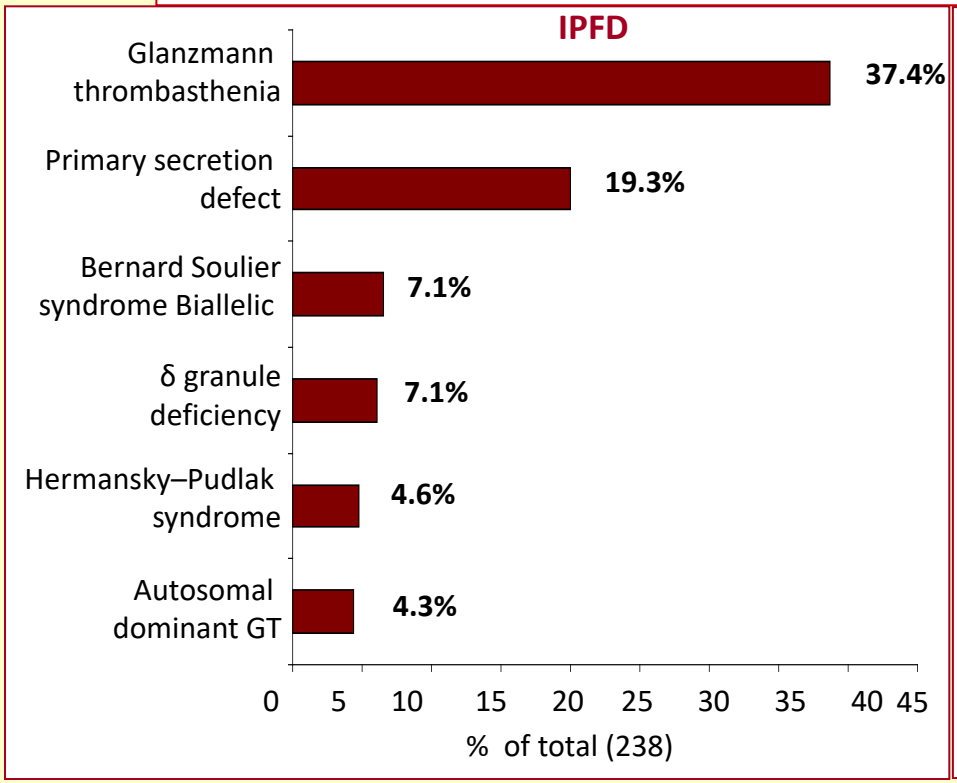
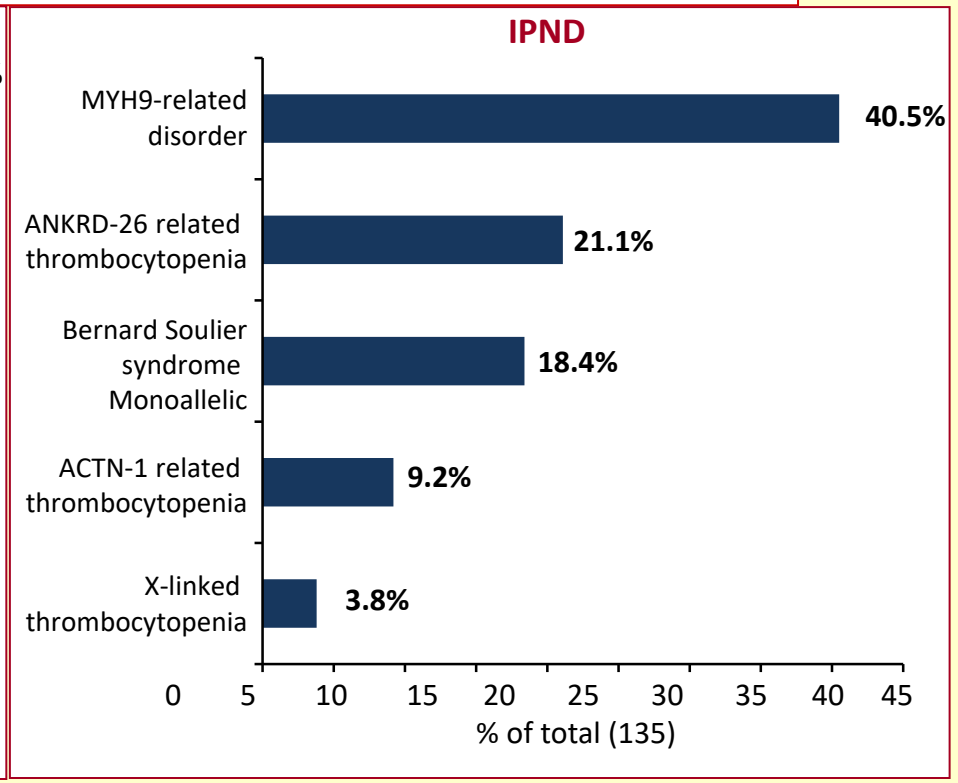
Orsini S et al., Haematologica 2017, 102:1192.

# Patients' characteristics

## The SPATA Study

**49** centers, **17** countries.
**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%




Thrombocytopenia in IPNDs was on average mild (microscopic platelet count: **median 68x10$^9$/L**; IQR 40-102x10$^9$/L) (counter platelet count: median 50x10$^9$/L; IQR 30-81x10$^9$/L).

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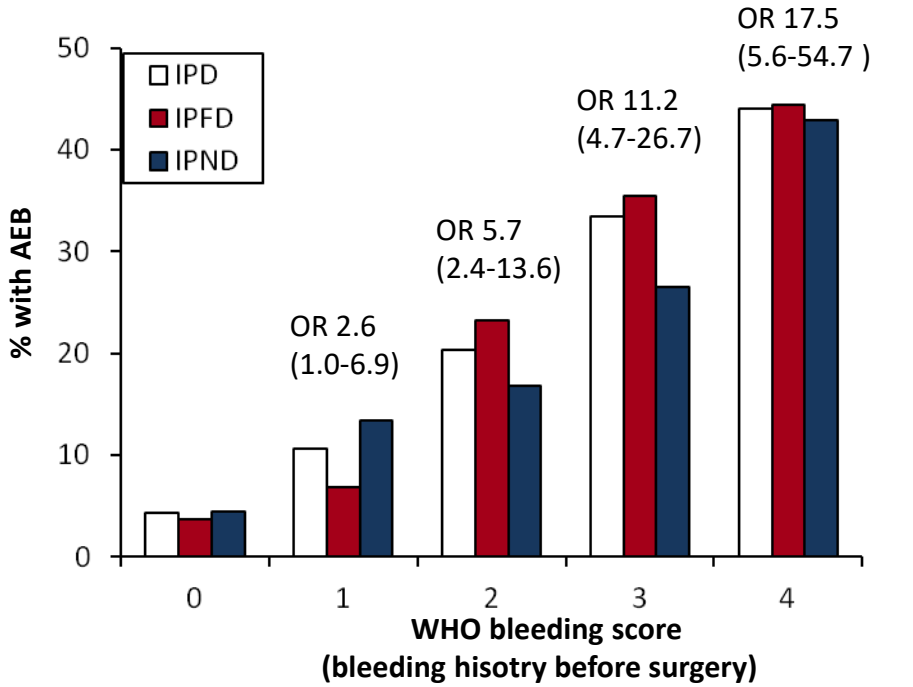
Orsini S et al., Haematologica 2017, 102:1192.

# Parameters associated with post surgical bleeding




| | IPFD (n=455) | IPND (n=374) |
|---|---|---|
| | OR (95% CI) | OR (95%CI) |
| **Female gender** | 1.8 (1.1-2.9) | - |
| **Platelet count <68x10$^9$/L** | - | 2.04 (1.01-4.12) |
| **Prophylaxis** | 0.38 (0.23-0.63) | - |

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Orsini S et al., Haematologica 2017, 102:1192.

# Treatment of bleeding for inherited platelet function disorders

| treatment | evidence | |
|---|---|---|
| | laboratory effect on platelets | clinic |
| local haemostatic measures | no data | amelioration of<br>• epistaxis<br>• surgical wound bleeding<br>• gingival bleeding<br>• dental extractions |
| desmopressin (DDAVP) | • increased platelet adhesion and aggregation under flow conditions<br>• enhanced platelet procoagulant activity<br>• shortening of the bleeding time | • management of bleeding during surgery and delivery<br>• efficacy for: δ-SPD, disorders of granule secretion, signal transduction defects, TP receptor deficiency, MYH9-RD<br>• equivocal evidence for BSS, HPS and COX-1 deficiency<br>• poor response for GT |
| antifibrinolytic agents | no data | amelioration of<br>• epistaxis<br>• gingival bleeding<br>• menorrhagia<br>prevention of bleeding following minor surgery |
| activated recombinant FVII (rFVIIa) | • increased generation of thrombin<br>• enhanced adhesion of platelets to extracellular matrix<br>• restoration of platelet aggregation | • approved by FDA for the treatment of bleeding episodes and for perioperative management of GT<br>• management of bleeding during minor and major surgery in BSS, PT-VWD and δ-SPD (case reports) |
| female hormones | no data | amelioration of menorrhagia |
| platelet transfusions | no data | management of bleeding during minor and major surgery |
| HSC transplantation | successfully used in GT dogs | successfully used in GT and BSS patients |
| gene therapy | • correction of the bleeding phenotype in GT- and BSS-mice<br>• successfully used in GT dogs | amelioration of the haematological and immunological condition in 7 WAS patients |

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Gresele P et al, Haemostaseologie 2016; 36:265

# INHERITED PLATELET FUNCTION DISORDERS
## A revised classification

**1-ADHESIVE PROTEIN RECEPTOR DEFECTS**

BSS, GT, PT-VWD, VCF, $\alpha_2\beta_1$, GPVI, GPIV

**2-ABNORMALITIES OF G-PROTEIN COUPLED RECEPTORS**

$P_2$ purinergic receptors, TP-receptor, $\alpha_2$ adrenergic receptor, PAR-1

**3-DEFECT OF PLATELET GRANULES (isolated/syndromic)**

α-granules, δ-granules, α+δ granules

**4-SIGNAL TRANSDUCTION PROTEINS DEFECTS**

Gs, Tx-synthase, $cPLA_2$, PKA, LADIII, CalDAG-GEFI, COX-1, SRC

**5-DEFECTS OF MEMBRANE PHOSPHOLIPIDS**

Scott syndrome, Stormorken syndrome

**6- DEFECTS OF TRANSCRIPTION FACTORS**

FPD/AML/MDS, FLI1-related dense granule defect,

Paris-Trousseau syndrome, GATA1

**7- DEFECTS OF CYTOSKELETAL PROTEINS**

Filaminopathy, WAS/XLT, Cytosolic adaptor protein (ADAP)

**8- ENHANCED PLATELET FIBRINOLITIC ACTIVITY**

**Quebec platelet disorder**

**9- UNCLASSIFIED**

SLFN14-related thrombocytopenia

Bury L, Falcinelli E, Gresele P, Wintrobe's Clinical Hematology 14° Edition, in press