In the name of God

Diagnosis of Inherited Platelet Disorders (IPDs) on a Blood Smear

A practical immunofluorescence approach

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Hematology and Blood banking

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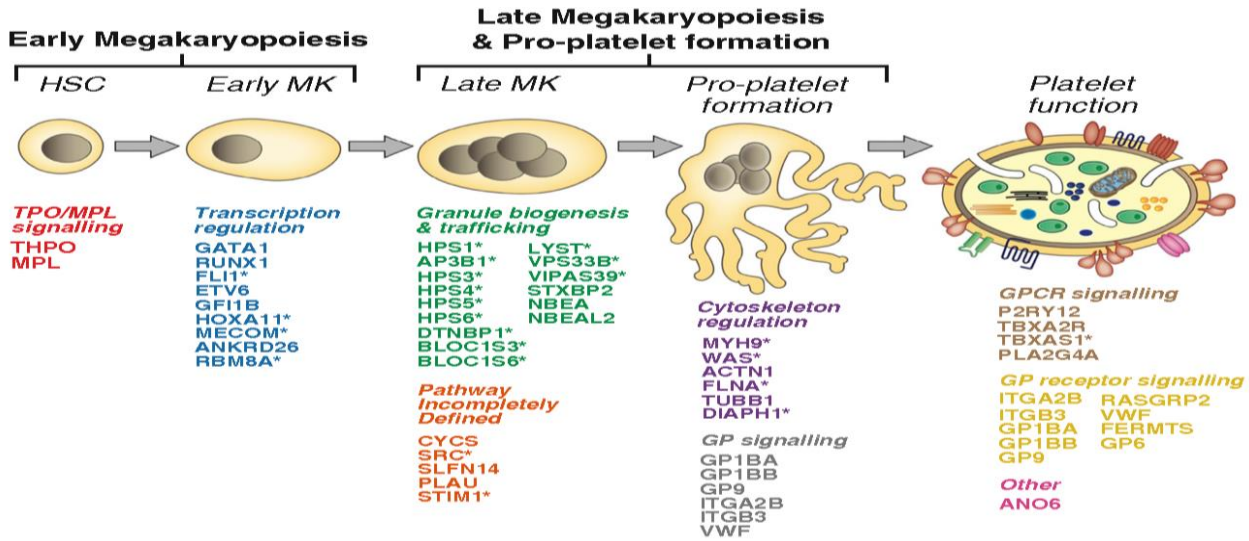
Inherited Platelet Disorders

- A heterogenous group of diseases caused by molecular anomalies in genes that are relevant of platelet number and/or function
- Variable bleeding tendency: vary widely, from asymptomatic cases to patients with severe bleeding, syndromic features, or early-onset blood cancers.
- At present, around 60 types of IPD due to molecular defects in about 75 different genes have been recognized.



Verónica Palma-Barqueros et al. Int J Mol Sci. 2021.

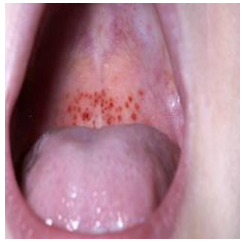
Why Diagnose IPDs Early?

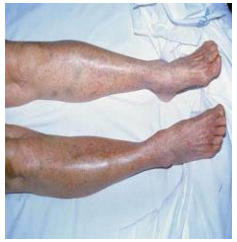
- Avoid misdiagnosis as ITP → prevent unnecessary treatments (steroids, splenectomy)
- Identify patients at risk of:
  - Leukemia (RUNX1, ANKRD26, ETV6)
  - Kidney failure (MYH9-RD)
  - Bone marrow failure (CAMT, RUSAT)
- Enable targeted genetic testing and family screening

Diagnostic Approach to IPFD

- Several surveys have demonstrated significant heterogeneity in diagnostic approaches.
- ***CLSI guideline. 2008***
- ***NASCOLA guideline . 2010***
- ***BCSH and BSH guideline. 2011***
- ***ISTH guideline. 2011***
- There was no consensus or standardized approach to the diagnosis of IPFD till 2015.

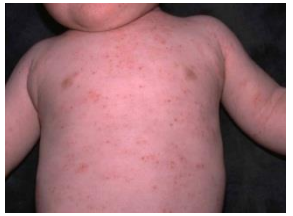
- **Latest ISTH guideline:** Gresele P et al. Diagnosis of Inherited Platelet Function Disorders: Guidance from the SSC of the ISTH. J Thromb Haemost 2015;13:314-22.

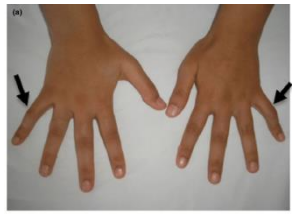




DIAGNOSTIC ALGORITHM
Flowchart

PROBAND





Clinical evaluation:

Personal and family history and bleeding score: bleeding manifestations typical of IPFD

Physical examination: bleeding manifestations typical of IPFD

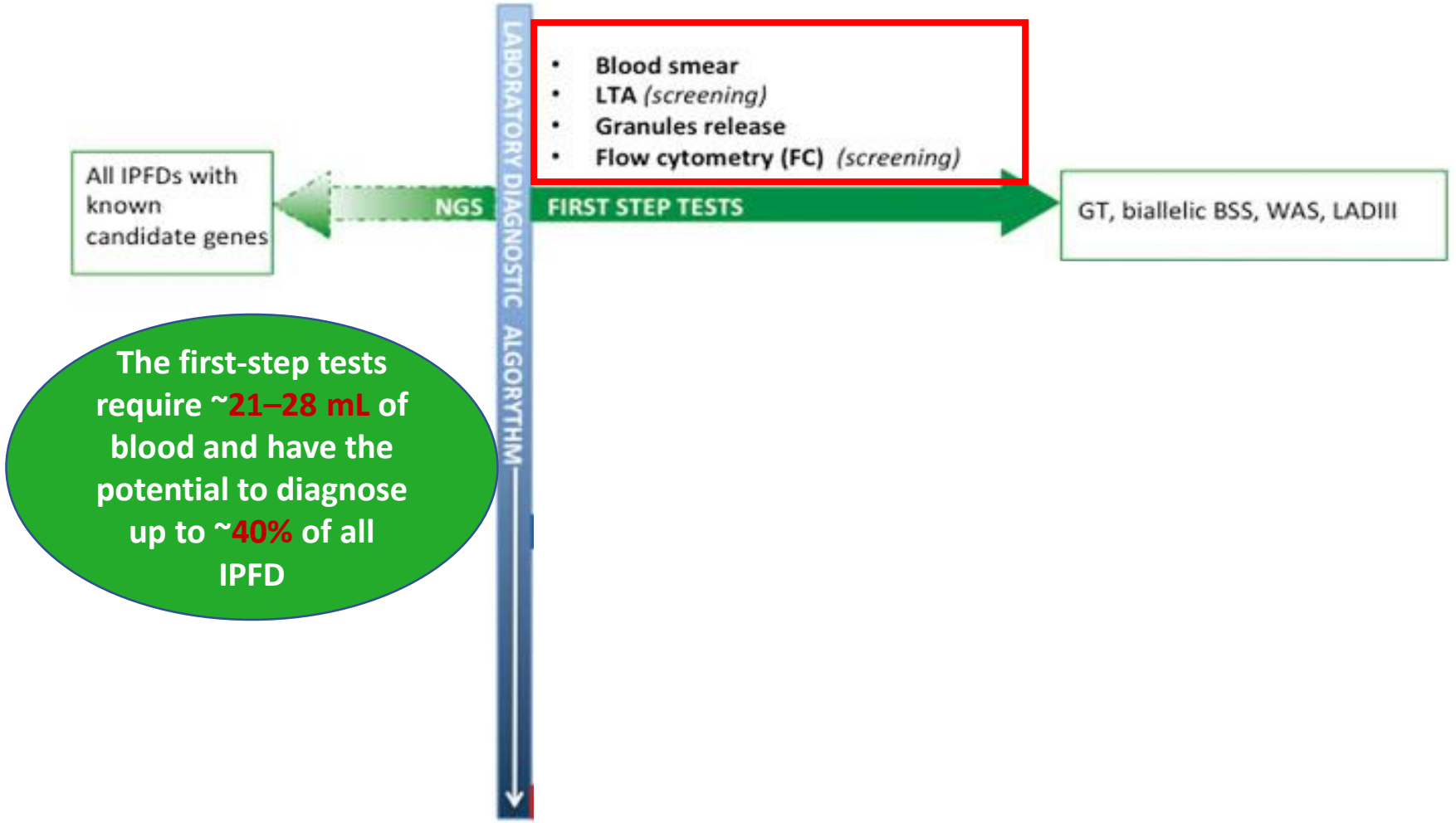
Syndromic forms: hearing loss; immundeficiency; renal function; cardiac function; mental redation; facial dysmorphism; eyes; bone; skin

NORMAL

No futher studies

JTH 2015:13:314-22

Phenotypic and genetic investigation algorithm for IPFDs
Step-by-step approach



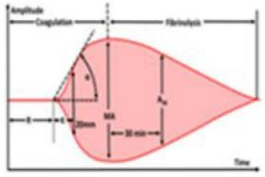
Gresele, 2016, Seminars in Thrombosis & Hemostasis

Stage 1: Initial laboratory testing

Coagulation screen
PT, aPTT, Fib, FVIII:C, VWF

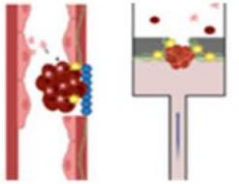
Complete blood count and smear

Stage 2: Global hemostasis and/or platelet function



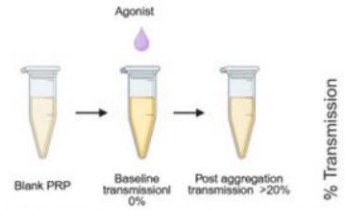
Tromboelastography

Total Thrombus Formation Analysis (T-TAS)



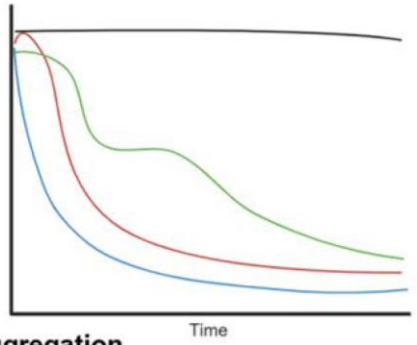
PFA-100/PFA-200

Stage 3:Basic studies of platelet phenotype

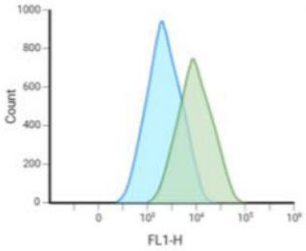


Agonists: ADP, epinephrine, collagen, ristocetin,AA, TRAP, U46619

Parameters: % max agg, % final agg, slope, area under the curve

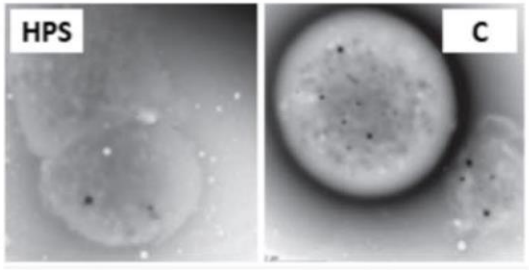


Platelet aggregation



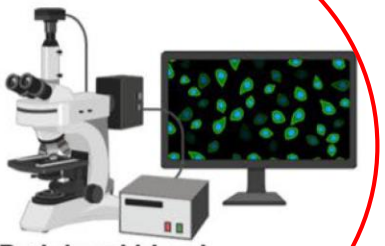
Flow cytometry

GPs, activation, granule secretion, CD34

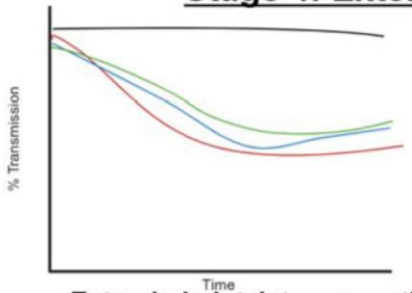


Electron microscopy (whole mount)

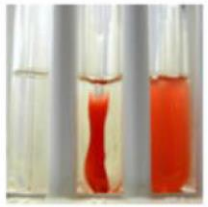
Stage 4: Extended studies of platelet phenotype



Peripheral blood immunofluorescence

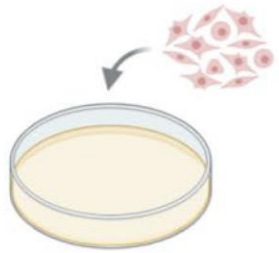


Extended platelet aggregation
(CRP, PAR4, PMA, rodocetin, etc,.)



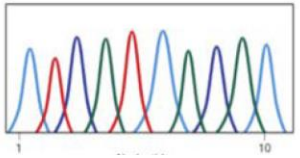
Clot retraction

Western blot



Cellular models

Stage 5: Molecular diagnosis - genetic studies



minION

GridION

Challenges and Limitations of Current Diagnostic Tools for IPDs

- **1. Genetic Testing (e.g., NGS):**
- **High cost**
- **Interpretation difficulties:**
  - Challenging correlation between genetic variants and platelet phenotype (count/function)
  - Particularly problematic when platelet phenotype is unclear
- **Limited accessibility:**
  - Available only in few specialized centers worldwide
  - Creates significant diagnostic barriers for patients in developing/underdeveloped countries
- **2. Platelet Function Tests (e.g., aggregometry):**
- **Technical limitations:**
  - Requires minimum platelet concentration (≥80,000/µL) for effective analysis
  - Challenging in patients with moderate/severe thrombocytopenia
- **Pediatric limitations:**
  - Difficult to obtain sufficient blood volume from neonates and young children
- **3. Overall Diagnostic Gap:**
- Current laboratory techniques remain inaccessible to many patients globally
- Creates disparity in diagnosis and management of IPDs worldwide

Blood Smear Analysis – A Novel Diagnostic Tool

- First proposed by Prof. Greinacher et al. 2017, for **immune-morphological** platelet analysis from peripheral blood smears.
- Uses **fluorescence microscopy** to diagnose a wide spectrum of platelet disorders.
- **Advantages:**

✓Minimal blood volume (< 100 µL)

✓Suitable for newborns and children

✓Samples can be shipped to reference labs

✓Fast (~45 min) and cost-effective

✓High diagnostic yield in selected IPDs

Diagnostic Algorithm (Practical Approach)

1. Clinical history + physical exam
2. Blood smear → platelet size, staining, clumps
3. Immunofluorescence for key markers (MYH9, GpIb/IX, GpIIb/IIIa, granule proteins)
4. Targeted NGS based on smear findings
5. Confirm diagnosis and plan follow-up

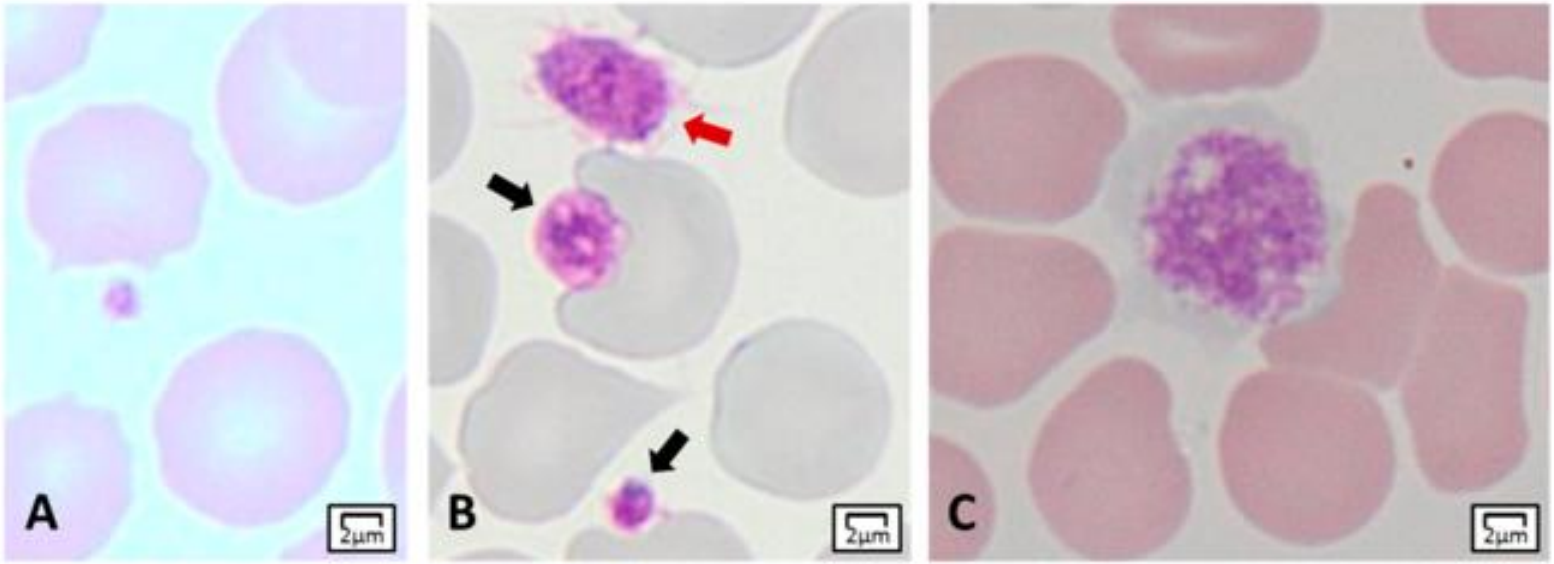
Light Microscopy

❖MGG- or Wright-stained blood film analysis is the first step, allowing:

- ➢Platelet **count** estimation
- ➢Platelet **size** (micro vs macro)
- ➢Platelet **staining** (granules)
- ➢Platelet **clumping** (artifact or clue)

Platelet Size Abnormalities

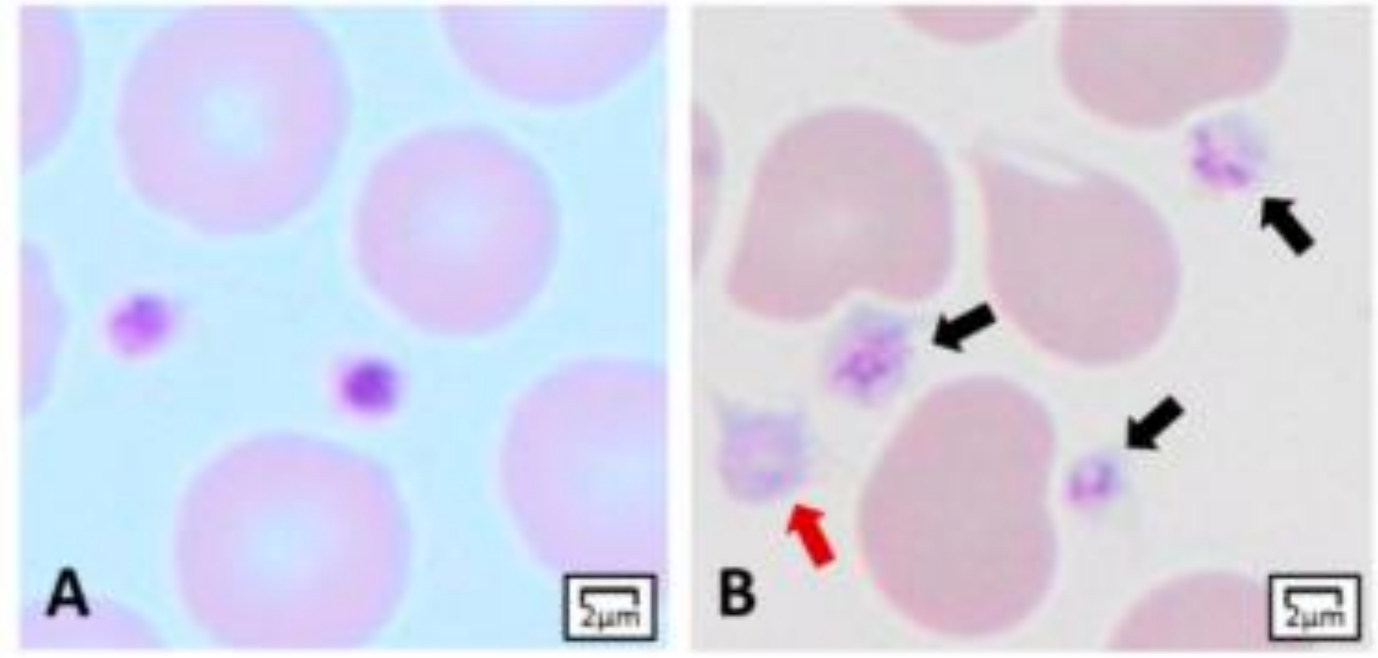
- **Giant platelets**: MYH9-RD, BSS
- **Small platelets**: WAS, CAMT
- **Normal size**: GT (Glanzmann)



Mean platelet diameter (MPD) assessment is crucial. A collaborative study classified IPDs by MPD: enlarged (>3.2 µm), decreased (<2.6 µm), and normal (2.6–3.2 µm). MPD > 3.9 µm or < 2.6 µm effectively differentiates IPDs with giant platelets (MYH9-RD, BSS) from those with small platelets (TAR, CAMT, WAS/XLT).

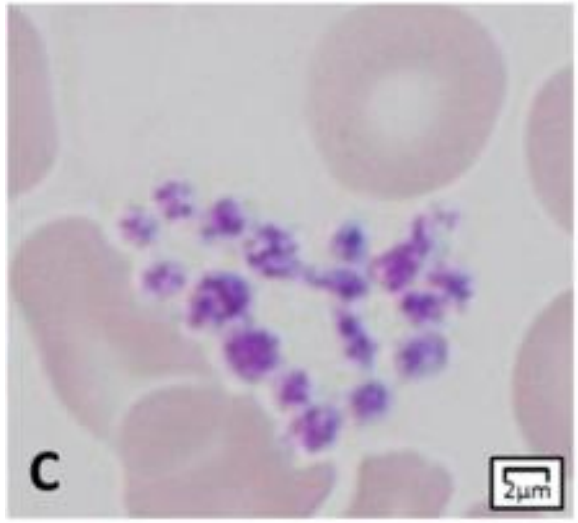
Platelet Staining Abnormalities

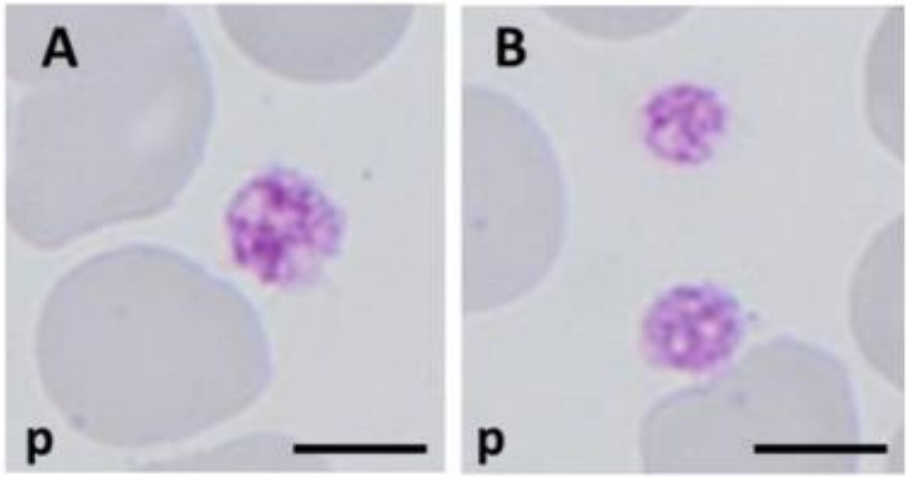
- Pale/gray platelets → Grey Platelet Syndrome (GPS)
- Reduced granules → GATA1-RT, GFI1B-RT

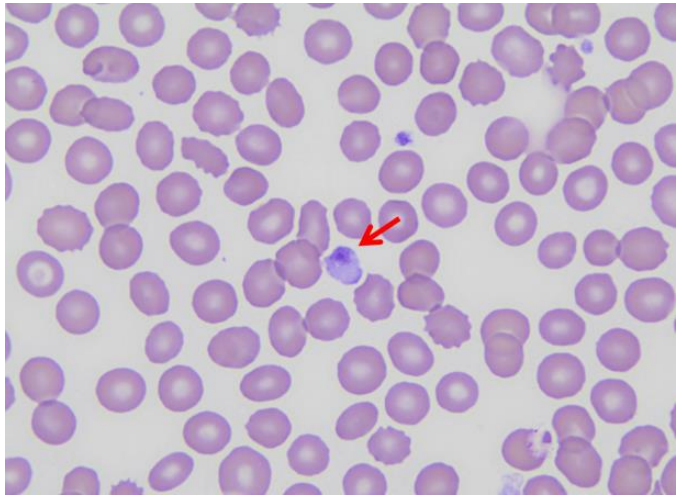


Other Morphologic Clues

- **Platelet clumps** → Type 2B von Willebrand disease
- **Giant alpha granules** → Paris-Trousseau syndrome
- **Vacuoles** → GATA1-RT

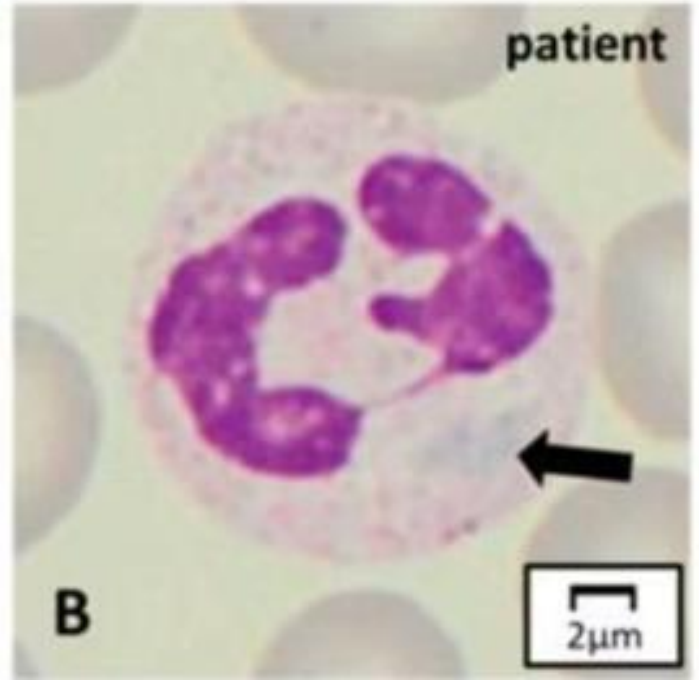


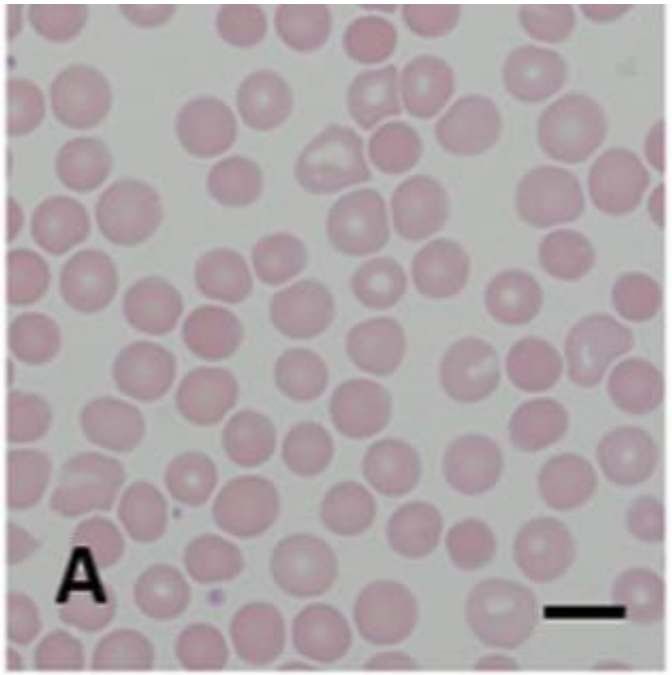




Other Blood Cells

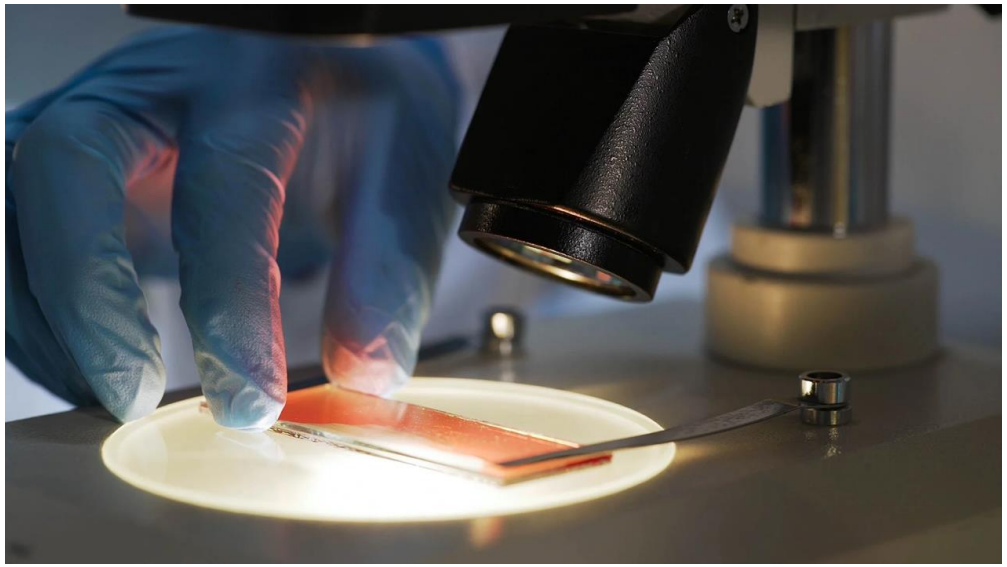
- Döhle-like bodies in neutrophils → MYH9-RD
- RBC anisopoikilocytosis → GATA1-RT





Immunofluorescence Microscopy

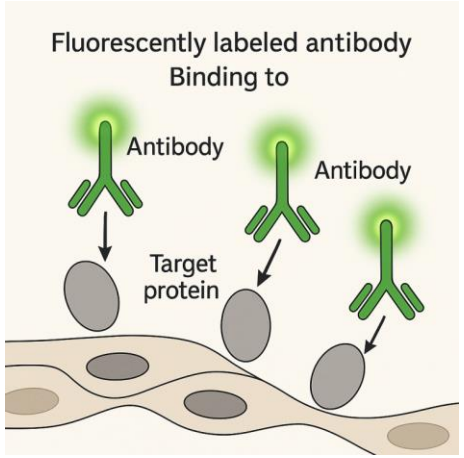
- Detects **protein distribution** inside platelets
- Confirms **specific IPD patterns**



Technical Considerations

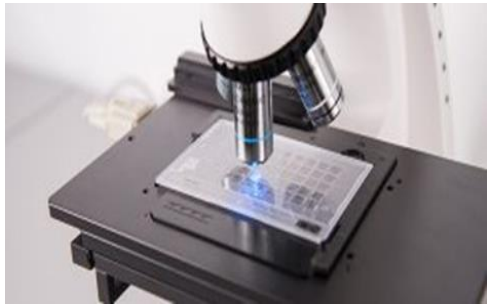
2.1. Preparation of Blood Smears

Capillary or anticoagulated blood (EDTA preferred for stability) can be used.
Slides should be prepared within 4 hours if anticoagulated and stored above 20 °C to prevent platelet size increase and microtubule depolymerization.
At least 15-20 slides are recommended per patient for immunofluorescence staining.



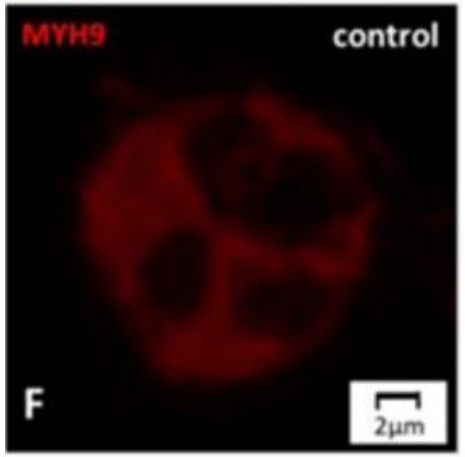
2.2. Immunofluorescence Labelling

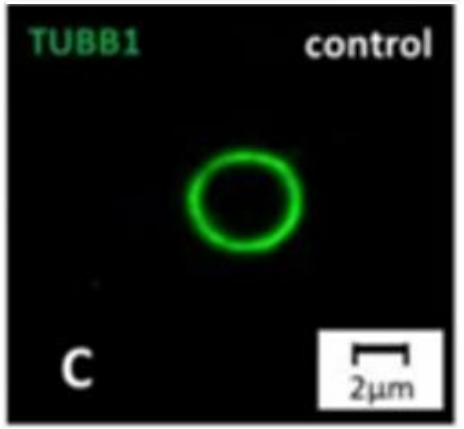
Slides are fixed and permeabilized
Covered with monoclonal or polyclonal antibodies against target proteins.
Fluorescence is achieved via direct or secondary antibody labeling.
Staining for two platelet structures is highly advisable for interpretation.
Comparison with a normal control smear helps detect abnormal protein distribution.

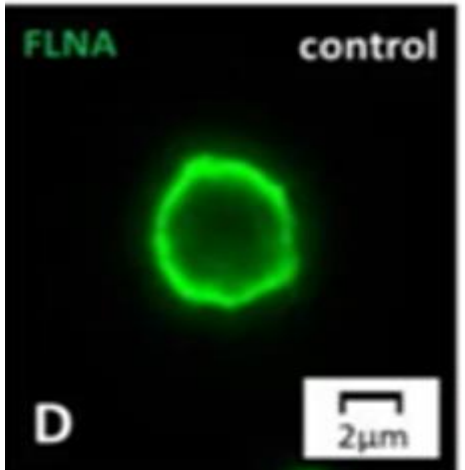


Cytoskeleton Markers

- **MYH9 aggregates** → MYH9-RD
- **Disrupted β1-tubulin ring** → TUBB1-RT
- **Loss of filamin A** → FLNA-RT

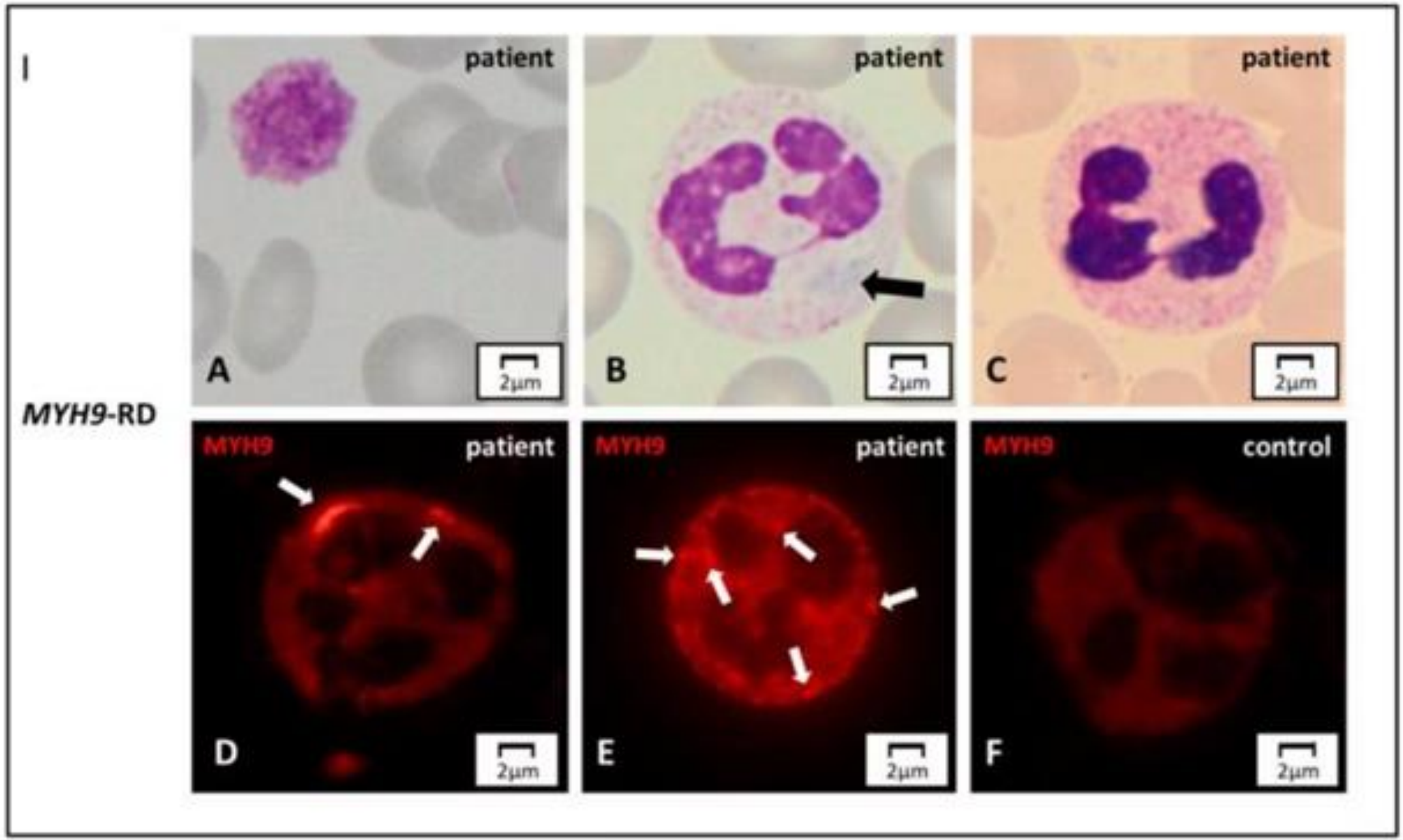


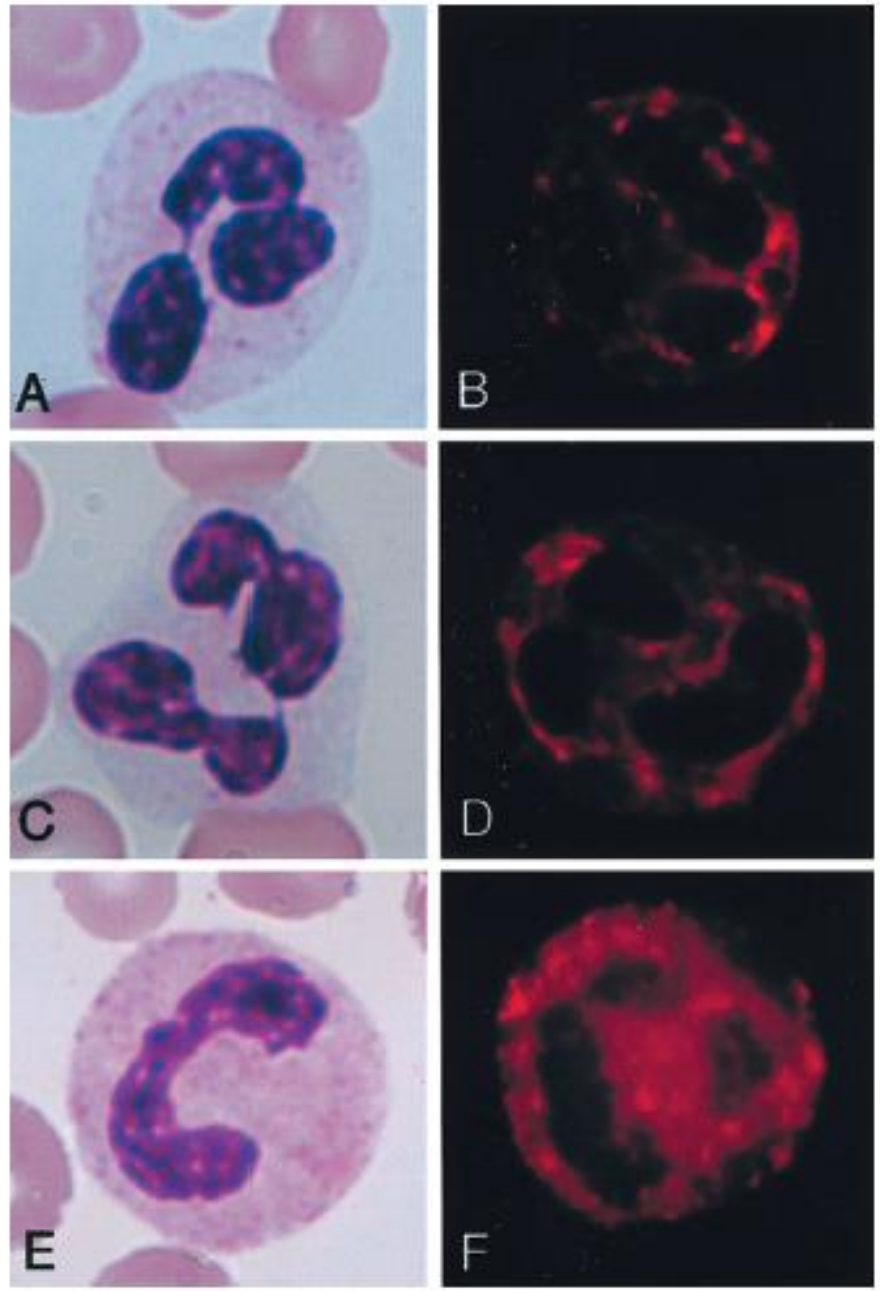




MYH9-related disorders

- MYH9-related disorders are:
- Autosomal dominant macrothrombocytopenia's
- Characterized by giant platelets, thrombocytopenia, and. Döhle-like bodies in neutrophils
- Conventional blood smear examination may fail to detect these inclusions.
- Neutrophil NMMHCA immunofluorescence is a **sensitive and reliable screening method** for MYH9 disorders


I
MYH9-RD
patient
patient
patient
A
B
C
2µm
2µm
2µm
MYH9
patient
MYH9
patient
MYH9
control
D
E
F
2µm
2µm
2µm


A
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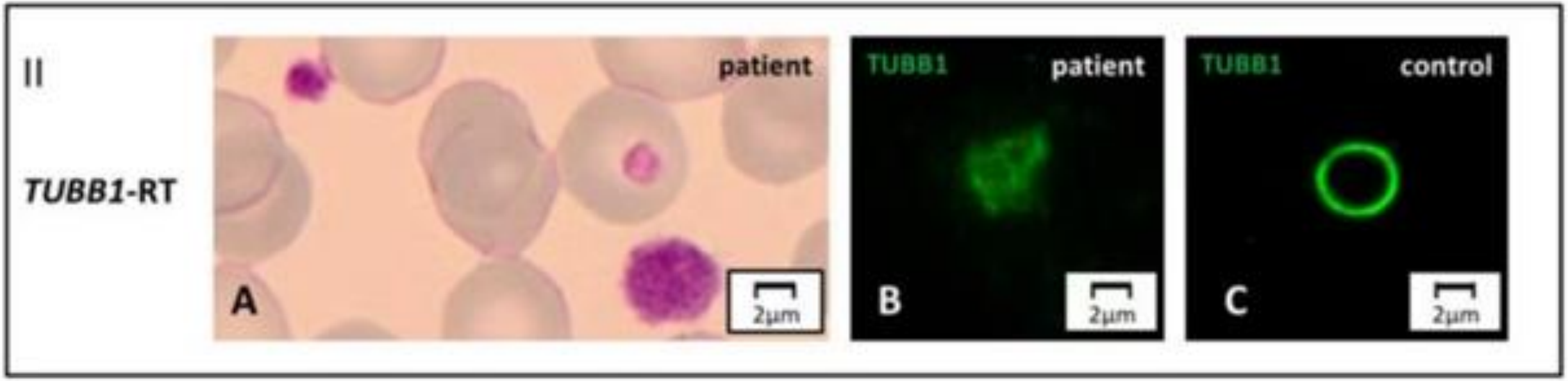
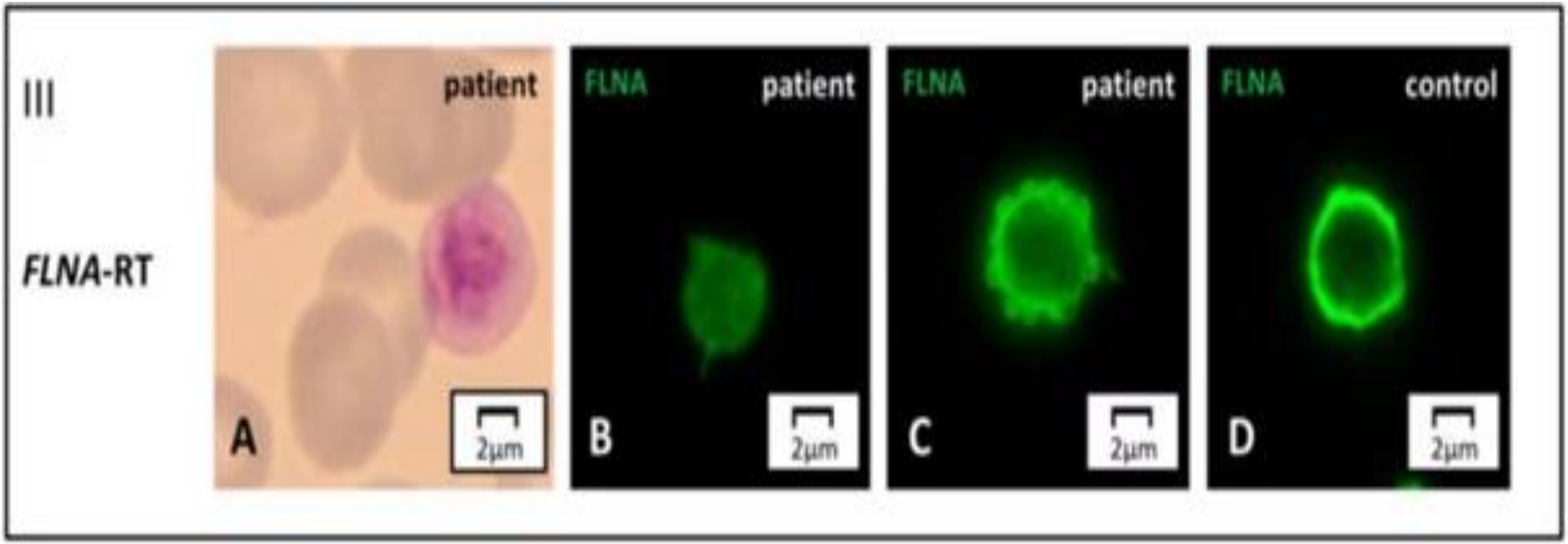
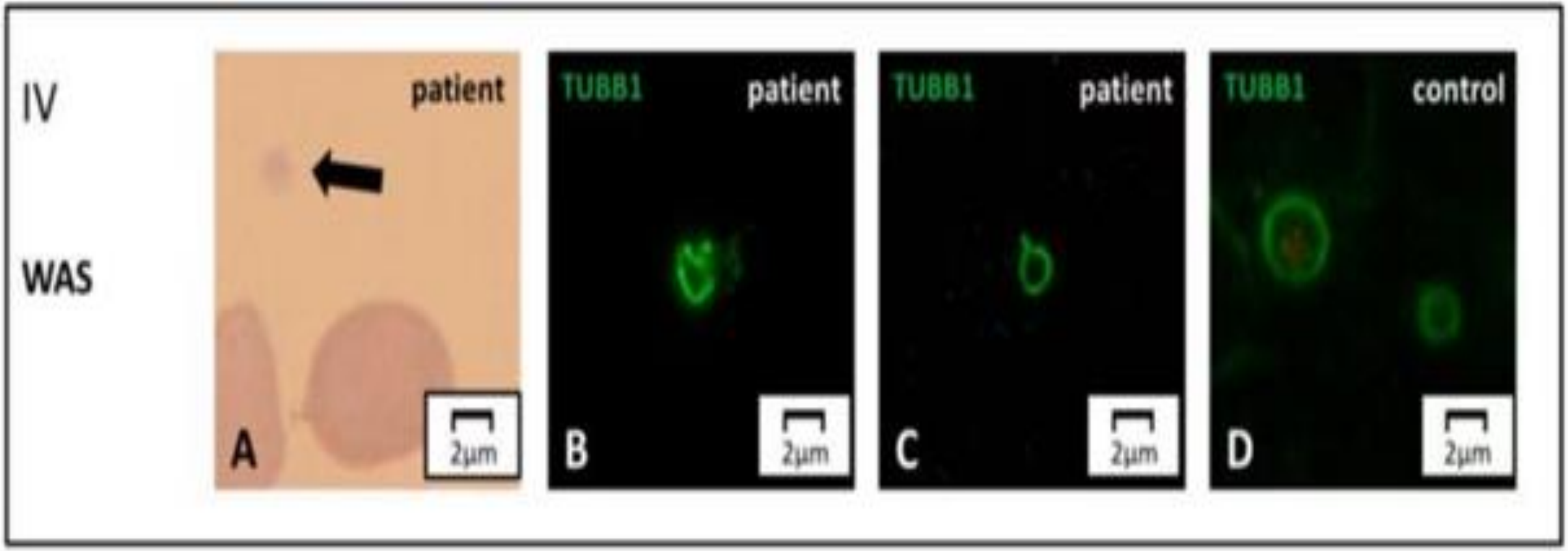
Cytoskeleton Markers



Cytoskeleton Markers

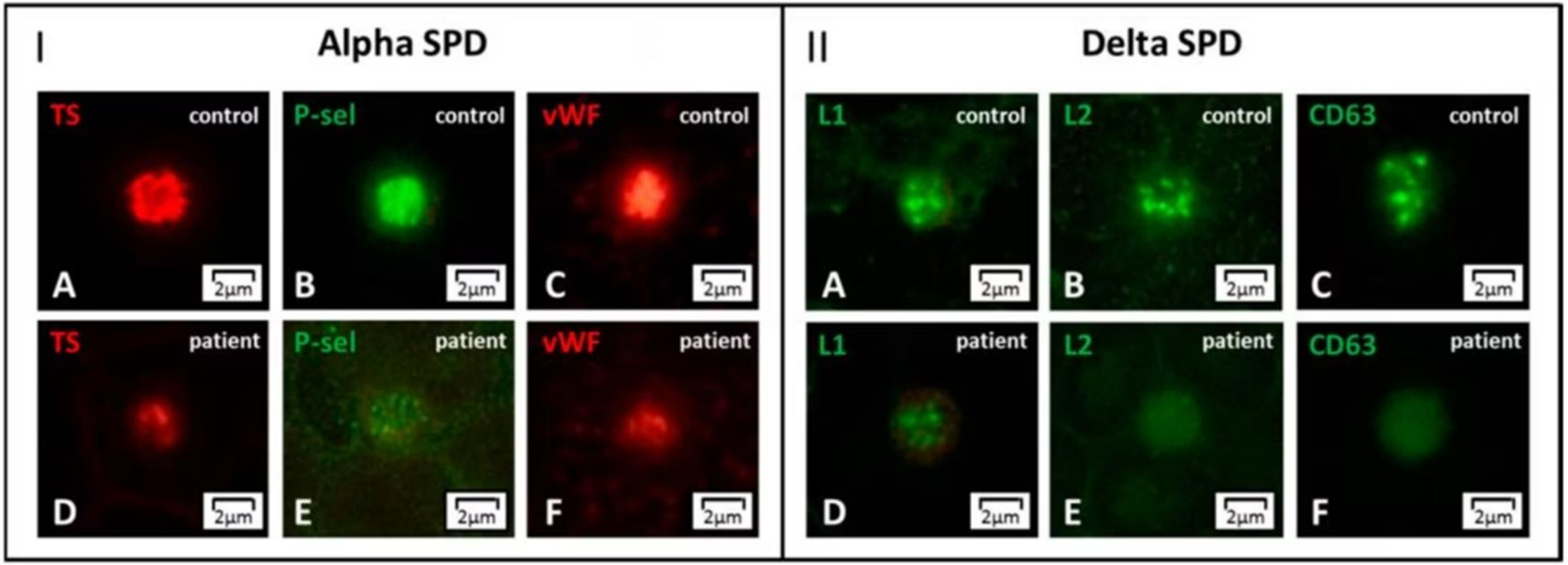


Cytoskeleton Markers



Alpha & Delta Granules

- ↓ α-granules → GPS, α-SPD
- ↓ δ-granules → δ-SPD, Hermansky–Pudlak syndrome



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Datum: 02.02.2023

Name of Patient: **Kiarash Sarlaki**

Date of Investigation: **27.01.2023**

Light microscopy:

	Finding	Interpretation
Staining (blood smear)	normal	normal
Platelet size (blood smear)	heterogeneuos	pnormal
Inclusion bodies (blood smear)	no inclusion bodies	normal

Immunofluorescence microscopy:

	Marker	Finding	Interpretation
Membrane-proteins	GP IbIX	regular expression	normal
	GP IIbIIIa	regular expression	normal
Alpha-Granule-markers	vWF	regular expression	normal
	Thrombospondin	regular expression	normal
	P-Selectin	regular expression	normal
Delta Granule-marker	Lamp 2	reduced, partally absent	
	CD63	nearly absent or diffusely expressed	
Lysosomes	Lamp 1	regular expression	normal
Cytoskeleton	NMMIIa	no inclusion bodies	normal
	ß1-Tubulin	nearly normal, only disturbed in the large platelets	
	α-Tubulin	nearly normal, only disturbed in the large platelets	

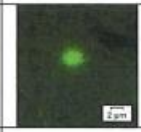
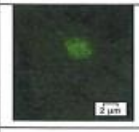
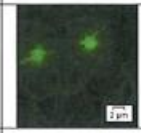
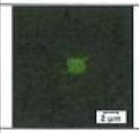
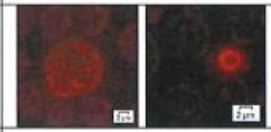
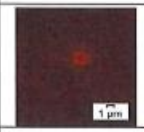
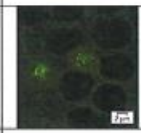
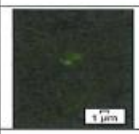
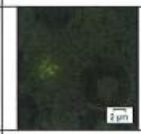
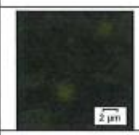
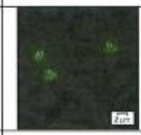
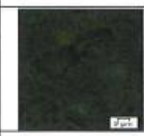
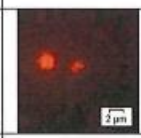
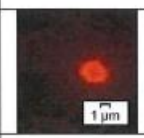
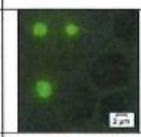
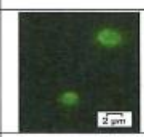
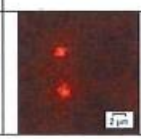
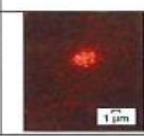
In the peripheral blood smear platelets were enlarged with normally staining.

Immunfluorescence LAMP1 is present, non-muscular myosin IIa is present, von Willebrand factor is normal, GP IbIX is present, LAMP2 is reduced and absent in some platelets but not in all platelets, Thrombospondin is normal, P selecting is normal, Beta one tubulin is normal, CD63 is nearly absent or diffusely expressed on the membrane, GP IIbIIIa is present, alpha tubulin is present but disturbed in the larger platelets

interpretation The platelet phenotype indicates a Delta storage pool deficiency primarily with alteration of CD63. If albinism is present Hermansky Pudlak syndrome might be the underlying cause

Prof. Dr. A. Greinacher

Dr. C. Zaninetti

antibodies against	control	Kiarash Sarlaki	
IbIX			normal
IIbIIIa			normal
Myosin			normal
Lamp1			normal
Lamp2			reduced, partly absent
CD63			nearly absent or diffusely expressed
Thrombospondin			normal
P-Selektin			normal
vWf			normal

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In Notfällen erreichen Sie uns unter: +49 3834 86 6074
Datum: 08.02.2023

Name of Patient: **Solaleh, Kawiani**

Date of Investigation: **27.01.2023**

Light microscopy:

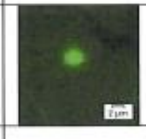
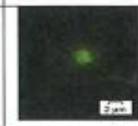
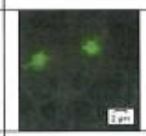
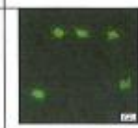
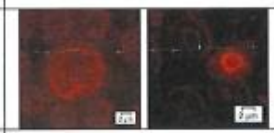
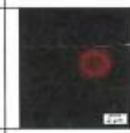
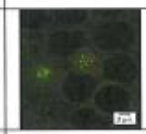
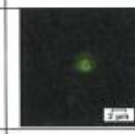
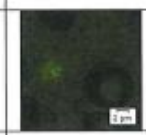
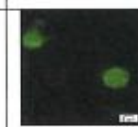
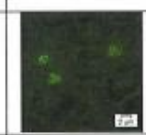
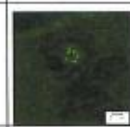
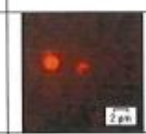
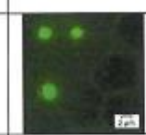
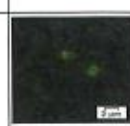
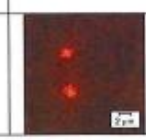
	Finding	Interpretation
Staining (blood smear)	normal, partial incomplete	normal
Platelet size (blood smear)	many platelets were enlarged	pathological
Inclusion bodies (blood smear)	no inclusion bodies	normal

Immunofluorescence microscopy:

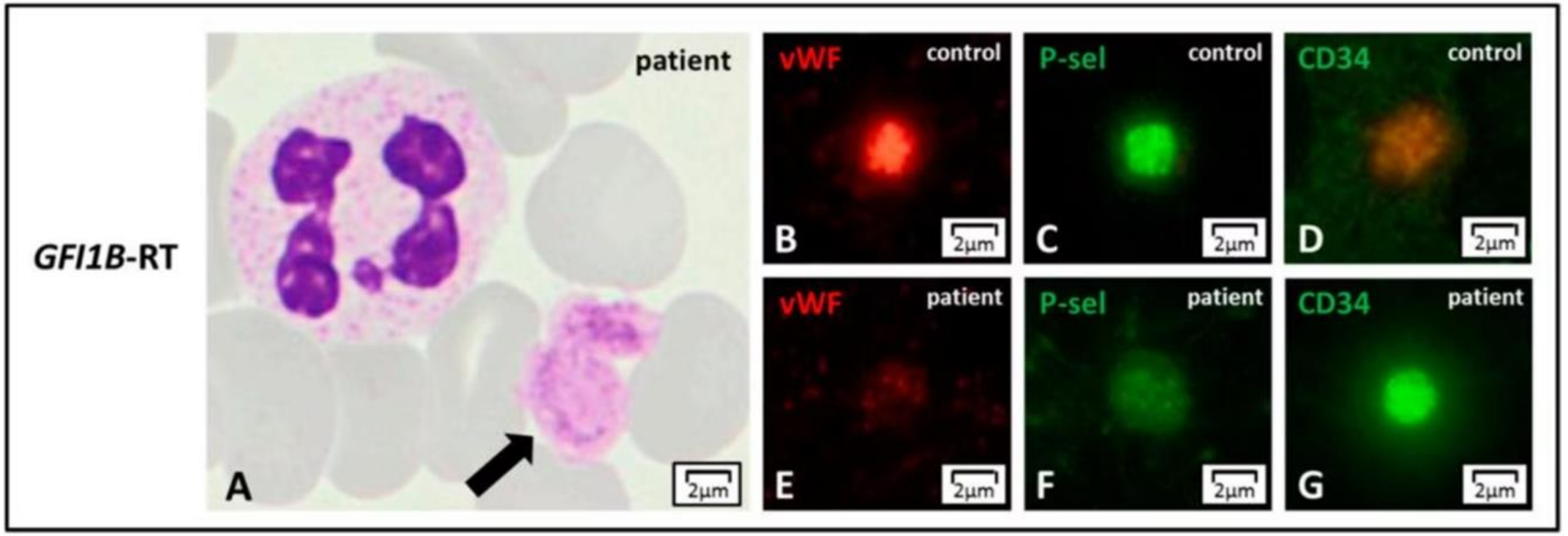
	Marker	Finding	Interpretation
Membrane-proteins	GP IbIX	regular expression	normal
	GP IIbIIIa	regular expression	normal
Alpha-Granule-markers	vWF	strongly reduced, absent	
	Thrombospondin	reduced	
	P-Selectin	reduced	
Delta Granule-marker	Lamp 2	regular expression	normal
	CD63	regular expression	normal
Lysosomes	Lamp 1	regular expression	normal
Cytoskeleton	NMMIIa	no inclusion bodies	normal
	ß1-Tubulin	normally expressed in small the platelets but disturbed in the large platelets	normal
	α-Tubulin	present in ring form in the small of platelets but strongly disturbed in the large platelets	normal
	CD34	non-detectable	normal

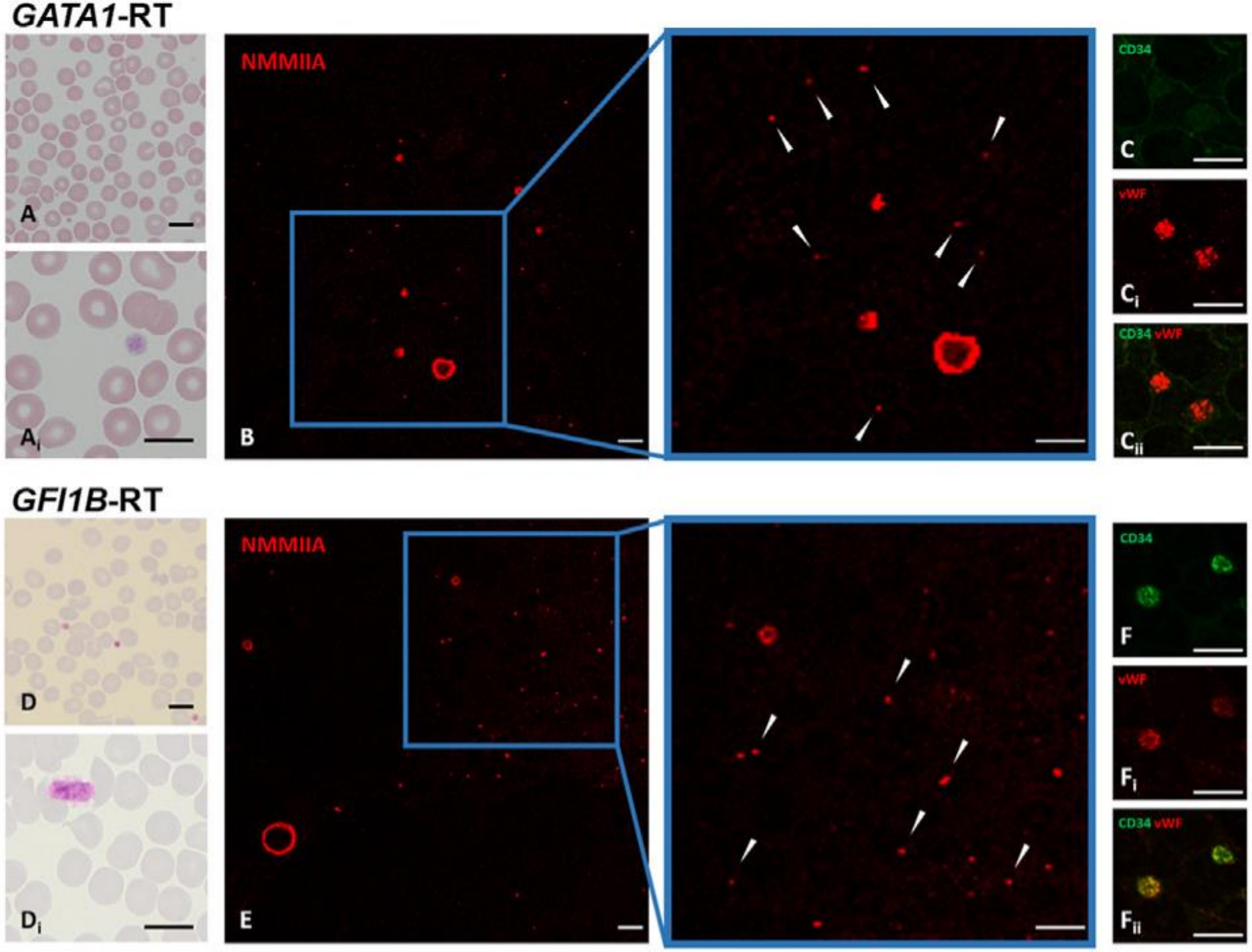
In the peripheral blood smear platelets were enlarged, several platelets had less intense staining.

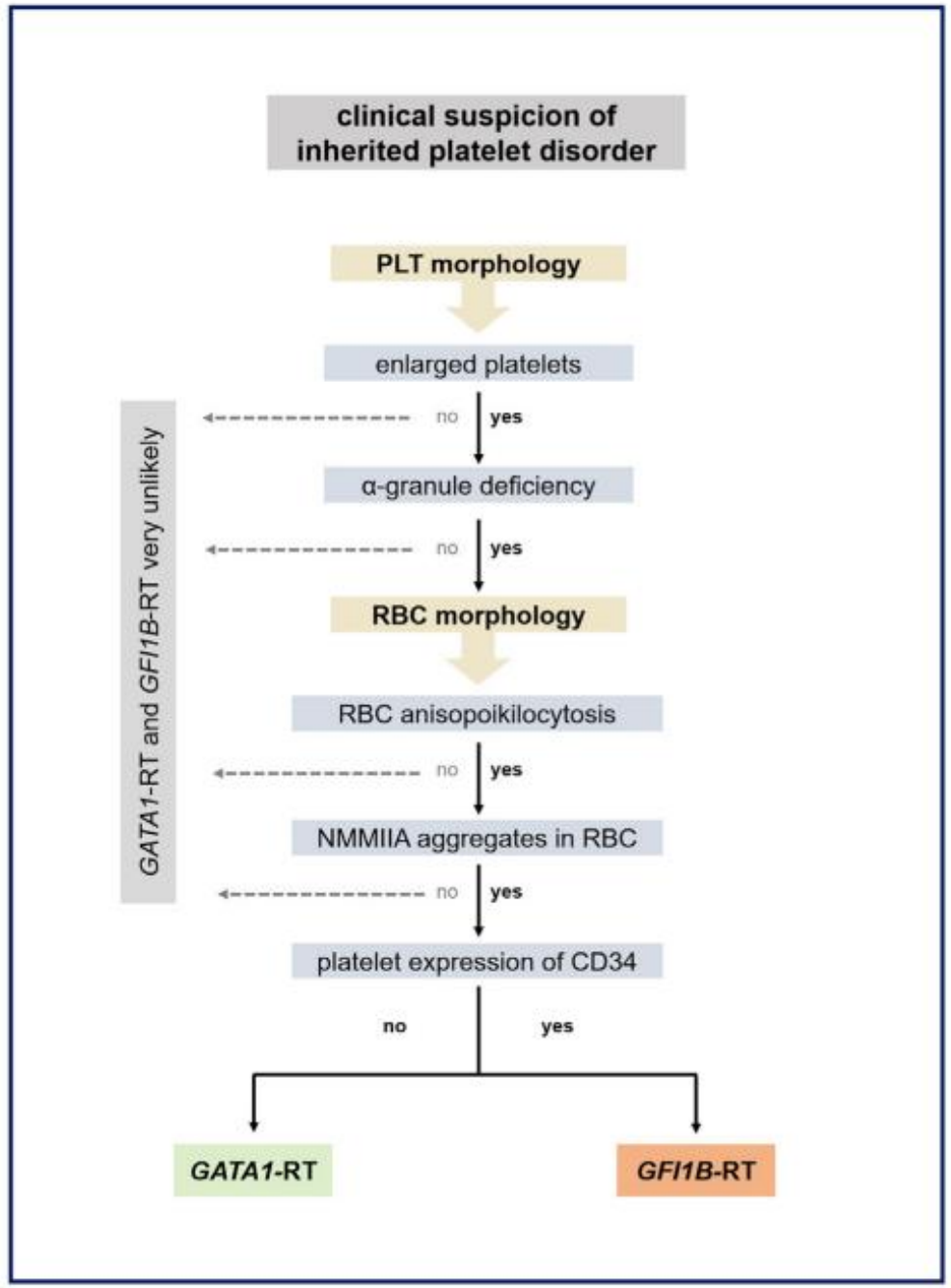
LAMP1 is present, non-muscular myosin IIa is present in ring form no inclusion bodies in the leukocytes. Von Willebrandt factor is strongly reduced and present in only 10% of platelets GP IbIX is normally expressed
LAMP2 is present, thrombospondin is present but slightly reduced and absent in about 30 % of platelets
P selecting is strongly reduced and if present often located at the membrane. Beta one tubulin is normally expressed in small the platelets but disturbed in the large platelets, CD63 is present in most platelets but in the larger platelets probably somewhat reduced but nearly all platelets

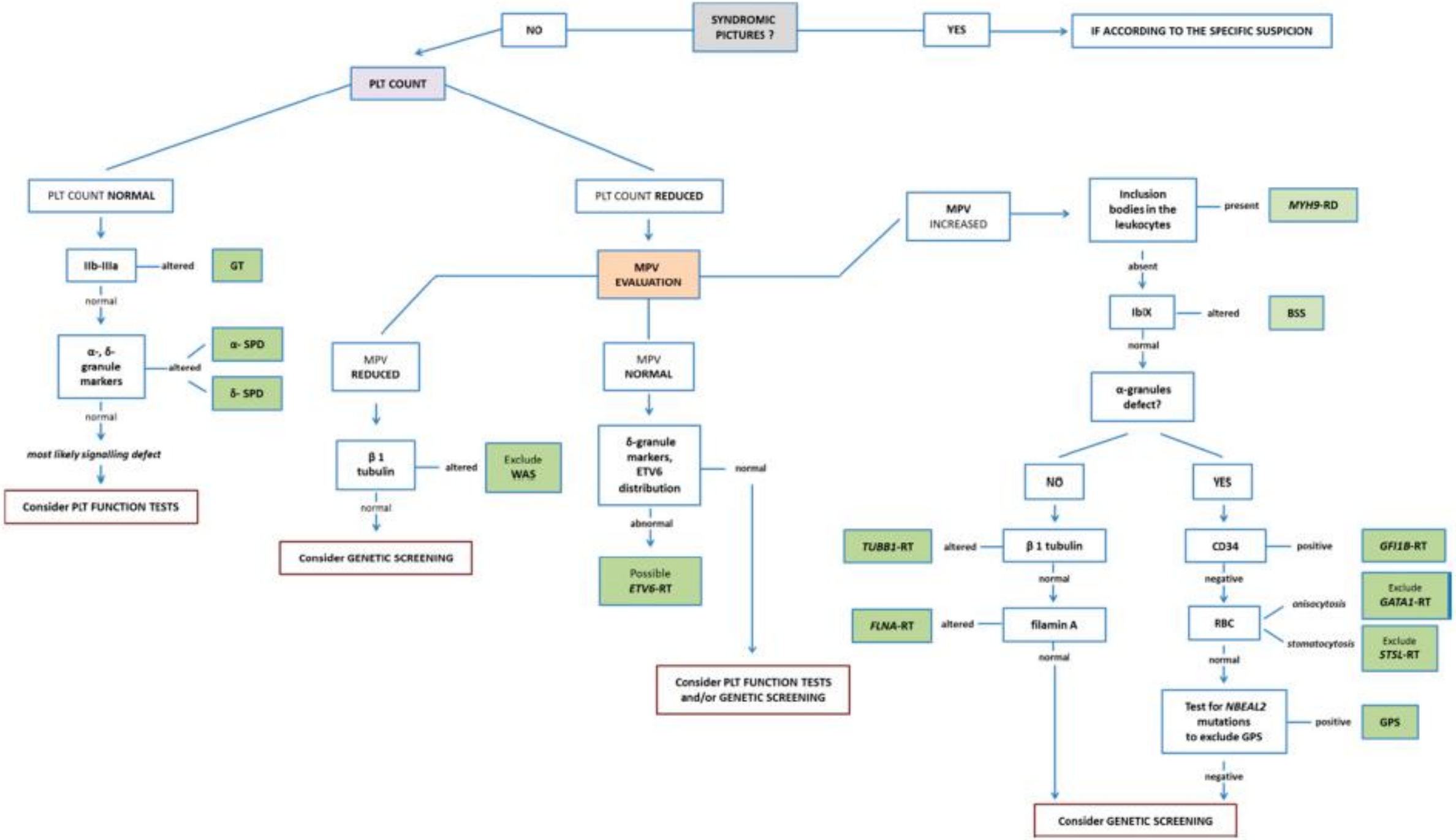
antibodies against	control	Solaleh, Kaviani	
IbIX			normal
IIbIIIa			normal
Myosin			normal
Lamp1			normal
Lamp2			normal
CD63			normal
Thrombospondin			slightly reduced
P-Selektin			strongly reduced
vWf			Absent

Alpha Granules




GATA1-RT
A
Ai
NMMIIA
B
CD34
C
vWF
Ci
CD34 vWF
Cii
GFI1B-RT
D
Di
NMMIIA
E
CD34
F
vWF
Fi
CD34 vWF
Fii


clinical suspicion of inherited platelet disorder
PLT morphology
enlarged platelets
no
yes
α-granule deficiency
no
yes
RBC morphology
RBC anisopoikilocytosis
no
yes
NMMIIA aggregates in RBC
no
yes
platelet expression of CD34
no
yes
GATA1-RT
GFI1B-RT
GATA1-RT and GFI1B-RT very unlikely


SYNDROMIC PICTURES ?
NO
YES
IF ACCORDING TO THE SPECIFIC SUSPICION
PLT COUNT
PLT COUNT NORMAL
PLT COUNT REDUCED
IIb-IIIa
altered
GT
normal
α-, δ-granule markers
altered
α- SPD
δ- SPD
normal
most likely signalling defect
Consider PLT FUNCTION TESTS
MPV EVALUATION
MPV REDUCED
β 1 tubulin
altered
Exclude WAS
normal
Consider GENETIC SCREENING
MPV NORMAL
δ-granule markers, ETV6 distribution
normal
abnormal
Possible ETV6-RT
Consider PLT FUNCTION TESTS and/or GENETIC SCREENING
MPV INCREASED
Inclusion bodies in the leukocytes
present
MYH9-RD
absent
IbIX
altered
BSS
normal
α-granules defect?
NO
YES
TUBB1-RT
altered
β 1 tubulin
normal
FLNA-RT
altered
filamin A
normal
CD34
positive
GFI1B-RT
negative
RBC
anisocytosis
Exclude GATA1-RT
stomatocytosis
Exclude STSL-RT
normal
Test for NBEAL2 mutations to exclude GPS
positive
GPS
negative
Consider GENETIC SCREENING

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ORIGINAL ARTICLE

Validation of immunofluorescence analysis of blood smears in patients with inherited platelet disorders

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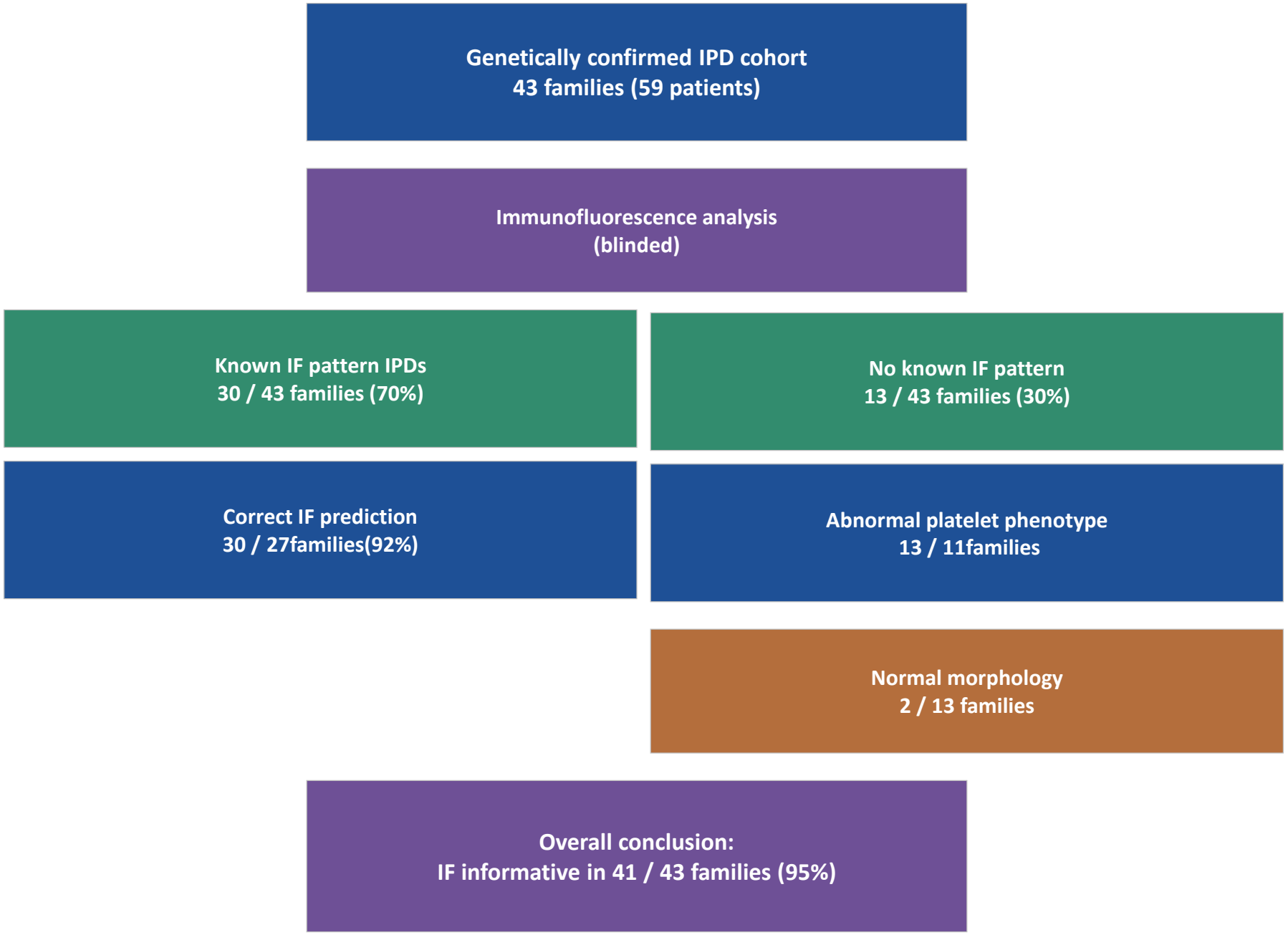
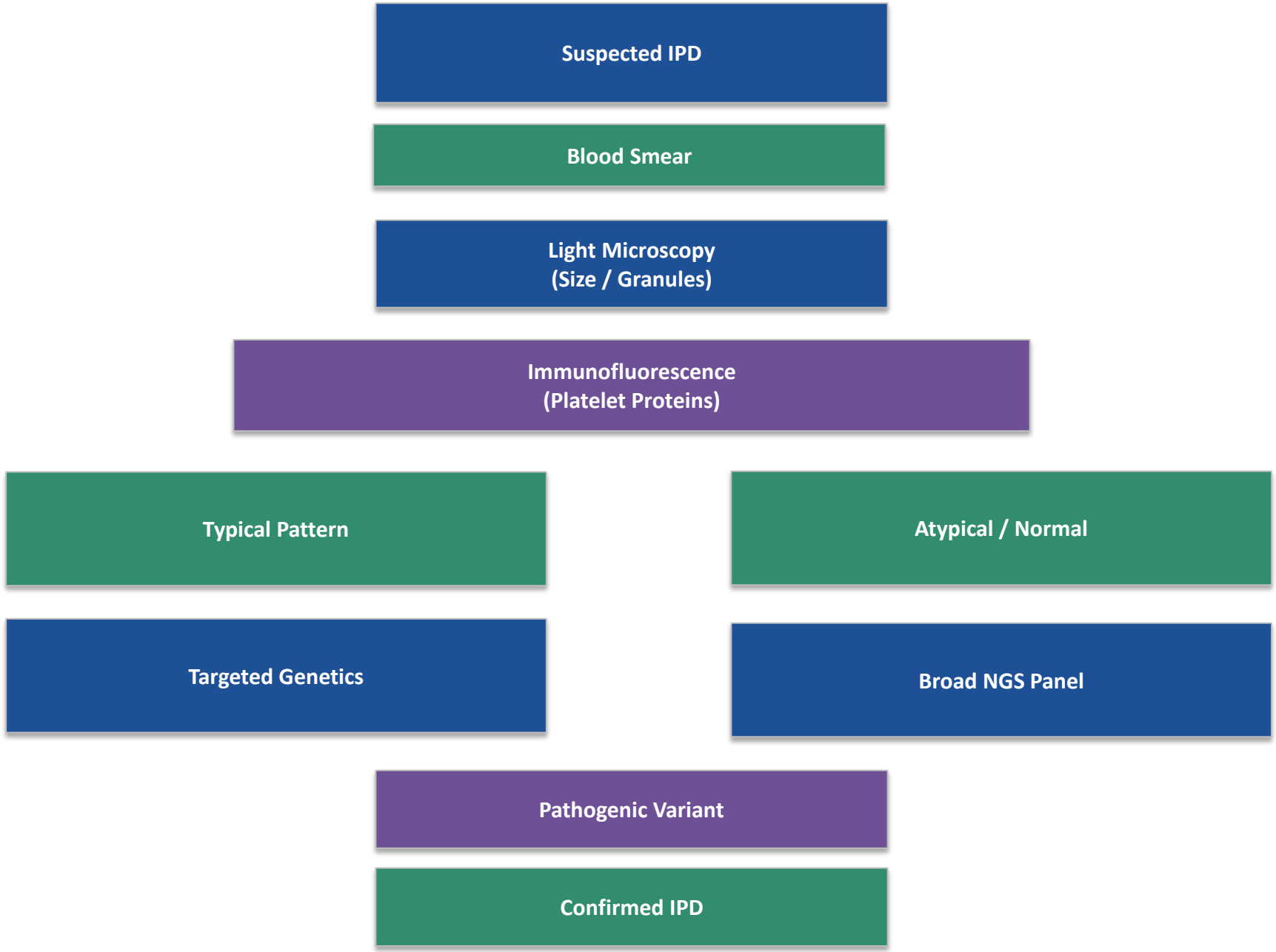
Main Idea of the Study

- Can **immunofluorescence microscopy on blood smears** be used as a **screening tool** for IPDs?
- How well does it match the **genetic diagnosis (gold standard)?**


Genetically confirmed IPD cohort
43 families (59 patients)
Immunofluorescence analysis
(blinded)
Known IF pattern IPDs
30 / 43 families (70%)
No known IF pattern
13 / 43 families (30%)
Correct IF prediction
30 / 27families(92%)
Abnormal platelet phenotype
13 / 11families
Normal morphology
2 / 13 families
Overall conclusion:
IF informative in 41 / 43 families (95%)



Key Message: Use immunofluorescence for early phenotypic stratification; confirm diagnosis by genetic testing.

genes

MDPI

Article

Automated Quantitative Immunofluorescence Microscopy Approach for Diagnosis of Hereditary Thrombopathies: A Proof of Concept Using Bernard–Soulier Syndrome and Glanzmann Thrombasthenia

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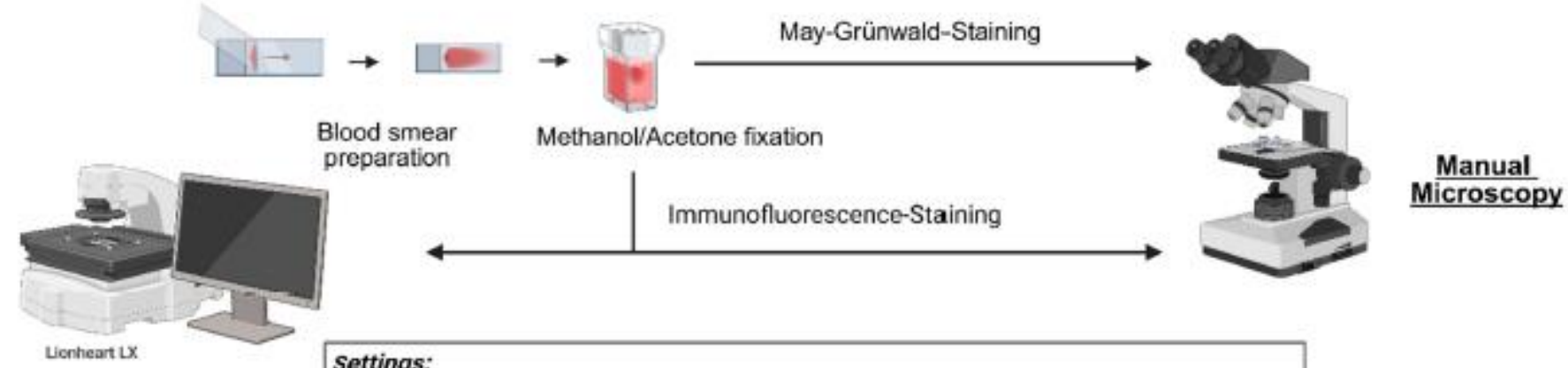
* Correspondence: behnaz.pezeshkpoor@ukbonn.de

† These authors contributed equally to this work.



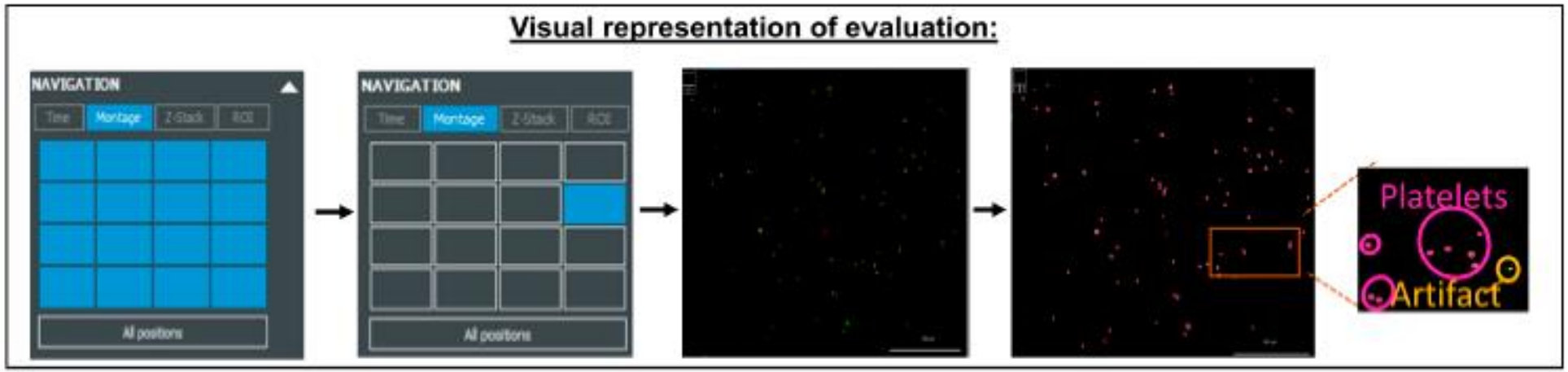


Figure 1. Workflow for automated immunofluorescence microscopy in the diagnosis of inherited platelet disorders.

- ✓ Clear discrimination between patients and controls
- ✓ **BSS:** Absent/reduced CD42b (GPIbα), macrothrombocytopenia
- ✓ **GT:** Deficiency of CD41/CD61 (GPIIb/IIIa)
- ✓ High concordance with flow cytometry and genetics

- ➢ **Complements genetic testing** and can guide diagnosis when flow cytometry is unavailable

Limitations

- Requires **expertise** in interpretation
- **Pre-analytical artifacts** (clumping, storage)
- **Normal smear does not exclude IPD**

Conclusion

- **Blood smear is a powerful diagnostic tool**

➢ **Minimal blood volume required** (< 100 µL)
➢ **Fast and cost-effective** (~45 minutes)
➢ **Suitable for neonates and children**
➢ **Samples can be shipped to reference laboratories**

-


thank you for your attention