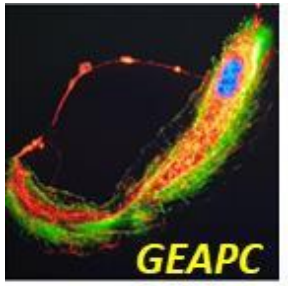
Centro de Hemodonación



Session 2:
Platelet aggregation - investigating a suspected platelet function disorder

José Rivera
Centro Regional de Hemodonación, Hospital General Universitario Morales Meseguer, University of Murcia, Spain
Grupo Español de Alteraciones Plaquetarias Congénitas

November 12th, 202

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

Co-funded by the Health Programme of the European Union

CRPP MHEMO

European Reference Network for rare or low prevalence complex diseases

Network Hematological Diseases (ERN EuroBloodNet)

Centro de Hemodonación

Conflicts of interest

Research support/PI	-
Employee	-
Consultant / Honoraria	-
Major stockholder	-
Speaker's fees	-
Scientific advisory board	-

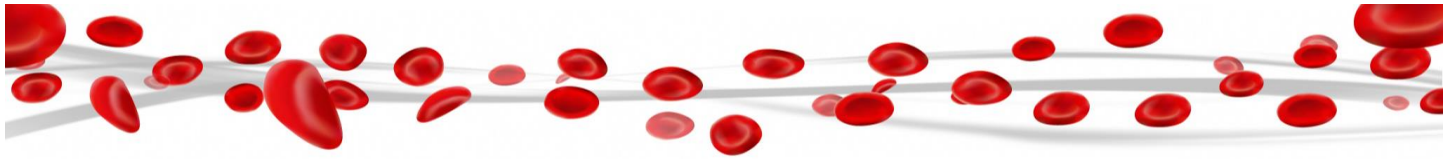
Co-funded by the Health Programme of the European Union

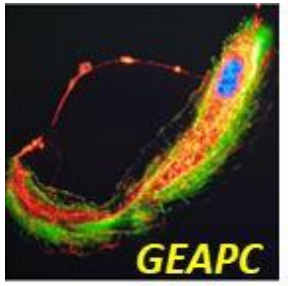
CRPP MHEMO

European Reference Network for rare or low prevalence complex diseases

Network Hematological Diseases (ERN EuroBloodNet)

Centro de Hemodonación





Learning objetives

1. IPFDs overview

2. Light transmisssion aggregometry (LTA): Principle and recommendations for performance and interpretation in the diagnosis of IPFDs

3. Using & interpreting LTA: Case examples from the GEAPC project

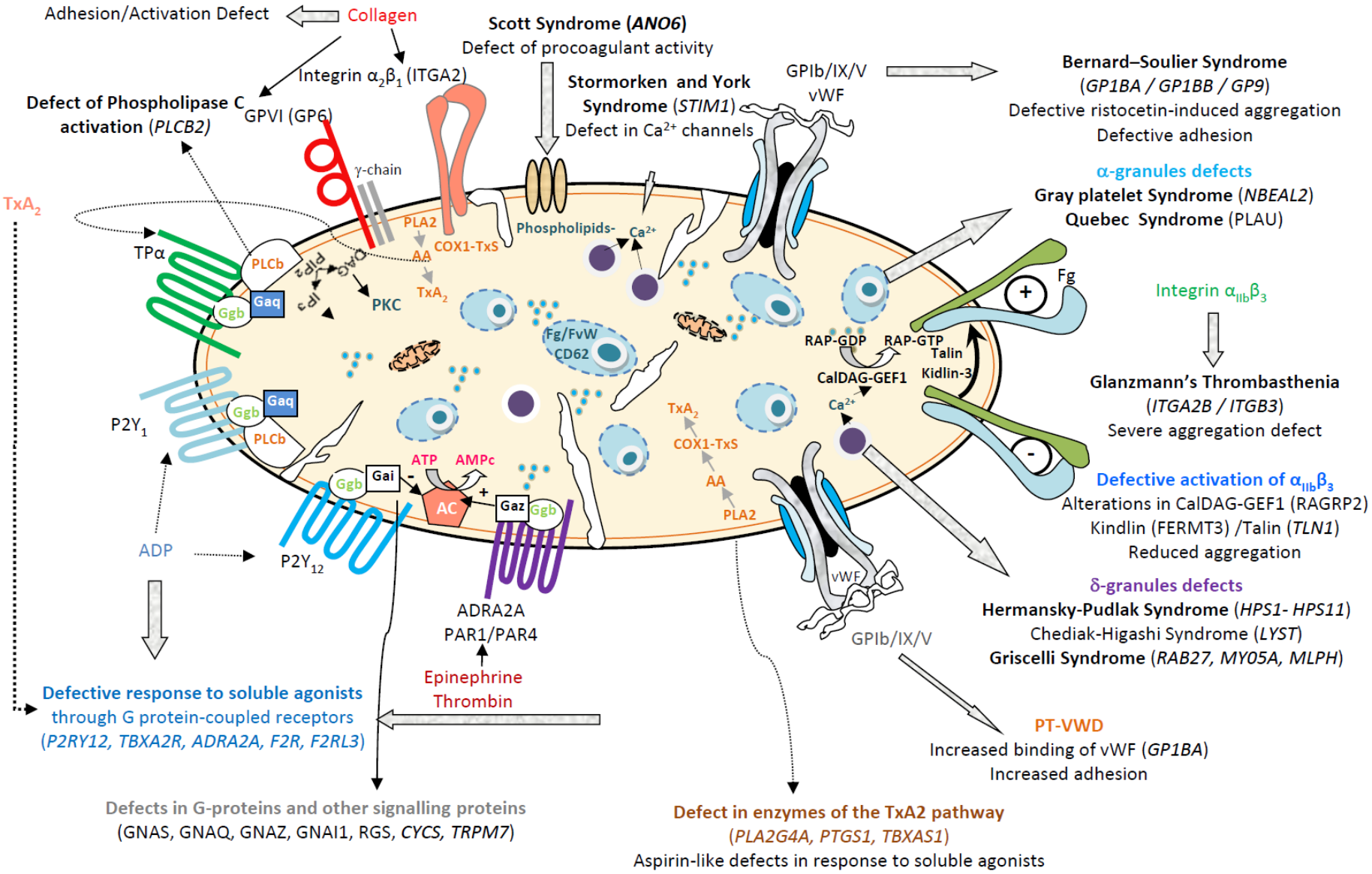
Co-funded by the Health Programme of the European Union

CRPP MHEMO

European Reference Network for rare or low prevalence complex diseases

Network Hematological Diseases (ERN EuroBloodNet)

Inherited Platelet Function Disorders (IPFD)



IPFD types

- Receptors:
  - ✓ Adhesive proteins
  - ✓ Soluble agonists
- Enzymes
- Signal transduction
- Granules
- Membrane Phospholipids

Clinical impact

- Bleeding
- Blood malignancy
- Sydromes or extra-hematological additional disease. (Lung fibrosis in HPS)

IPDs ≈ 60 types, 75 genes; IPFDs≈ 15 types, 30 genes; ≈ 1:10.000 – 1:1.000.000;
Likely underdiagnosed; ≈ 6000 new diagnosis/yr; 0.3-2.5% based in recent DNA senquencing data

Rodríguez-Alén Biomolecules 2025; https-doi.org10.3390biom15111528

Grupo Español de Alteraciones Plaquetarias Congénitas (GEAPC) (Spanish Group of IPD)

Multicentric Project of Functional and Molecular Characterization of IPDs (2008-2025)

https://seth.es/investigacion/geapc/

Coordinators

Dr. José Rivera: jose.rivera@carm.es

Dr. José María Bastida: jmbastida@saludcastillayleon.es

Comprehensive approach to IPD diagnosis

Genetic diagnosis

Final Report

Spanish IPD Registry (RETPLAC)

DNA/RNA analysis

Blood Sampling

Patients (P) & controls (pC)

Functional diagnosis: Platelet Phenotyping

Blood smear, LTA, FC, IF, WB, EM, etc.

P + pC + fresh Control

Limitation:

Time from extraction (<24h)

Review of clinical & laboratory data

Study Proposal

Elegibility Criteria

IPFDs:

- No acquired platelet dysfunction, vWD or coagulation disorder
- Bleeding
  - Adults: BS ≥3 in males or ≥5 in females, or ≥3 different bleeding symptoms
  - Pediatric population: BS ≥2
- Consider syndromics events (albinism, organ malformations , infections, etc.)

ITs:

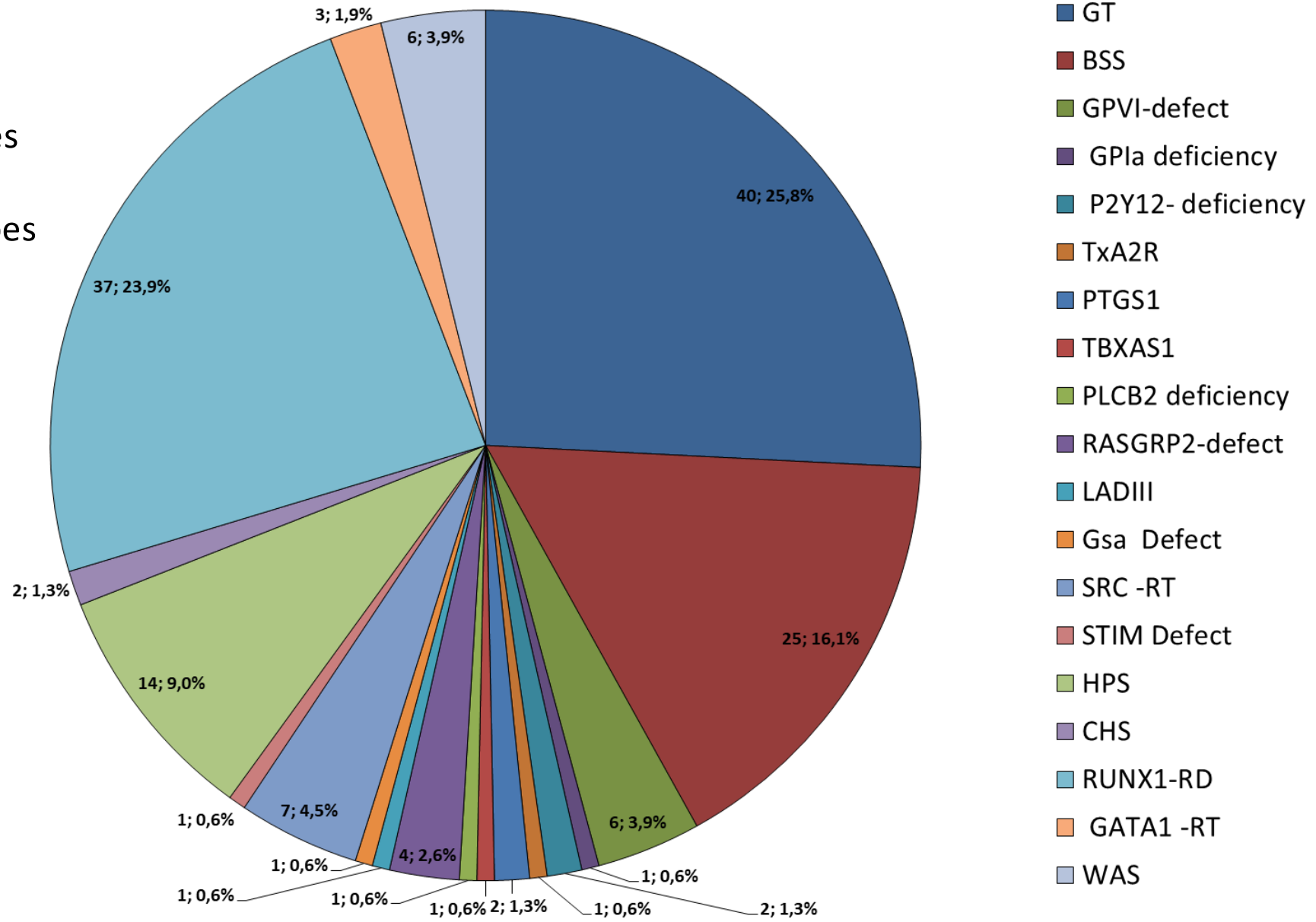
- No acquired; No previous normal platelet, (irrespective of age and family background)
- Bleeding history in adults with severe thrombocytopenia of unknown origen.
- Demonstration of Giant/Large /Small platelet size
- Consider syndromics events (deafness, renal disease, malformations, infections, family history of neoplasm)

GEAPC- IPFDs Casuistry

2008-2025

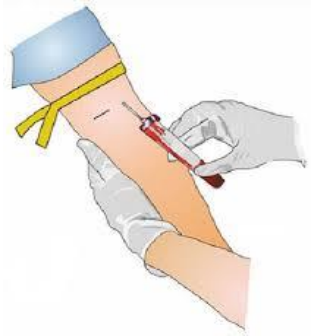
IPDs: n=500, 45 types

IPFDs: n=155, 19 types



Standard Approach to diagnosis of IPD

Session 3: P Gresele



Sessions 2,4,6-9

In Vitro

In Vivo

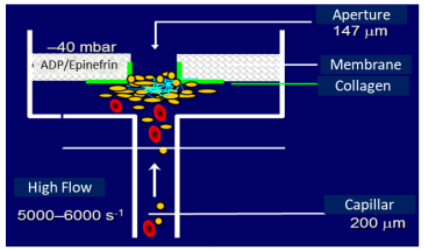
Session 5: K Freson

Clinical Evaluation → **Blood drawn Preliminary tests** → **First&second steps: Biochemical & functional characterization** → **Third step: molecular characterization** → **Diagnosis & treatment**

Gresele .. J Thromb Haemost 2015; Rabbolini.. Pathology 2020; Gomez.. Br J Haematol 2021; Bourguignon.. Crit Rev Clin Lab Sci 2022; Sánchez Fuentes.. Biomolecules 2025

Screening methods in IPFD diagnosis

PFA-100: Bleeding time *in vitro*



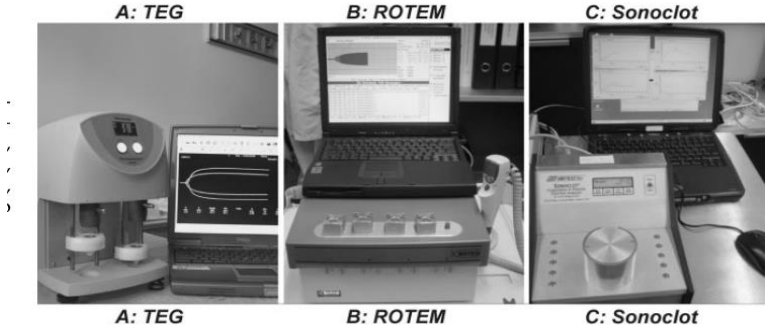
Normal closure time

Col-ADP	Col-Epi	ADP [P2Y, PFA-Innovance]
57-100s	76-131s	53-91s

Advantages: Non-invasive, Simple, fast, automatic, small blood volume
Limitations: influenced by many variables (platelet function & count, Hct, VWF, anticoagulant used, drugs, foods)

Low specificity, poor sensitivity for moderate platelet disorders

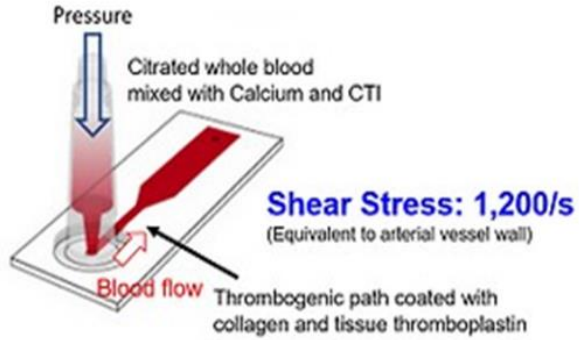
Widely used: ≈50% of centers that do platelet studies(Gresele et al. JTH 2014; 12:1562-69)

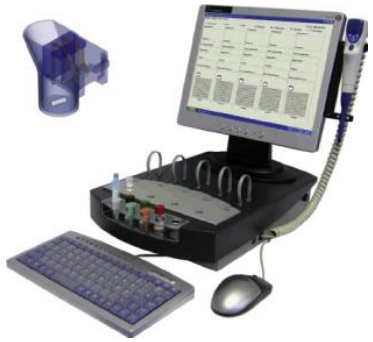


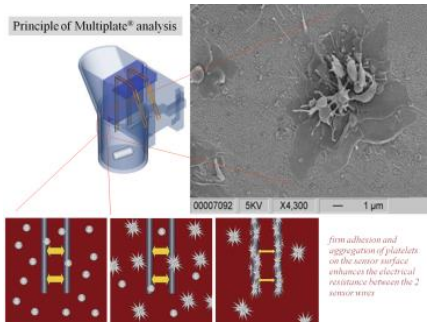
Test viscoelastic test
Thrombus physical properties

Total-Thrombus Analyzer System (T-TAS)

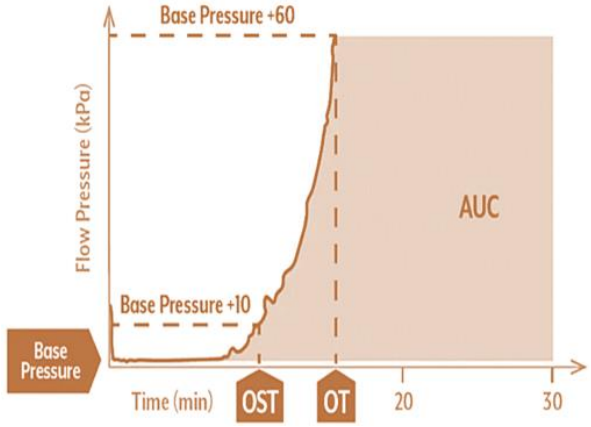
AR, HD, PL







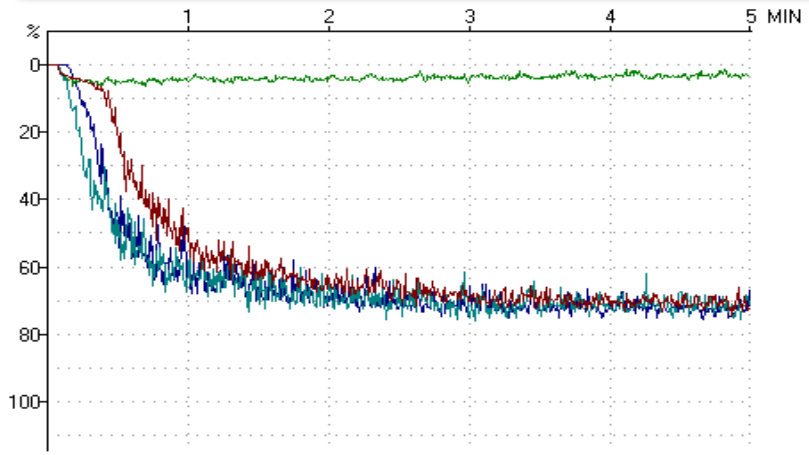
Test: ADP, ASPI[arachid. acid], TRAP, PGE1 [+ADP], IIb/IIIa antagonist



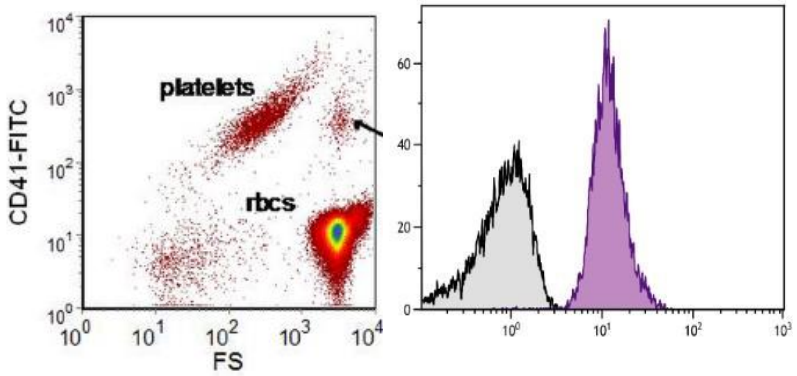
Studies have shown that Multiplate is less sensitive than LTA for diagnosing moderate TPCs.

Diagnostic methods for IPFDs

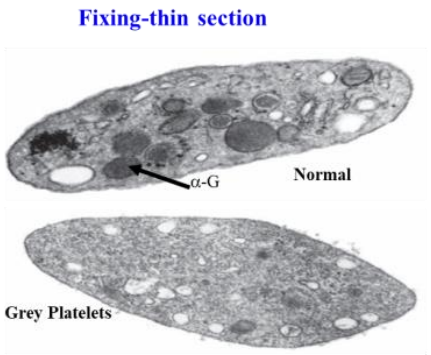
Light Transmission aggregometry

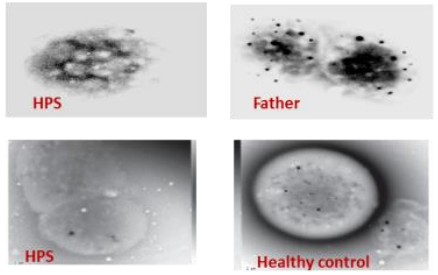


Flow cytometry



Electron Microscopy

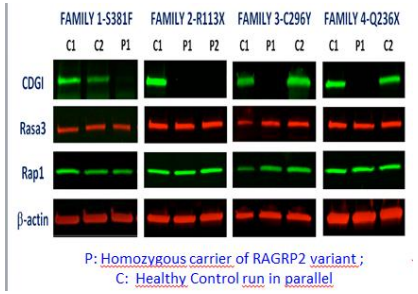




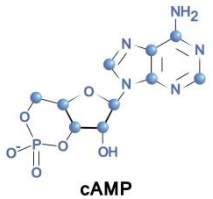
González-Conejero et al. Br J Haematol. 2003 123:132-8
Sánchez-Guiu I, et al. Hamostaseologie. 2014;34:301-9

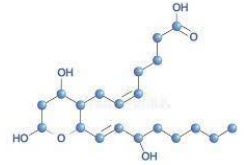
Limitations: Laborious; highly specialized personnel, expensive and sophisticated equipment.
Methods: Total mount, fixation-fine section EM (scanning electron microscopy).
Uses: Granule deficiencies (GPS, HPS, CHS, GATA1, etc),

Biochemical methods



P: Homozygous carrier of RAGRP2 variant ;
C: Healthy Control run in parallel





Immunoblotting; cAMP level, TxB2 level (serum, LTA supernatant, Whole blood), β-TG, etc.

Light transmission aggregometry (LTA) in PRP

GVR BORN- JR O'BRIEN

AGGREGATION OF BLOOD PLATELETS BY ADENOSINE DIPHOSPHATE AND ITS REVERSAL

By Prof. G. V. R. BORN

Department of Pharmacology, Royal College of Surgeons of England, London

IN 1956 it was shown that blood platelets contain adenosine triphosphate (ATP) in extraordinarily high concentrations[1] and that most of this ATP breaks down rapidly when platelets are suspended in plasma which is clotting[2,3] or whenever platelets undergo viscous metamorphosis[4]. The suggestion was made[5] that if this breakdown involved ATP situated on the surface of the platelets, the large net negative charge on platelets[6] might be diminished and their tendency towards adhering to each other enhanced.

Recently, it has been found that platelets can be made to aggregate in plasma and to adhere to other surfaces by adding adenosine diphosphate (ADP) (ref. 7). Twenty-two nucleotides were tested for this effect; it was brought about only by ADP and by deoxyadenosine diphosphate. Since it is likely that ADP is produced by platelets themselves whenever their ATP breaks down, the adhesion and aggregation of platelets that occur in the initial stages of thrombosis may well be initiated by ADP. This effect has therefore been investigated by a method, recently described[8], with which the rate at and extent to which platelets aggregate can be determined quantitatively.

About 80 ml. of human blood was mixed with just enough sodium citrate (3·8 mgm./ml. blood) or heparin (0·01 mgm./ml. blood) to prevent clotting. As cooling increases the tendency of platelets to aggregate, the experiments were done at room temperature of 20°–22° C. The blood was centrifuged at 500*g* for 20 min. The plasma, usually about 35 ml. and containing 10^8–10^9 platelets/ml., was transferred with a siliconed capillary pipette into a plastic measuring cylinder.

A sample of 3 ml. was pipetted into a Spinco centrifuge tube, made of transparent plastic, which was inserted into a Unicam *SP* 400 absorptiometer. Light at a wave-length of 600 mμ was passed through the tube. The dark current was set at infinity and the optical density of distilled water at zero. The optical density of platelet-rich plasma was proportional to the concentration of platelets in it, provided the optical density of platelet-free plasma was subtracted.

The platelet-rich plasma was stirred by a small iron rod covered in polythene which was rotated magnetically. Stirring was stopped when readings were taken. When plasma was stirred gently there was no significant change in the concentration of platelets or in the optical density; this showed that the platelets did not stick to the tube or to the stirrer.

When platelet-rich plasma was stirred vigorously the concentration of platelets usually decreased slightly and so did the optical density (Fig. 1). The decrease was mostly in the first half-hour; after that it was very slow or absent. Presumably a small proportion of the platelets was broken up by vigorous stirring.

All additions were made to the plasma while it was being stirred. When substances were added which caused the platelets to aggregate, the rate and extent of aggregation increased as the rate of stirring was increased up to about 1,000 r.p.m. In the experiments described here stirring was always at this rate.

BORN GV. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature. 1962 Jun 9;194:927-9.

J. clin. Path. (1962), **15**, 446

Platelet aggregation

Part I Some effects of the adenosine phosphates, thrombin, and cocaine upon platelet adhesiveness

J. R. O'BRIEN

From the Portsmouth and Isle of Wight Area Pathological Service

SYNOPSIS Platelets in native blood adhere spontaneously to glass independently of temperature: if adenosine diphosphate is added to the blood the adhesiveness of the platelets is increased and this effect is largely independent of temperature. The mono- and triphosphates decrease adhesiveness at 20°C. and 37°C. but have no effect at 0°C.; cocaine inhibits adhesion at 37°C. and at 0°C.

Aggregation and viscous metamorphosis of platelets in native plasma is induced at 37°C. by adenosine diphosphate or by thrombin; these reactions do not occur at 0°C. Cocaine and all the other anti-adhesive drugs inhibit thrombin or adenosine diphosphate-induced aggregation. The mono- and tri-phosphates appear to compete with adenosine diphosphate and inhibit aggregation; they also inhibit thrombin-induced aggregation. Aggregation induced by adenosine diphosphate or thrombin is not prevented by any of the usual enzyme inhibitors or uncoupling agents at the appropriate strength. At 37°C. aggregation and viscous metamorphosis induced by adenosine diphosphate or thrombin are reversible, and the addition of more adenosine diphosphate or of thrombin again produces aggregation and viscous metamorphosis.

Platelets incubated with adenosine diphosphate but not agitated lose their power to aggregate but when more adenosine diphosphate is added with agitation, then aggregation is again produced. These observations are presumably explained by the finding that intact platelets, but not fragmented platelets, can inactivate adenosine diphosphate. From these results it is tentatively concluded that adhesion may involve intrinsic adenosine diphosphate in the platelet which may be activated by thrombin and inhibited by the added mono- or triphosphate. The anti-adhesive drugs act in a different manner. These phenomena have a remarkable similarity to those concerning mitochondrial swelling.

It is not known why a platelet sticks, but Hellem (1960) reported that a factor R isolated from red cells caused platelets to stick to glass; Øllgaard (1961) also studied a non-protein extract from platelets and red cells that caused platelet aggregation. Gaarder, Jonsen, Laland, Hellem, and Owren (1961) showed that their factor is adenosine diphosphate, that it is highly specific, and that it induces platelet aggregation in citrated platelet-rich plasma. O'Brien (1961) showed that the adhesion of native platelets to glass and to damaged cells *in vitro* and *in vivo* was inhibited by many anti-malarial, anti-histaminic and local anaesthetic and some other drugs which will be called the 'anti-adhesive' drugs. These findings stimulated the present study of the effects of adenosine diphosphate and thrombin on platelet adhesiveness to glass and on platelet aggregation and viscous metamorphosis. The effect of the anti-adhesive drugs and adenosine monophosphate and the triphosphate and enzyme inhibitors were also studied in an attempt to understand the processes involved in adhesion, aggregation, and viscous metamorphosis.

Received for publication 5 February 1962.

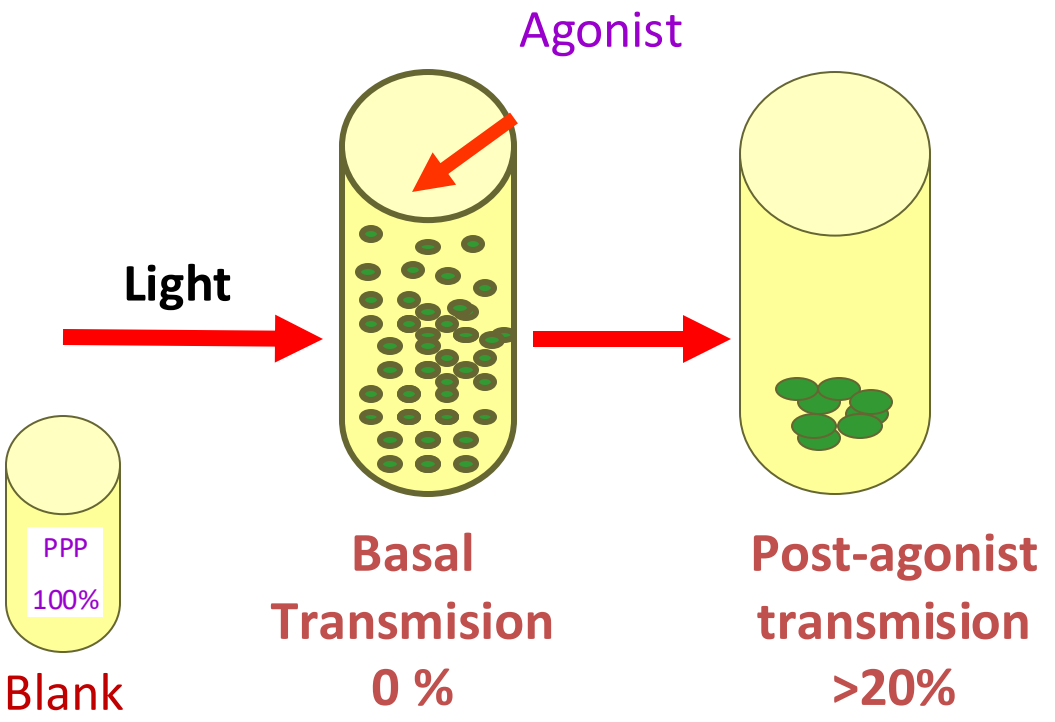
METHODS

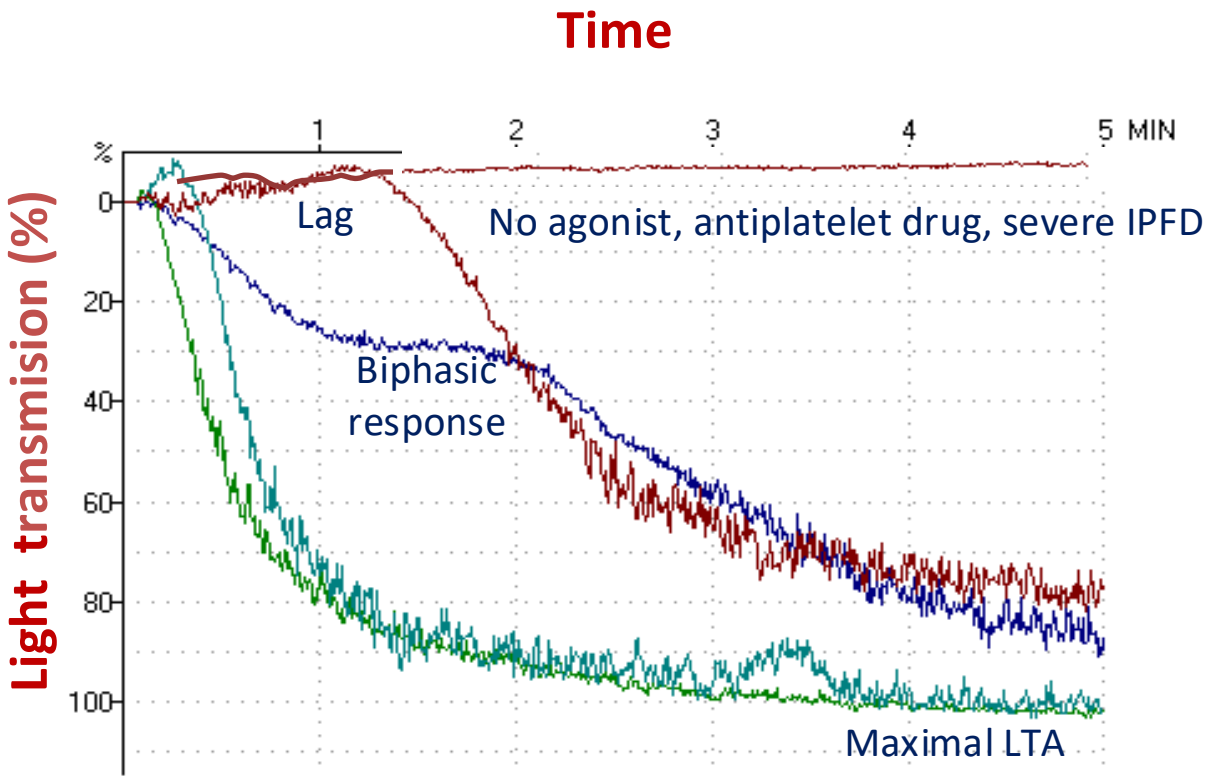
ADHESION OF PLATELETS TO GLASS Native blood collected in a plastic tube was used at once or after cooling for five minutes in ice-water; the blood was mixed with barbitone buffered saline as a control or with the material under study and was passed immediately through 'filter units' consisting of a standard quantity of glass beads in a

O'brien JR. Platelet aggregation: Part I Some effects of the adenosine phosphates, thrombin, and cocaine upon platelet adhesiveness. J Clin Pathol. 1962 Sep;15(5):446-52.

Light transmission aggregometry (LTA) in PRP

Gold standard of PFT





Agonists: ADP, epinephrine, collagen, ristocetin, arachidonic acid, Trap, U46619, etc.

Parameters: %Shape change, Lag, slope, % maximal aggregation, % final aggregation, area under curve.

(Born GV Nature 1962; 194:927-9; O'Brien JR. J Clin Pathol 1961; 14:140-9)

Advantages and limitations of LTA

Advantages:

- Flexible: Multiple agonists and doses.
- Information on different aspects of platelet function (receptors, secretion, activation)

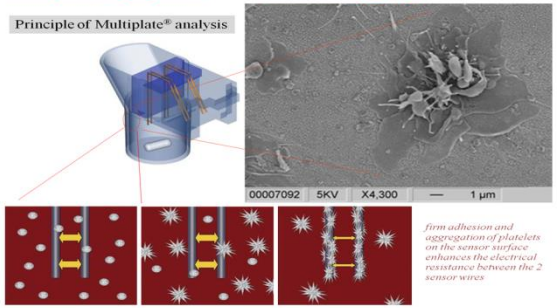
Limitations:

- Response test under non-physiological conditions
- Laborious, time consuming, qualified personnel required
- High sample volume (limitation in pediatrics)
- Not applicable in cases of thrombocytopenia or in the presence of interfering medications or foods
- Influenced by many pre- and analytical variables (anticoagulants, extraction, PRP making, etc.)
- Poorly standardised

Moffat et al, T&H 2005,Cattaneo M et al JTJ, 2013

Alternative methods to measure platelet aggregation

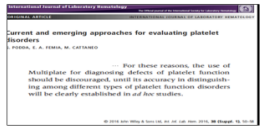
Multiplate: agregación semi-automátic aggregation in WB en ST



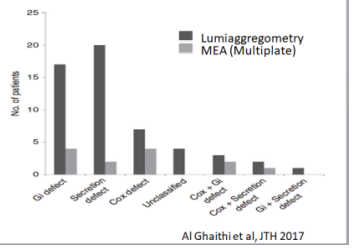
Diagnostic performance for PFD in 109 children with bleeding history: LTA *vs* Multiplate

Test	N. of patients with abnormal results
LTA	15
Multiplate	3

Haas et al, Platelets 2018

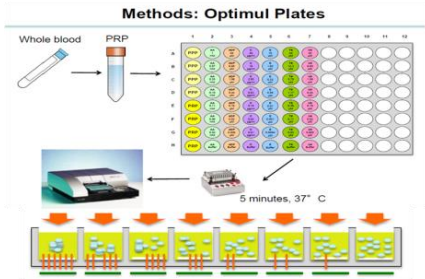


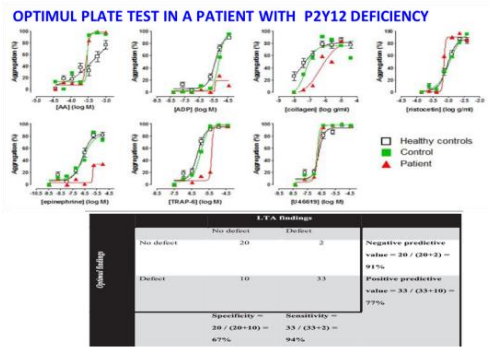
Comparison of the diagnostic efficacy for PFD of MEA (Multiplate) and Lumiaggregometry



Al Ghaithi et al, JTH 2017

Aggregation in 96-well plates





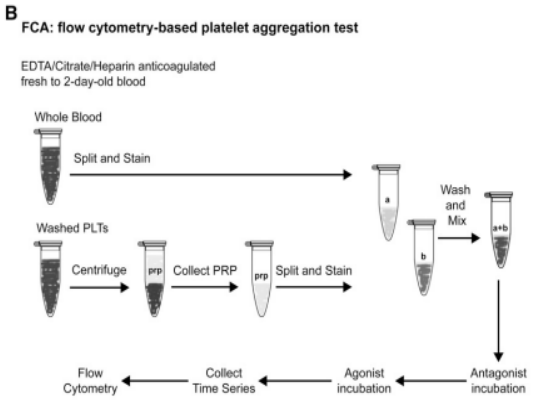
- 96-well plates with freeze-dried agonists at different doses.
- Only requires adding PPP or PRP; 40 µL/well.
- Orbital shaking (BioShale IQ) 1200 rpm, 5 min, 37°C.
- Abs reading at 595 nm in a standard ELISA reader
- The % LTA is calculated as a function of ABS of PRP and PPR
- Multiple LTA in minutes
- Less PRP volume than in LTA
- Possibility of collecting supernatants and measuring TXA2, ATP, etc.

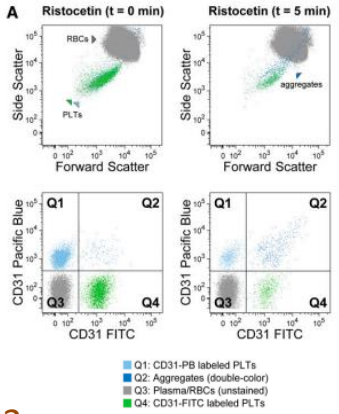
Lordkipanidzé et al. Blood 2014; 123:e11-22

- ✓ **Optimul plates not commercially available.**
- ✓ **LTA and aggregometry in 96-well plates are not completely interchangeable; the response to some agonists or the effect of drugs varies.**

Chan & Warner. Platelet 2012; 23:404-8; Lordkipanidzé et al. Blood. 2014; Chan et al. Methods Mol Biol. 2023

Flow cytometry–based platelet aggregation assay





De Cuyper et al. Blood 2013

Automated LTA in New coagulation Analyzers

Siemens Healthineers	COAG 360[b]
Sysmex	CS-2500 CS-5100 CN-3000 CN-6000
Behnk Elektronik	Thrombomate XRA

Lechhi A et al. Blood Transfus. 2024; 22:350-9

2025 ISTH-MEETING. **Automate study –UK**

OC 42.2 - Evaluation of national guidelines for light transmission aggregometry in adults. Automate study. Mr. Sean Platton

Sysmex CS series devices.

1021 patients; IPD: Sensitivity 87%; Specificity 94%

Standardization of LTA

J Thromb Haemost 2013; **11**: 1183–9. DOI: 10.1111/jth.12231

OFFICIAL COMMUNICATION OF THE SSC

Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH

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Update of the recommendations for the standardization of light transmission aggregometry of the ISTH SSC on platelet physiology

Objective

- Update the ISTH SSC recommendations with regard to LTA pre-analytical, analytical and post-analytical issues in the light of the studies published within the last ten years as well as based on international expert consensus

Members

- PI: Georges Jourdi; Co-PI: Emma Josefsson, Eleonora Petito
- Interested experts: Paul Armstrong, Marina Camera, Scott Cameron, Rutvi Dave, Pierre Fontana, François Mullier, Fabio Pulcinelli, José Rivera, Carlo Zaninetti, Manal Ibrahim, Meenakshi Banerjee, Charlotte Gran

Status

- Approved project starting on June 16, 2025: 1st meeting PI/Co-PI roadmap for the project
- 2nd meeting 1st of July 2025 with all interested experts to start the literature review: Nov. 2009-July 2025
  - Three groups: pre-analytical, analytical, post-analytical & LTA indications

Scientific and Standardization on Platelets in Health and Disease

For updates, contact Georges Jourdi

Second manuscript

Consensus on Aggregometry for platelet function testing in thrombocytopenic patients: communication from the SSC of the ISTH

Ruchika SHARMA[1*], Georges JOURDI[2,3*], Ishac NAZY[4], Tamam BAKCHOUL[5], Marie LORDKIPANIDZÉ[6,7], Sofia RAMSTRÖM[8*], and the Aggregometry-PFT in TP study group

Link to the manuscript for review

Public Comment on Aggregometry for Platelet Function Testing

https://survey.alchemer.com/s3/8318565/Public-Comment-on-Aggregometry-for-Platelet-Function-Testing

Major specific considerations relative to platelet function testing using aggregometry in thrombocytopenic patients

Light transmission aggregometry	1	The expert panel consensus is that results for LTA should be interpreted with caution when platelet counts get lower than 75 X 10^9/L in whole blood.
	2	In case of macrothrombocytopenia in patients undergoing light transmission aggregometry, if difficulties are encountered to get enough platelets by centrifugation, sedimentation by gravity should be considered instead.
	3	In case of severe thrombocytopenia (≤ 80 X 10^9/L in PRP), high concentration of collagen and ristocetin agonists are considered most informative for assessing platelet function using light transmission aggregometry.
	4	When considering testing other agonists in mild to moderate thrombocytopenia (140-249 X 10^9/L in PRP), laboratories are advised to use internal validation data, or at least published literature to reliably interpret the results.
	5	The use of a platelet count adjusted control may be considered for LTA in patients with thrombocytopenia. The use of a non-adjusted normal sample is considered good practice as a control for instruments and reagents.
Whole blood impedence aggregometry	6	In the case of whole blood impedance based aggregometry in patients with low platelet counts, the use of 2 concentrations of ADP (low and high) should be considered to account for the endogenous ADP release from platelets and red cells.
	7	For whole blood impedance based aggregometry, there is very little data to reliably interpret results for testing in undiluted samples with a platelet count below 50 X 10^9/L.
	8	There is insufficient evidence to suggest adjusting the platelet counts in the control samples when using whole blood impedance aggregometry.

Recommendations for the standardization of LTA: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH

70 statements grouped into:

1. Clinical usefulness of LTA
2. Pre-analytical variables
3. Blood collection
4. Preparation of PRP and platelet-poor plasma (PPP)
5. Assessment of PRP quality
6. Methodology
7. Choice of agonists
8. Evaluation and reporting of results

Cattaneo M et al. J Thromb Haemost 2013;11: 1183–9

LTA- SSC-ISTH recommendations. Performance

- **Drug use control (no use for 10 days);** rest period (30 min); no smoking (30 min); no caffeine (2h); not fasting
- Blood extraction: 21g needle; minimal stasis; First ml for blood count; plastic or siliconised glass tubes, 3.2% buffered citrate (0.102 M), 15 minutes rest
- PRP preparation: 150 x g 10 min, RT, no brake [isth: 200-250 x g]. Rest 15 min Not suitable for giant platelets (SBS) (sedimentation)
- **Platelet count not adjusted** in the PRP (normal range ... 150-600.000 pl/uL).
- **Samples from a normal subject run in parallel.**
- Test execution: 37°C, 1000 rpm, 5 minutes recording (10 min for some agonsits) , 250 µL final volume.
- **Completed (if possible) <4 hours from extraction.**
- Agonists: < 10% volume; concentration from-
  - ADP-2uM ; Epinephrin-5uM; Collagen-2ug/ml; PAR1-10uM; TxA2.. 10 uM; AA- 1mM; U46616.1um; Rsitocetin-1,2mg/ml (0,5-2mg/mL)
- Evaluation: Shape chage, Lag phase, Slope, Maximal %; Final % ; Secondary wave; Deaggregation

Cattaneo M et al. J Thromb Haemost 2013

Clinical Usefulness of LTA: ISTH recommendations

- **LTA is clinically useful for the study of subjects with bleeding disorders**

- LTA should not be used for the identification of subjects at risk of thrombosis, except in research (the experts agreed that this is an area that still needs further studies and standardization)

- LTA should not be used for monitoring subjects on antiplatelet therapy, except in research

Cattaneo M et al. J Thromb Haemost 2013;11: 1183–9

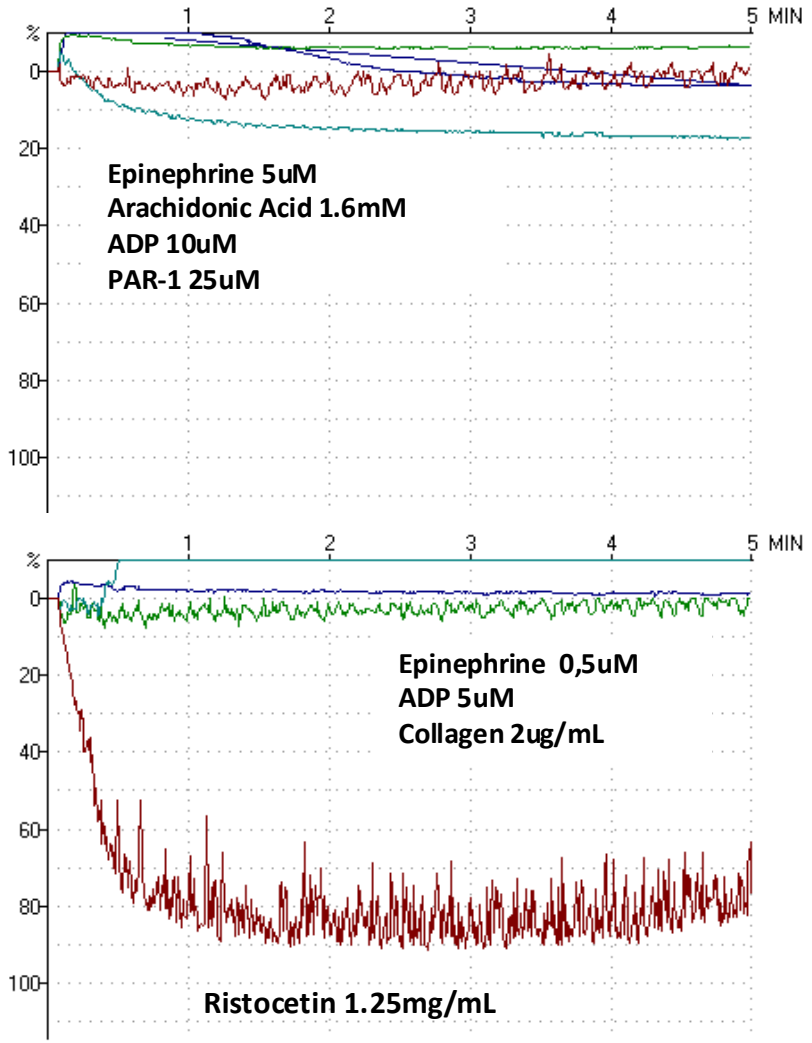
How useful is LTA for the diagnosis of IPFDs ?

It is very useful.. (If used properly)

LTA, alone, can be diagnostic for a few severe IPFDs

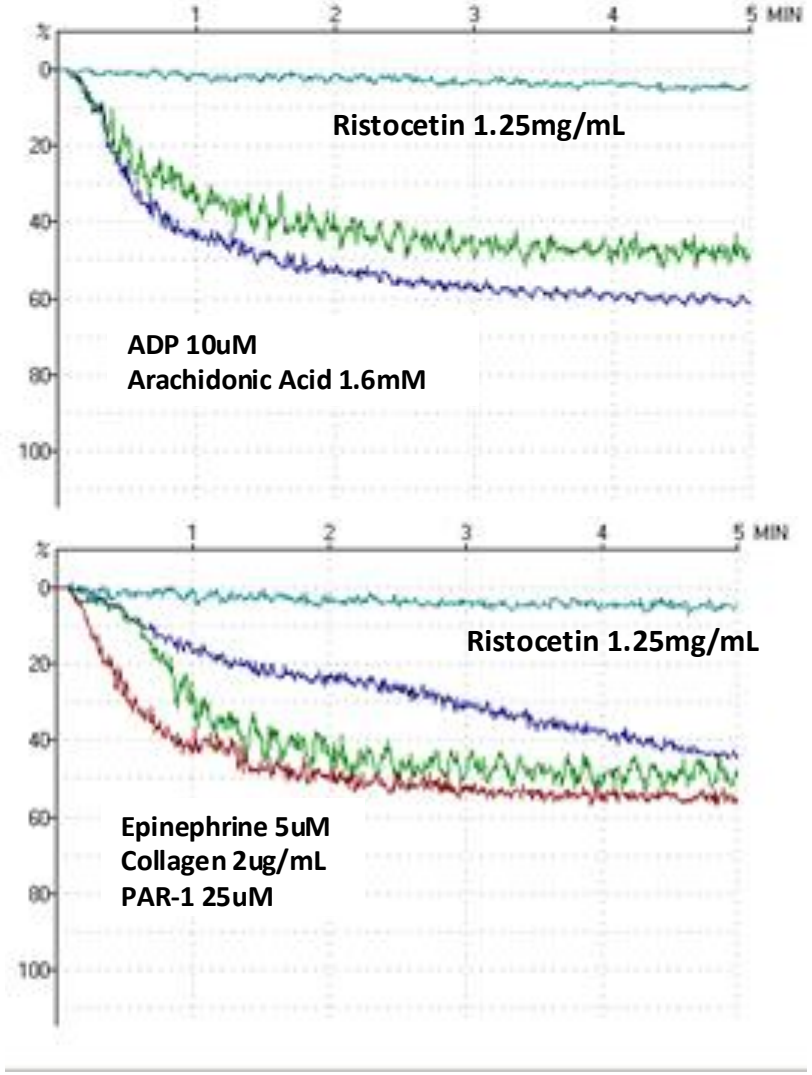
LTA (%)

Glanzmann Thrombastenia



Normal Platelet count

Bernard Soulier Syndrome



Severe macrothrombocytopenia ; Control with adjusted platelet count

LTA utility in diagnosis of mild/intermediate IPFDs is far less clear?

Hayward CPM et al. J Thromb Haemost. 2009; 7:676-84

Dawood BB et al. Blood. 2012;120: 5041-49

Alessi MC et al. J Clin Med. 2020;9:763

Bourguignon A et al. Crit Rev Clin Lab Sci. 2022;59405-44

Sánchez-Fuentes et al. Biomolecules . 2025; 15:846

Could We Use General Guidelines to Interpret LTA in moderate IPFDs?

Cues for interpreting LTA in moderate IPFDs

Evaluation of participants with suspected heritable platelet function disorders including recommendation and validation of a streamlined agonist panel

Ban B. Dawood,[1] Gillian C. Lowe,[1] Marie Lordkipanidzé,[1] Danai Bem,[1] Martina E. Daly,[2] Mike Makris,[2] Andrew Mumford,[3] Jonathan T. Wilde,[4] and Steve P. Watson[1]

Lumi-aggregometry (5 years studies)

- 111 unrelated cases with suspected PFDs
  (IT, BSS, GT or HPS excluded)
- 70 healthy volunteers.

Extended panel o f 9 agonists

ADP (3, 10, 30, and 100M)
Adrenaline (10, 30, and 100M)
Collagen (0.3, 1, and 3 g/mL)
CRP (1, 3, and 10 g/mL)
PAR-1 (10, 30, and 100M)
PAR-4 (100, 250, and 500M) peptides
Arachidonic acid (0.5, 1, and 1.5mM)
U46619 (1 and 3mM)
Ristocetin (1, 1.25, 1.5, and 2 mg/mL)

Table 1. Classification of participants with mild platelet-based bleeding defects

Type of platelet defect	No. of participants	% of participants
Membrane Gi signaling	21	32.8%
TxA_2 pathway	14	21.9%
GPVI	4	6.2%
Gq	1	1.6%
Dense granule	19	29.7%
Complex	5	7.8%
Total	64 **(58%)**	100%

Dawood BB et al. Blood. 2012;120(25): 5041-5049

Cues for LTA intrepretation in moderate IPFDs

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Gi-like defect. (32.8% of IPFDs)

Aggregation and secretion defect to the ADP and adrenaline.

Key diagnostic LTA features:

- Transient aggregation to ADP (10uM),
- Reduced or absent primary wave with no secondary aggregation wave to adrenaline
- Reduced aggregation and secretion to low concentrations of other platelet agonists, most notably collagen
- Robust response to 1mM arachidonic acid → distinguish from a defect in the TxA2 pathway

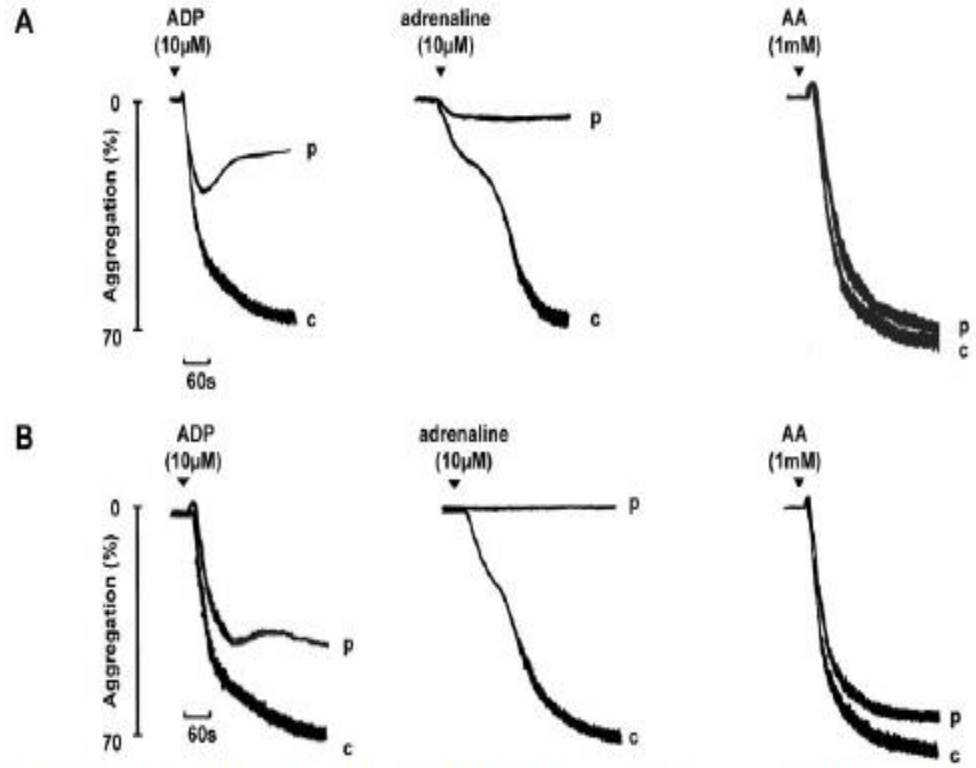

Figure 1. Aggregation to ADP and adrenaline in 2 participants diagnosed with a GI-like defect. Aggregation in 2 participants (p) diagnosed with a GI-like defect is shown. The participant in panel A shows a partial primary wave response to adrenaline, whereas for a second participant shown in panel B, the primary wave is absent. "c" indicates the control (healthy volunteer). Note that the biphasic aggregation to ADP shown in panel B would eventually decline. The patterns of aggregation are representative of other participants diagnosed with a GI-like defect.

Potential test of diagnosis confirmation: VAPS test (PGE1 + ADP/EPI)

Dawood BB et al. Blood. 2012

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Dense granule–secretion defect.

(30% of IPFDs)

Key diagnostic LTA features:

- Reduced aggregation and secretion to low concentrations of most agonists, most notably low dose of collagen (1-3ug/ml)
- Robust response to ADP 10uM →consistent minimal role of secretion in ADP-induced aggregation

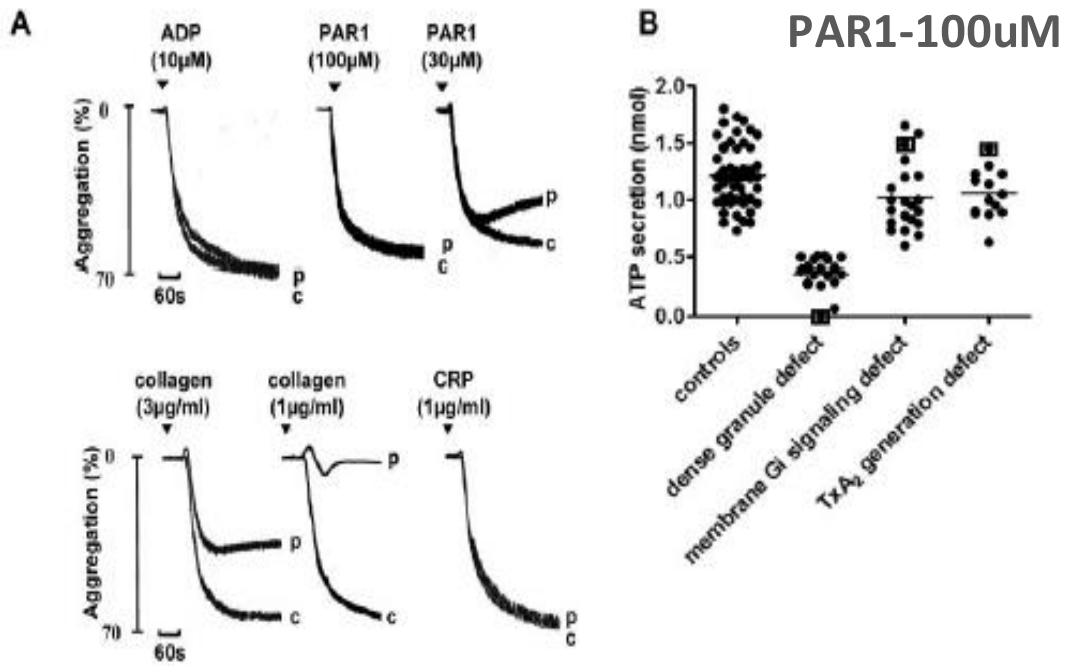

Figure 3. Aggregation and ATP secretion in a participant diagnosed with a dense granule defect. (A) Aggregation in a participant diagnosed with a defect in dense granule secretion on the basis of a significantly reduced level of secretion to high concentrations of PAR-1–specific peptide and other platelet agonists, including PAR-4–specific peptide and CRP relative to a panel of controls. "c" indicates control (healthy volunteer). The pattern of aggregation is representative of other participants diagnosed with a secretion disorder. (B) ATP secretion was measured alongside aggregation in a Born lumi-aggregometer in PRP using Chrono-Lume reagent for the detection of ATP. The degree of ATP secretion (after normalization to platelet count, supplemental Figure 2) to PAR-1–specific peptide (100μM) in healthy volunteers and participants diagnosed with defective dense granule secretion is shown. Participants identified with mutations in the $P2Y_{12}$ (present study), TxA_2 receptors,[10] and HPS-9[19] are identified by square brackets.

Diagnosis confirmation: granule/secretion assays (ATP, mepacrine, CD63, whole-mount EM)

Dawood BB et al. Blood. 2012

Cues for LTA interpretation in moderate IPFDs

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TxA_2 pathway defect (21,9 % of IPFDs)

Key diagnostic LTA features:

TxA2 synthesis defect

- Marked and selective defect in aggregation and
- secretion to arachidonic acid (1mM)
- Reduced or absent primary wave with no secondary aggregation wave to adrenaline.
- Reduced aggregation and secretion to low concentrations of most other agonist s, recovered at high dose, except for ADP and adrenalins which remains reduced/no second wave.
- Normal aggregation response to U46619

TxA2 receptor defect

- Reduced aggregation with both AA and U46619

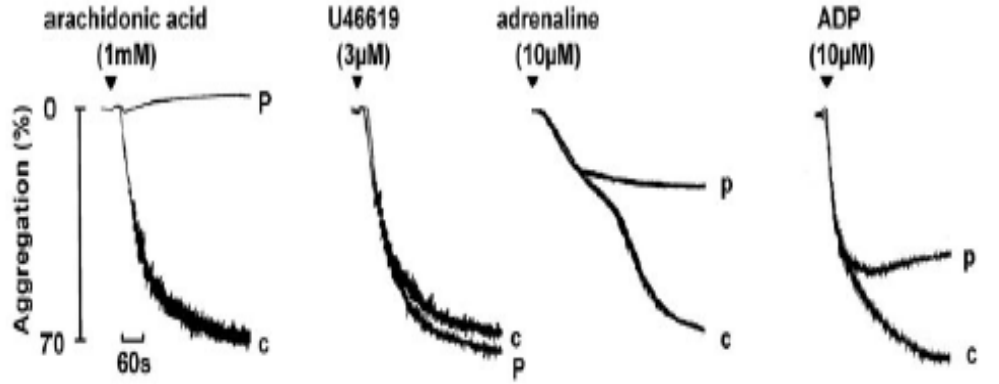

Figure 2. Aggregation in a participant diagnosed with a TxA_2 pathway defect. Aggregation in a participant (p) diagnosed with a TxA_2 pathway defect. The TxA_2 pathway defect also results in the abolition of response to arachidonic acid (1mM) and impairment in response to other agonists, including ADP and adrenaline, but not to U46619, indicating a defect in arachidonic acid metabolism. "c" indicates control (healthy volunteer). The pattern of aggregation is representative of other participants diagnosed with a defect in arachidonic metabolism.

Potential genetic defects in COX-1, TXAS1, PLCA2

Dawood BB et al. Blood. 2012;120(25): 5041-5049

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P2Y12 receptor defect

Key diagnostic LTA features:

- Transient aggregation (+absence of secretion) with high concentration of ADP (100M)
- Minor defect in adrenaline aggregation
- Reduced aggregation to low concentrations of other platelet agonists, most notably collagen
- No effect of P2Y12 receptor antagonist (AR-C67085)

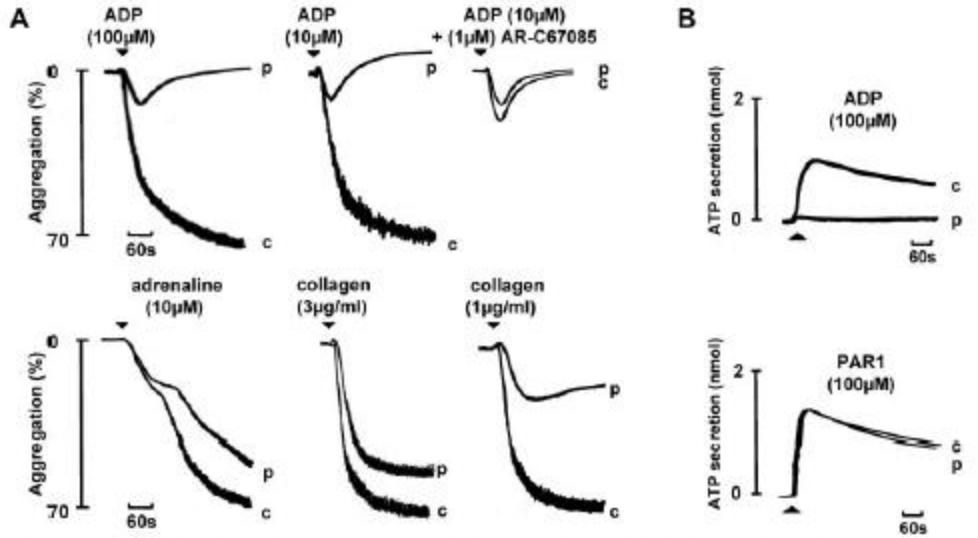

Figure 4. Aggregation and secretion in a participant with a homozygous P2Y$_{12}$ mutation that prevents receptor expression. Aggregation and secretion in a participant (p) with a homozygous mutation in P2Y$_{12}$ that introduces a frame-shift mutation early in the coding sequence (c.36delG, p.Gly12fs). Responses are shown alongside a control (c). The PRP platelet count in the control and participant were 4.1 × 10^8/mL and 3.9 × 10^8/mL, respectively.

Potentially useful functional assays

- VASP test, PGE1-induced cAMP synthesis.→ lack of ADP effect
- P2Y12 receptor level (Immunobloting/ FC)

Dawood BB et al. Blood. 2012;120(25): 5041-5049

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GPVI defect. LTA features:

- Selective reduction in GPVI mediated aggregation
- Reduced with low dose of collagen
- Normal LTA with other agonists (ADP, AA, PAR1)

- Reduced LTA also with Rhodocityn may discriminate GPVI receptor expression defect from GPVI signaling defect

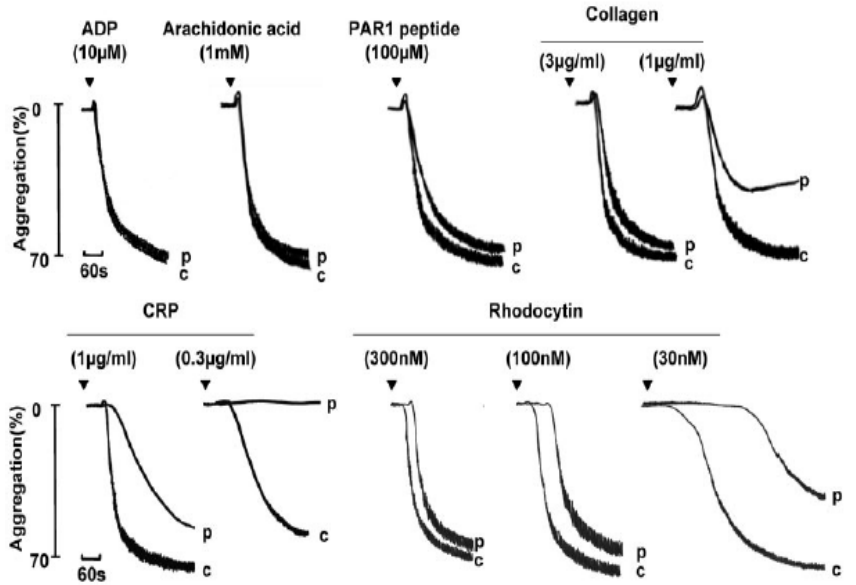
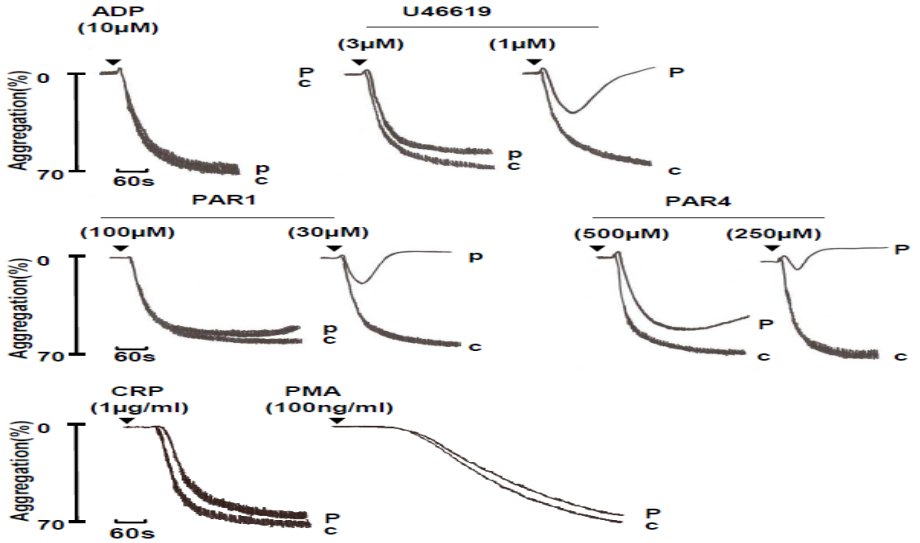


Figure 5. Aggregation and secretion in a participant with a GPVI-like defect. Aggregation in a participant (p) diagnosed with a GPVI-like defect on the basis of a reduced response to CRP and to rhodocytin. A similar pattern of aggregation was observed in other participants diagnosed with a GPVI-like defect. "c" indicates control.

Gq-like defect. LTA features:

- Reduced with low-intermediate dose of PAR1, PAR4, U46619
- Normal LTA with ADP, CRP or PMA



Dawood BB et al. Blood. 2012

LTA assay at CRH (1992-2025)

We, essentially, follow the general recommendations for LTA established by ISTH (currently under review)

Table 3. Panel of first-tier agonists recommended for the study of in vitro platelet aggregation in patients with a suspected IPFD [52].

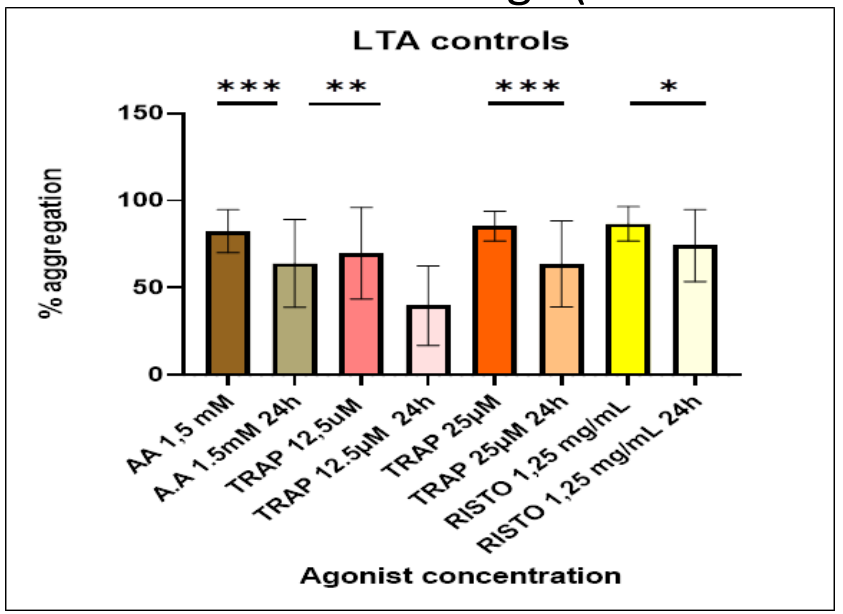
Agonist	Concentration	
	PRP Fresh Blood	PRP Blood 12–24 h *
Arachidonic Ac.	1.5 mM	1.5 mM
ADP	2.5 µM	5 µM
If altered at low dose	10 µM	10 µM
Epinephrine	5 µM	-
If altered at low dose	10 µM	
TRAP (PAR-1)	12.5 µM	25 µM
If altered at low dose	25 µM	
Ristocetin	1.25 mg/mL	1.25 mg/mL
If platelet type VWD is suspected	0.5–0.8 mg/mL	0.8 mg/mL
Collagen	2 µg/mL	5 µg/mL
If altered at low dose	5 µg/mL	10 µg/mL
CRP (If altered with collagen)	2 µg/mL	5 µg/mL
If altered at low dose	5 µg/mL	10 µg/mL
U46616 (If altered with arachidonic acid)	2 µM	5 µM
If altered at low dose	5 µM	10 µM

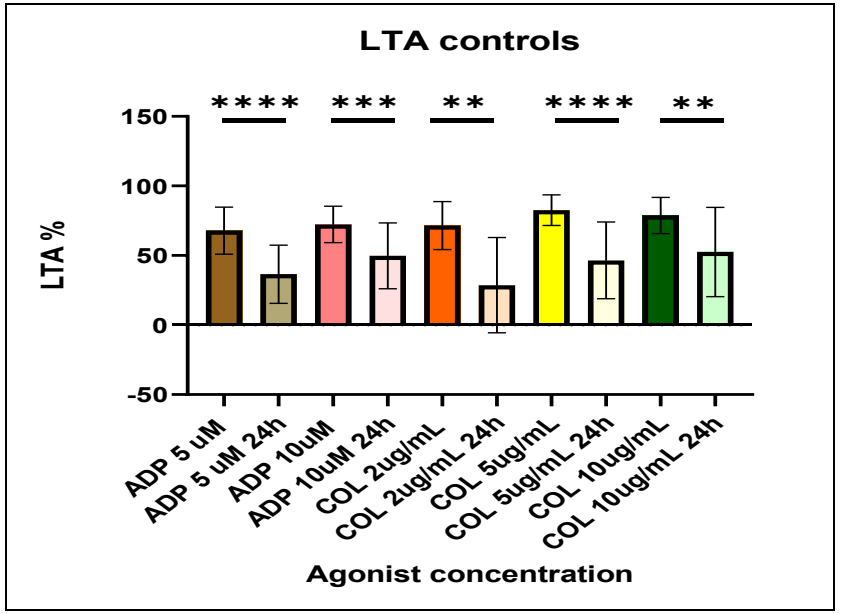
* Only assess if unable to obtain fresh whole-blood sample. PRP: platelet-rich plasma.

- Depending on the results of LTA obtained, consideration should be given to testing other doses and/or other agonists (PAR-4, A23187, PMA, convulxin, PAF, or others) in the same study, or in a subsequent study according to the diagnostic suspicion.
- If a patient's LTA study is abnormal, it should be repeated after at least one month to confirm the persistence of the abnormality.

Sánchez Fuentes et al. Biomolecules 2025

Normal range (n=25-





Using & interpreting LTA: Case examples from the GEAPC project

IPFD

IPFD& IT

Gi

GPVI

RUNX1

HPS

RASGRP2

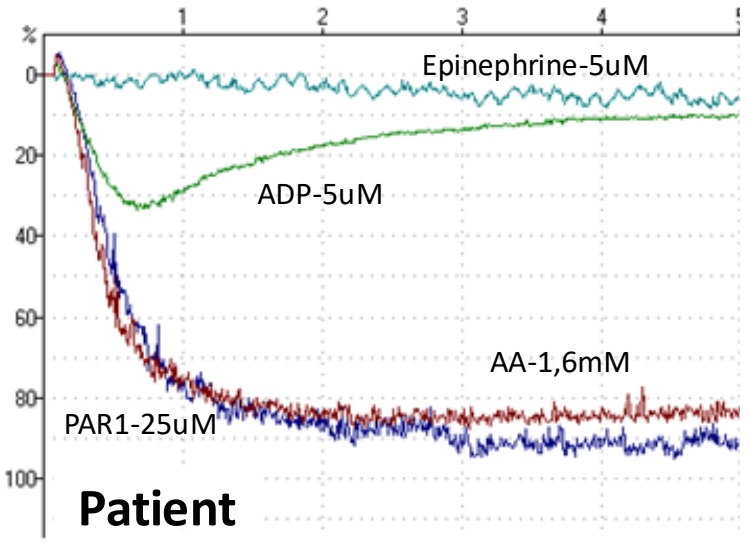
SRC

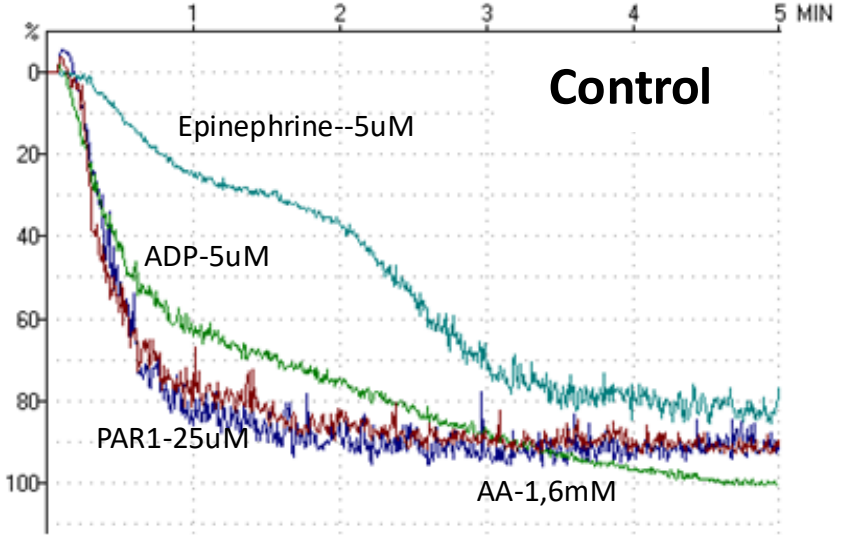
P2Y12

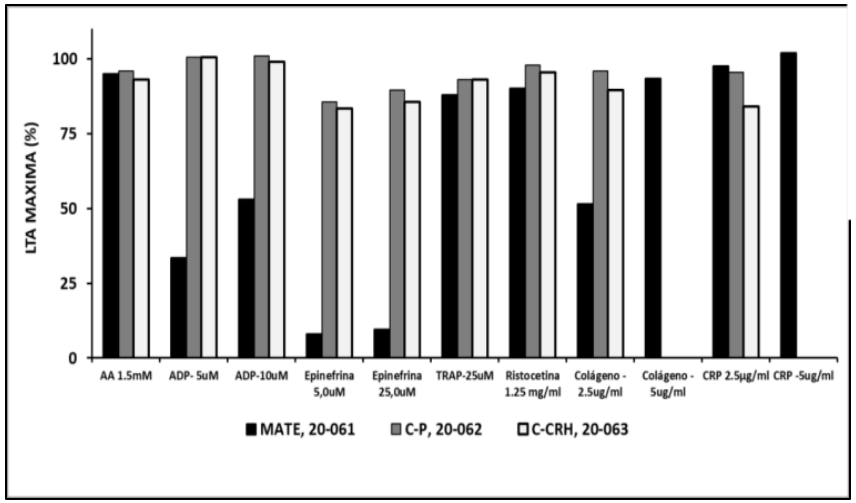
PTGS1

LTA findings in moderate IPFDs: Gi signalling defect

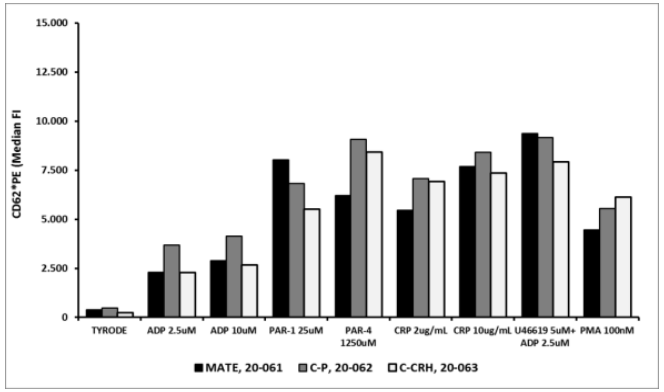
Woman 62yr
302 x 10^9pl/L, MPV: 11,1 fL
ISTH-BAT: 19
Four daughters; 1 with bleeding

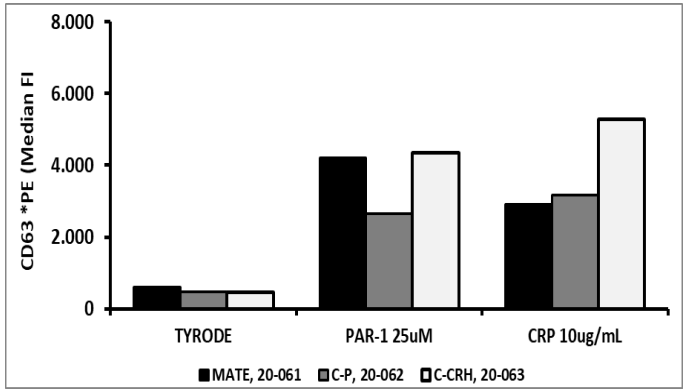






Key diagnostic LTA features:
-Transient aggregation to ADP (10uM),
- Absent primary wave with adrenaline,
- Reduced aggregation with collagen,
- Robust response to 1mM arachidonic acid



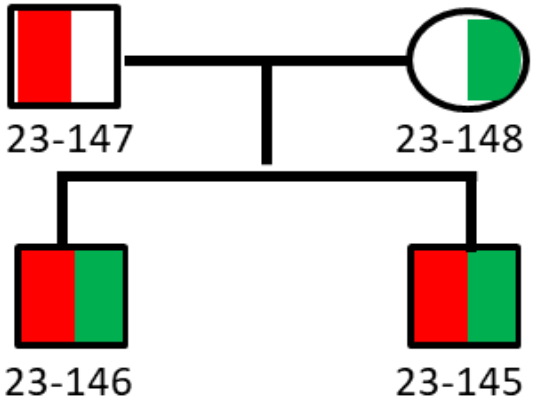


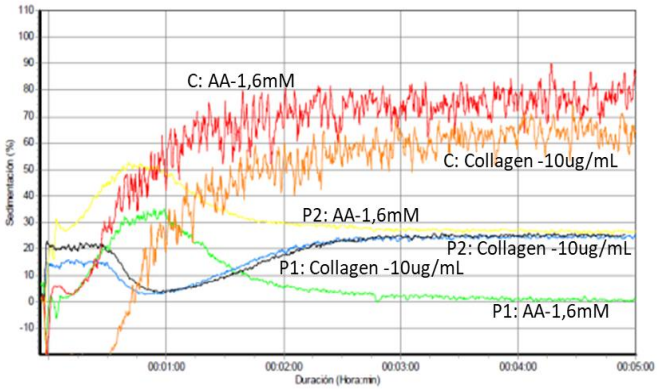
HTS Gene panel: Negative-→ WES

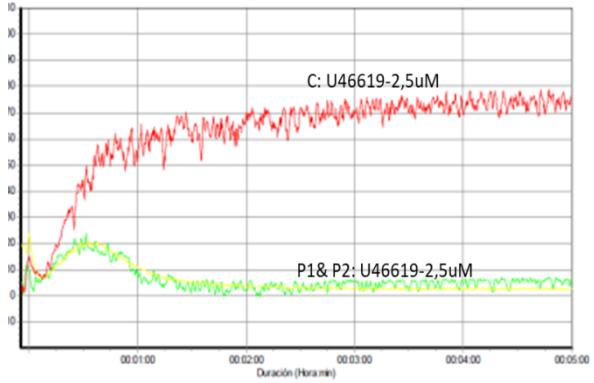
Normal α and δ granule secretion

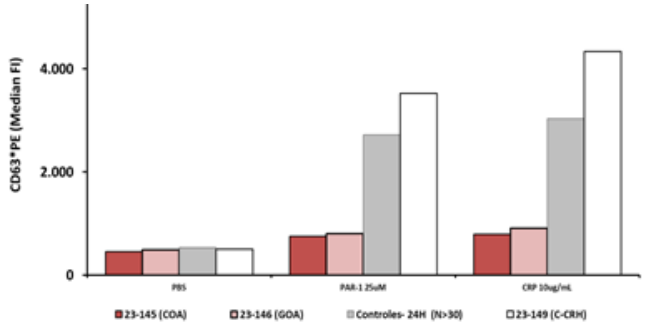
LTA findings in moderate IPFDs: HPS

Oculocutaneous albinism and mild bleeding

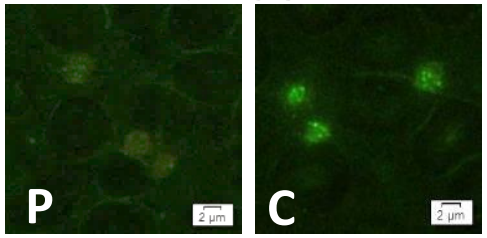








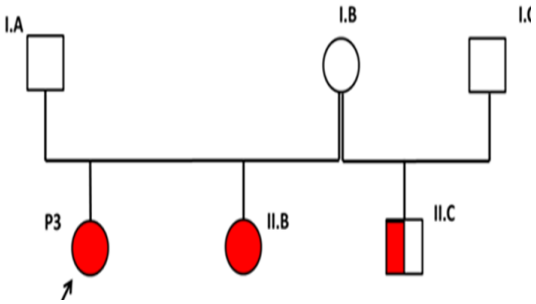
CD63

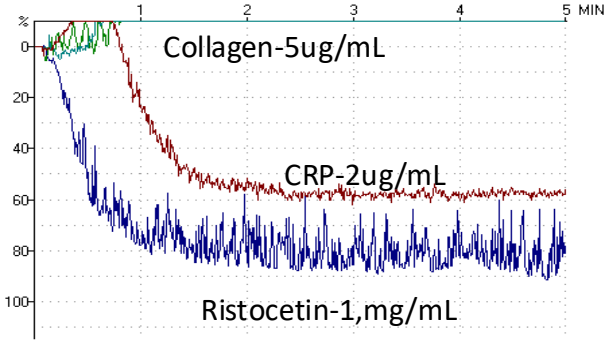


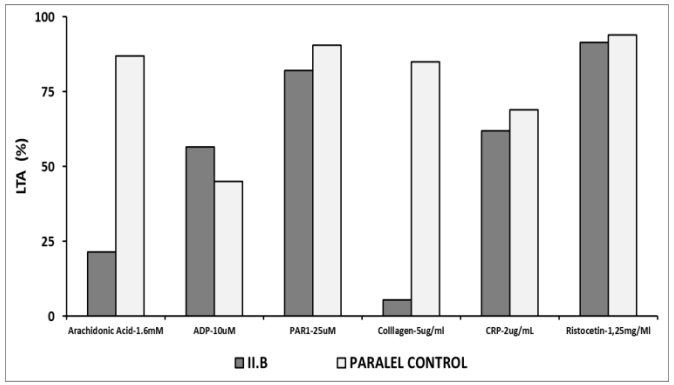
HTS Gene panel:

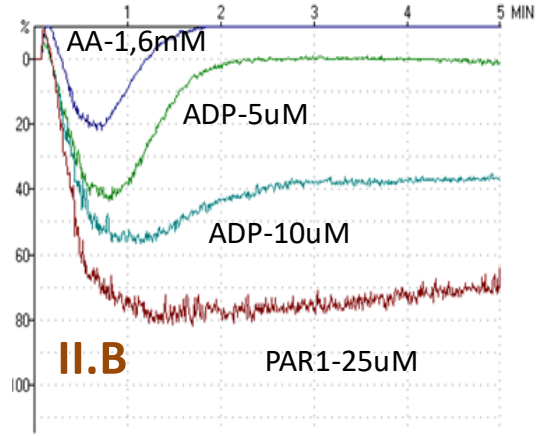
HPS5
c.191G>A
p.Trp64Ter

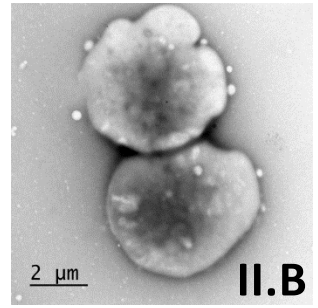
HPS5
c.1633_1634insT
p.Ser545MetfsTer4

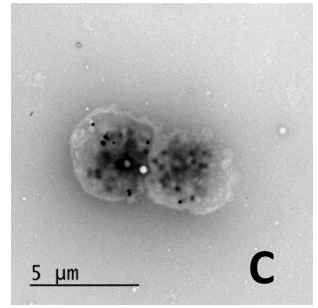








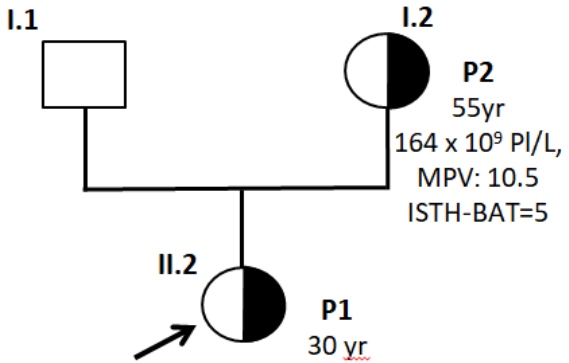




HTS gene panel Negative
Nanopore Sequencing positive

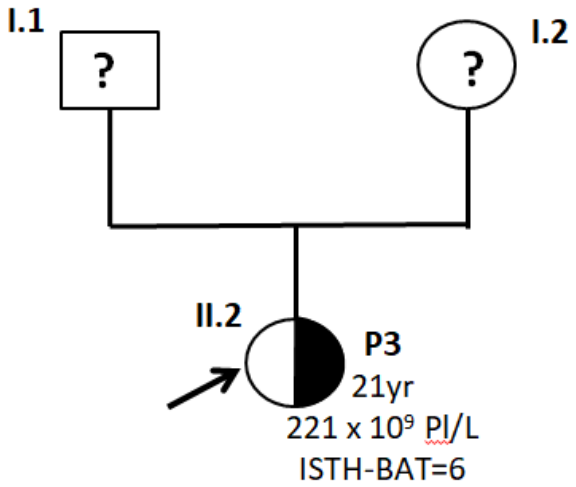
10kb deletion in *HPS5*

LTA findings in moderate IPFDs: P2Y12 defect

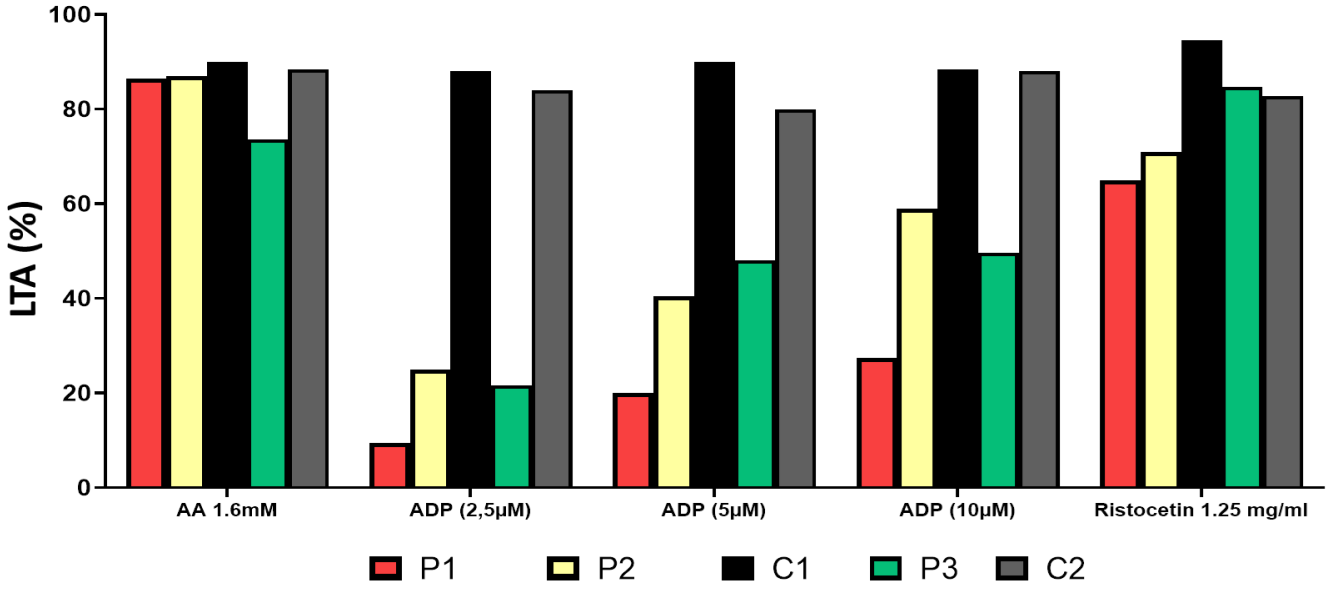


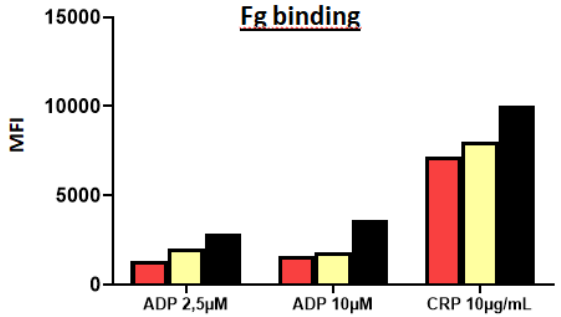
HTS Gene panel

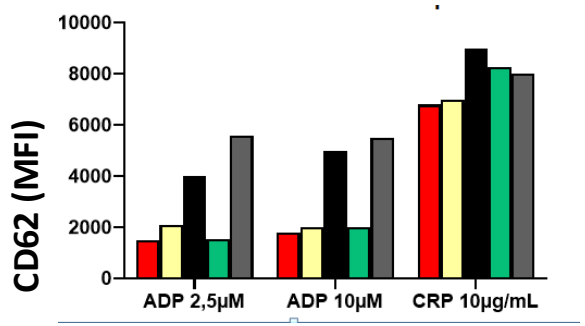
***P2Y12*: c.44delG (p.Ser15Ilefs*33)**

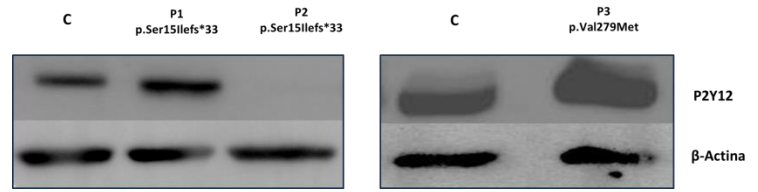


***P2Y12* c.835 G>A p.Val279Met**









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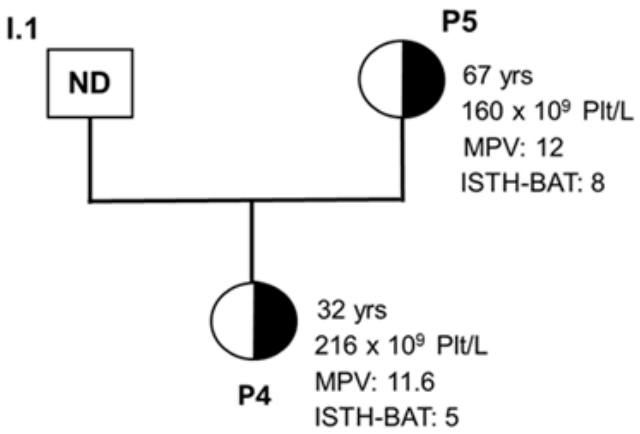
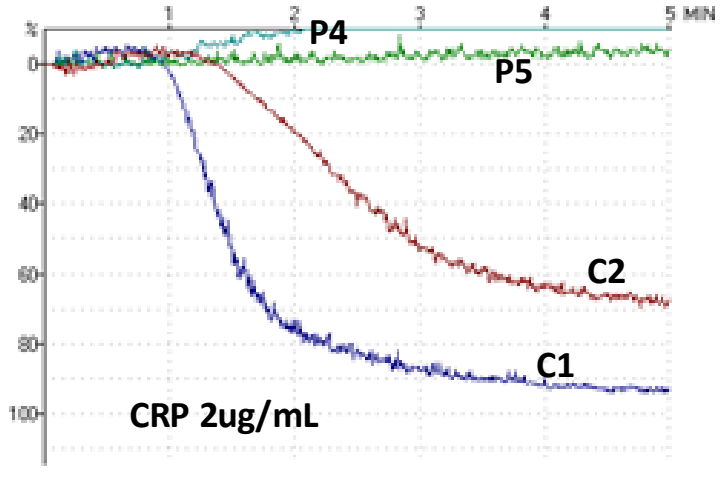
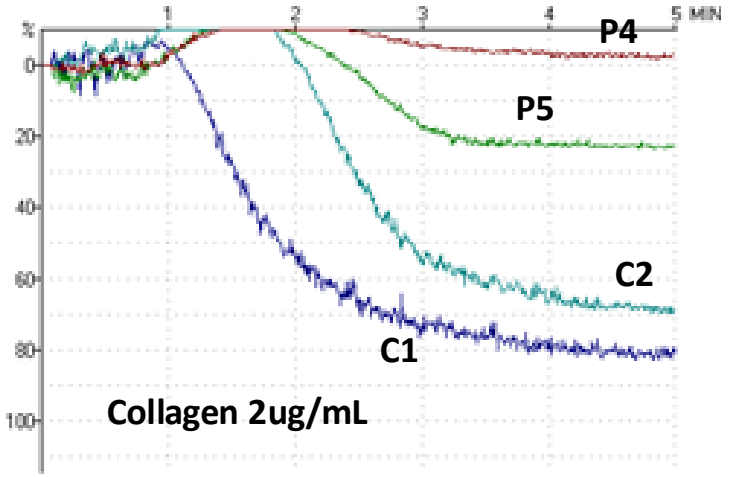
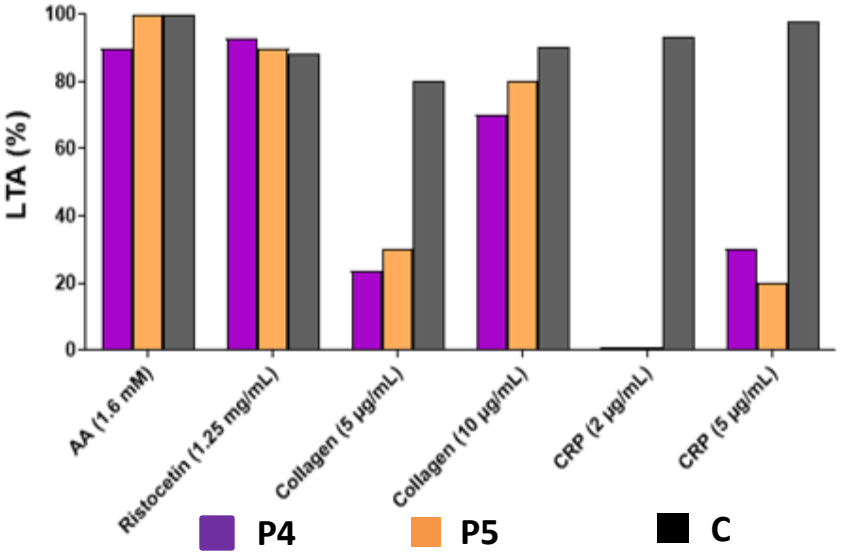
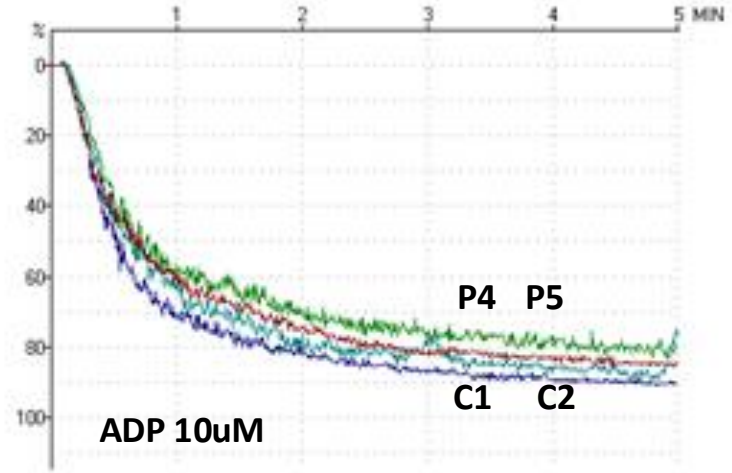
LTA findings in moderate IPFDs: GPVI defect

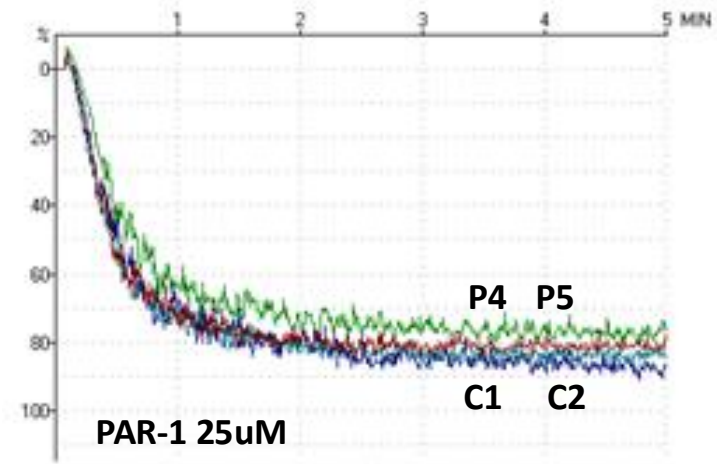












P4 *GP6* p.Asn236Lysfs*42

P5 *GP6* p.Asn236Lysfs*42

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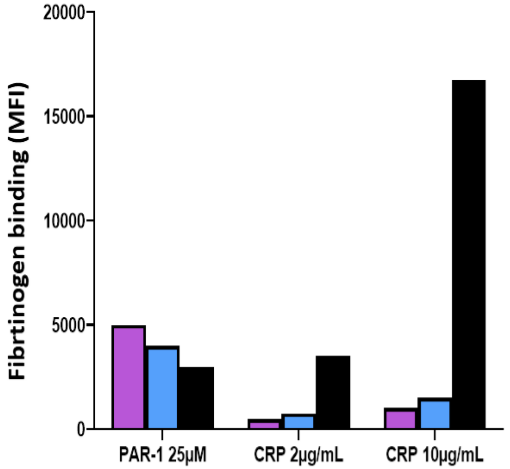
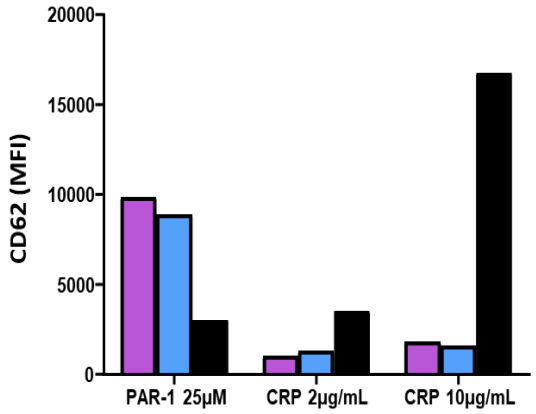
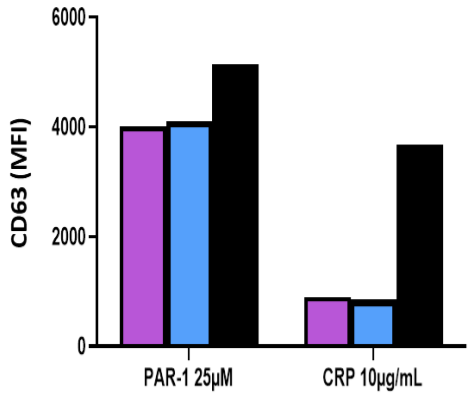
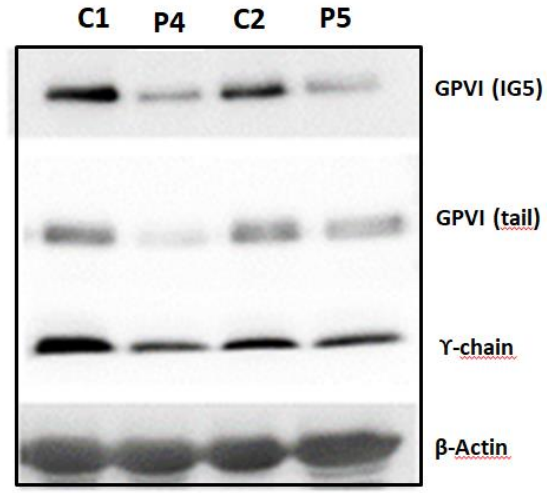
LTA guided diagnosis and characterization of a GPVI defect

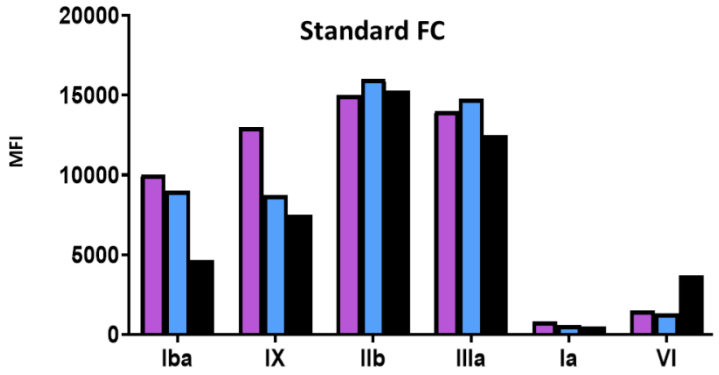


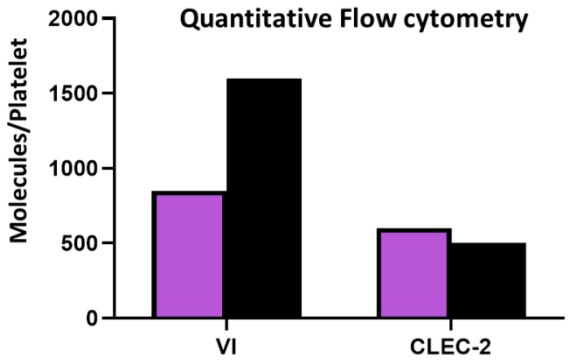


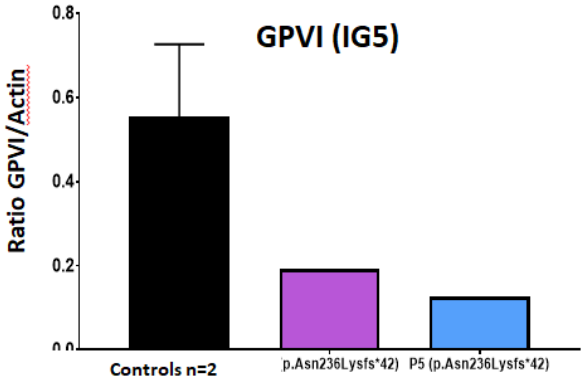




Reduced GPVI expression







P4 *GP6* p.Asn236Lysfs*42

P5 *GP6* p.Asn236Lysfs*42

C3

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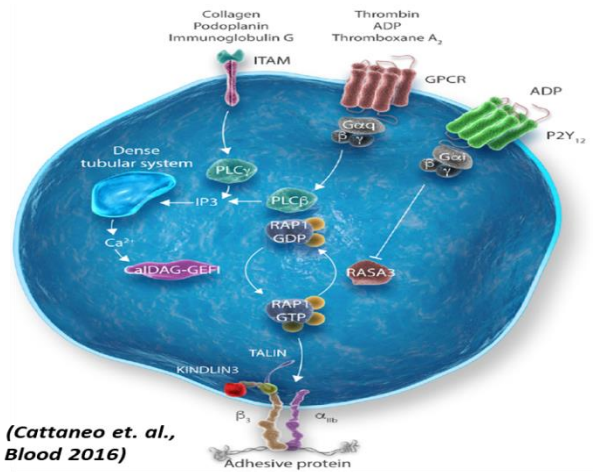
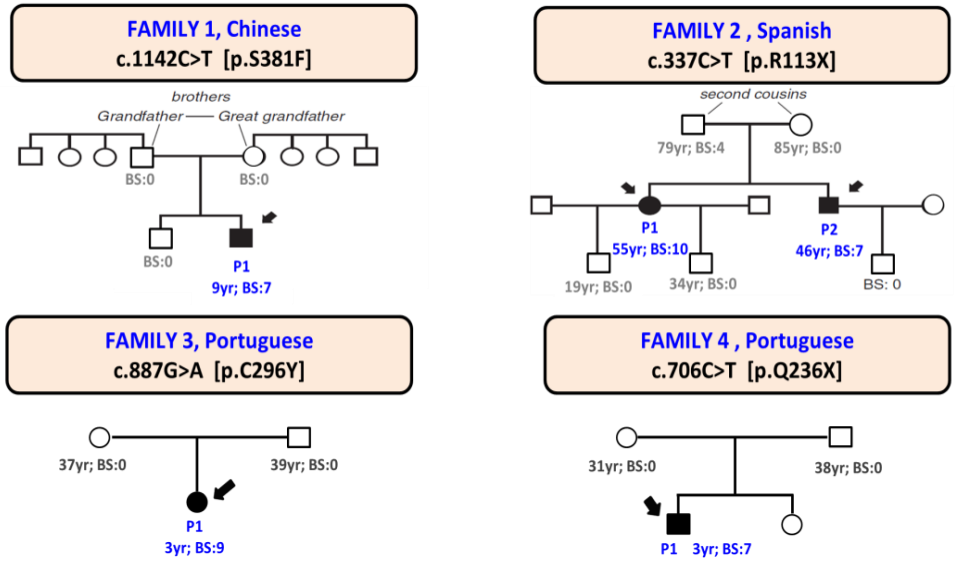
LTA findings in moderate/severe IPFDs: RASGRP2 defect

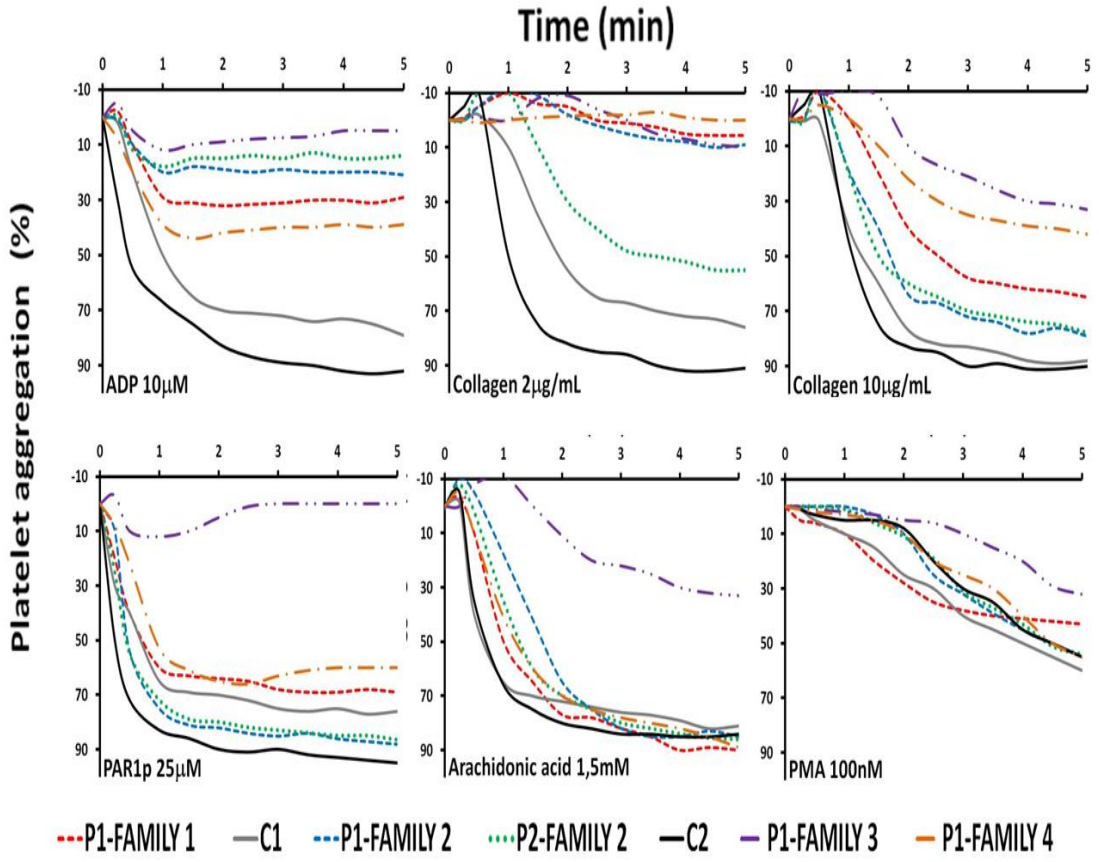


(Cattaneo et. al., Blood 2016)

GEAPC pedigrees



Lozano et al. Blood 2016; Sevivas et al. Platelets 2017



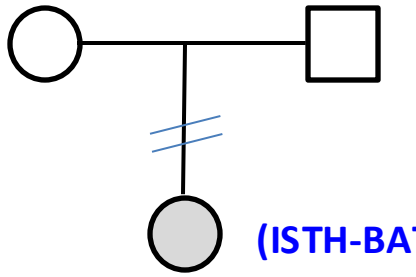
LTA features:

- Impaired aggregation with low dose agonsits (ADP, collagen & thrombin):No diagnostic of Glanzmann Thrombastenia
- Less affected response to strong agonists and PMA

Other features:

- Impaired Fibrinogen binding and granules secretion by FC , except with PMA

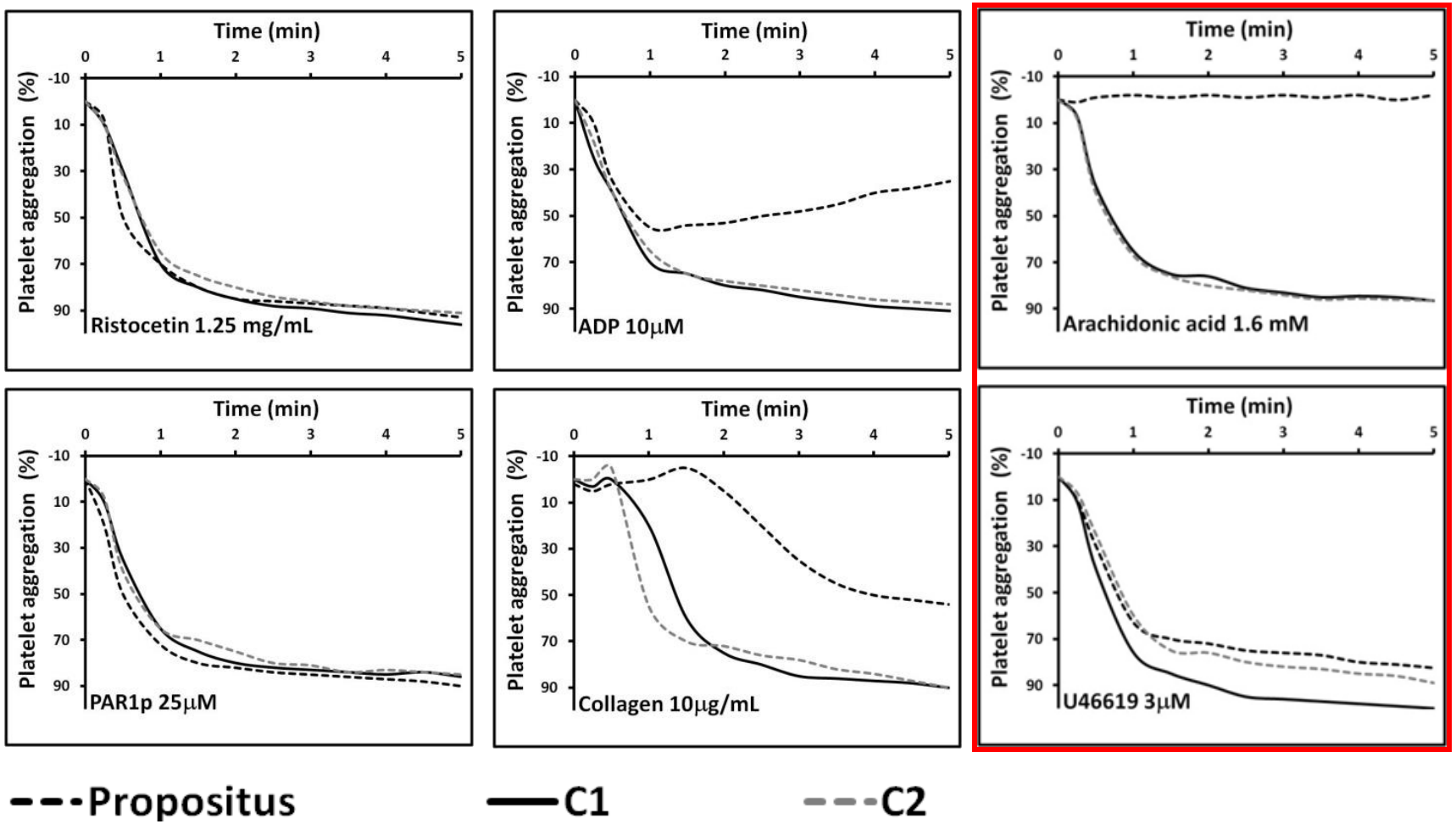
LTA findings in moderate/severe IPFDs: COX1 defect



(ISTH-BAT=6)

A 13-year-old girl of Asian origin
She has had a lifelong moderate bleeding diathesis, presenting as bruising and petechiae, which occur more frequently following minor trauma. Recurrent and prolonged epistaxis, sometimes associated with NSAID intake.
Menorrhagia, once requiring tranexamic acid and desmopressin treatment; excessive bleeding after tonsillectomy.

Normal platelet count (206 x10^9 pl/L) and volume (11,5 fL)

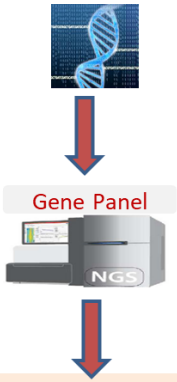


LTA normal with ristocetin and PAR-1; reduced with high dose ADP and collagen

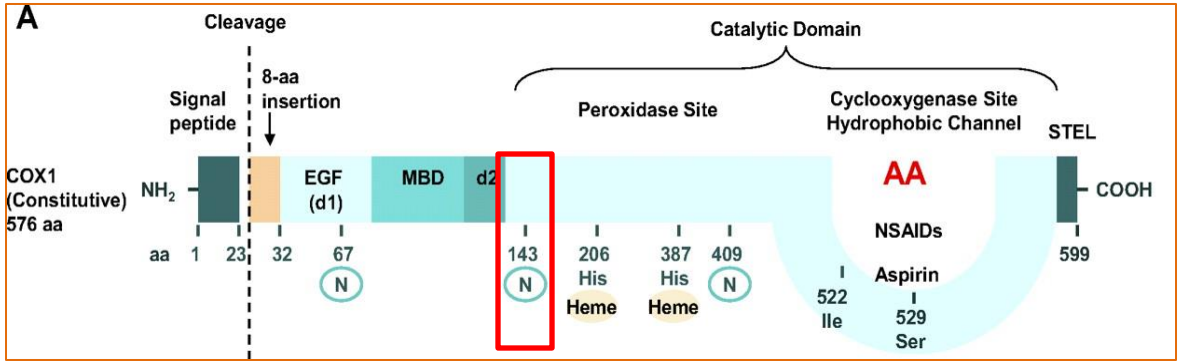
LTA absent arachidonic acid, but normal with U46619
A severe defect in TxA_2 level was found in LTA supernatant and collagen-stimulated whole blood

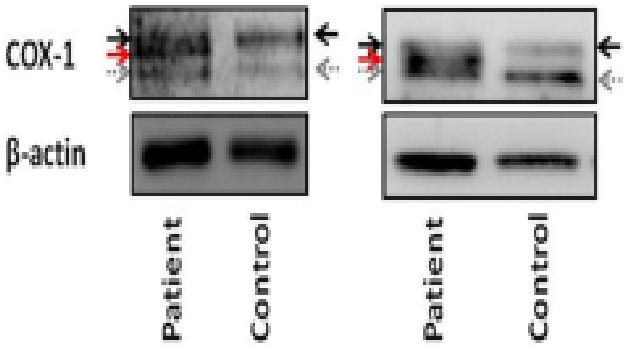
Palma-Barqueros V et al. Am J Hematol. 2021

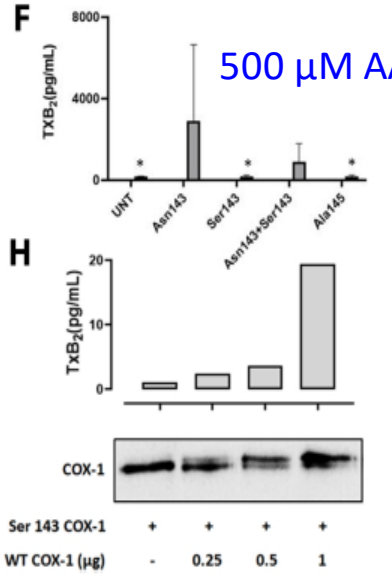
A few PTGS1 variants have been reported



PTGS1
c. 428A>G, [p.Asn143Ser] (Heterozygous) (Novel)





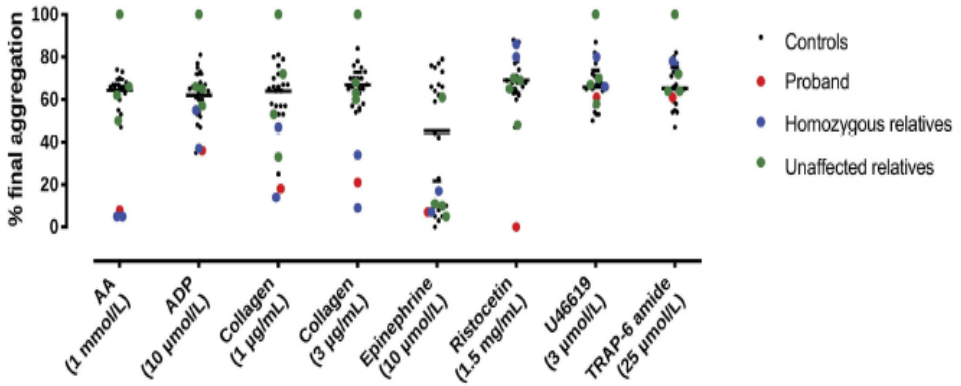


Previous reports of PTGS1 variants in bleeding patients

- Freson, Blood 2011 (Abstract ASH): A case with heterozygous variants in TBXA2R (R60H) & PTGS1 (p.Leu237Met),heterozygous (AD)→ no specific studies

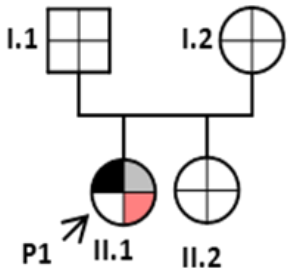
- Leinoe et al. BrJH2017; p.Arg113Cys (novel) + p.Val481Ile (0.77%). Low TxB2 in plasma

- Chan MV et al. Haematologica. 2021 965G>C[p.Trp322Ser];homozygous;R reduced TxB2 synthesis, COX1 absence in platelest (WB); reduced LTA

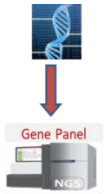


Palma-Barqueros V et al., Am J Hematol. 2021

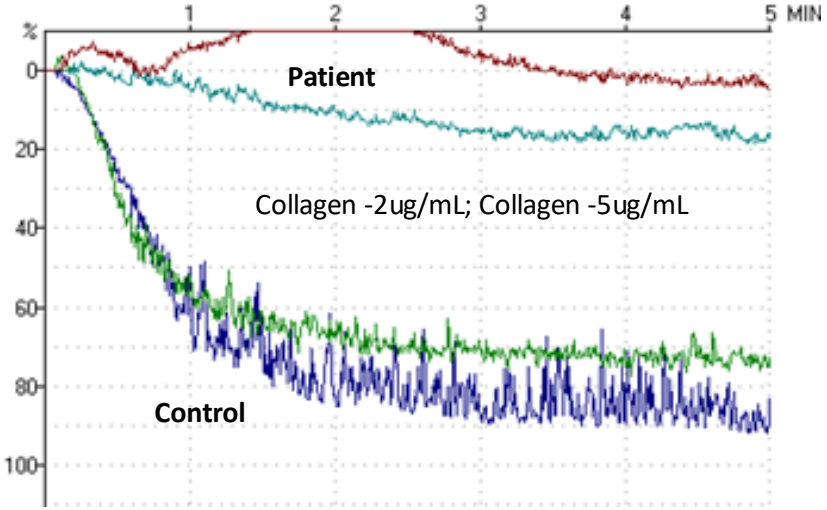
LTA findings in moderate IPFDs: RUNX1-RT

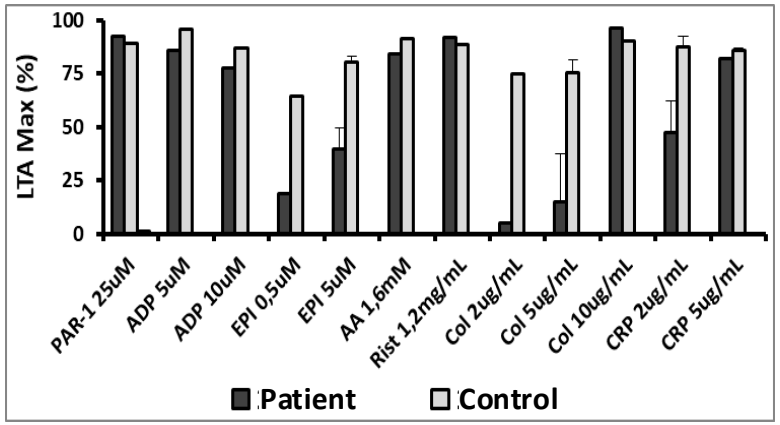


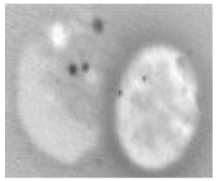
Women, 27yr
108 x 10^9 pl/L
MPV: 10.9
ISTH-BAT=5

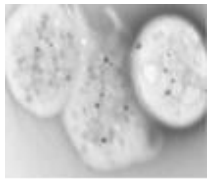


***RUNX1*:**
c.802C>T
[p.Gln268*]
De novo

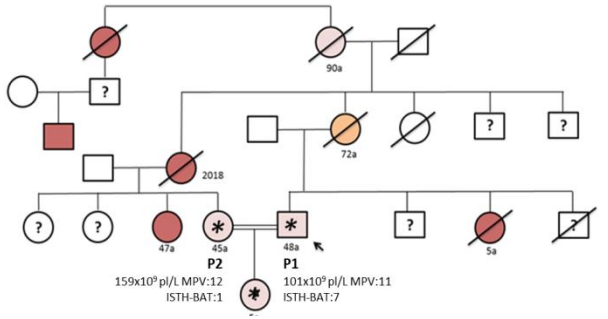


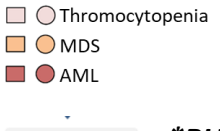




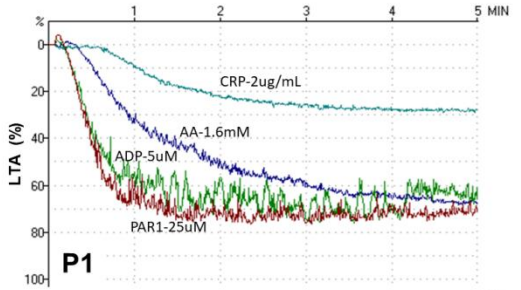


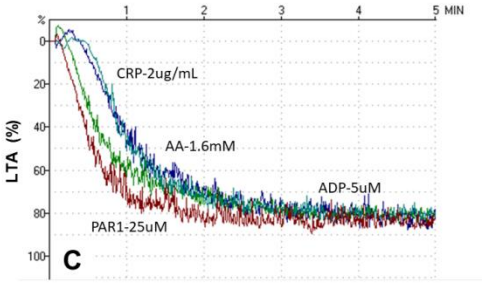
Bastida el Al. Haematologica 2017

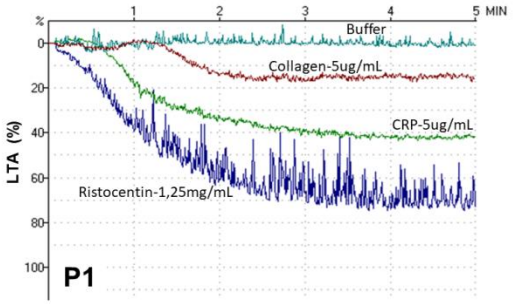


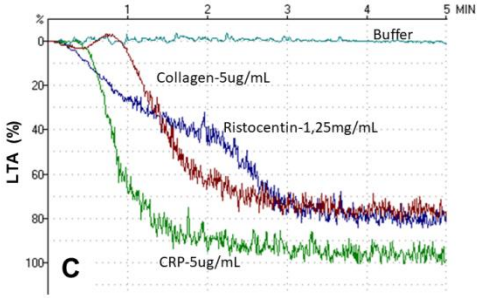


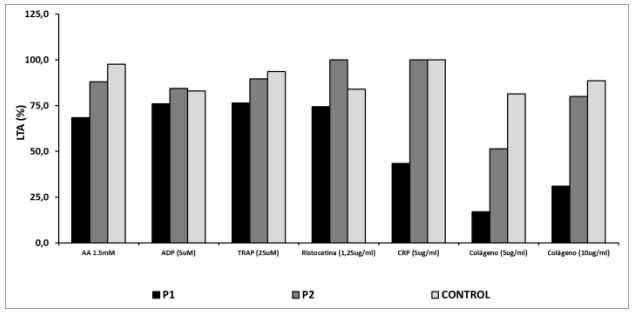
RUNX1:
c.416G>A
[p.Arg166Gln]

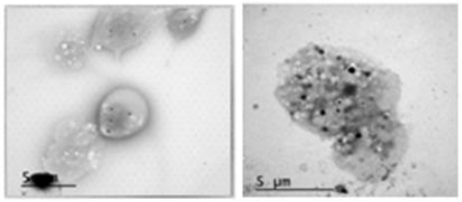




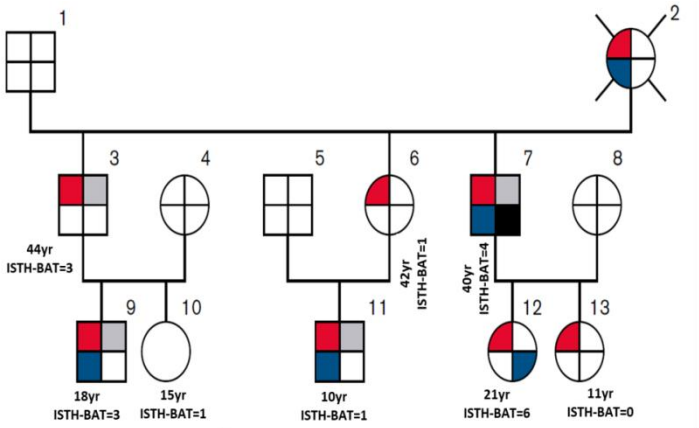




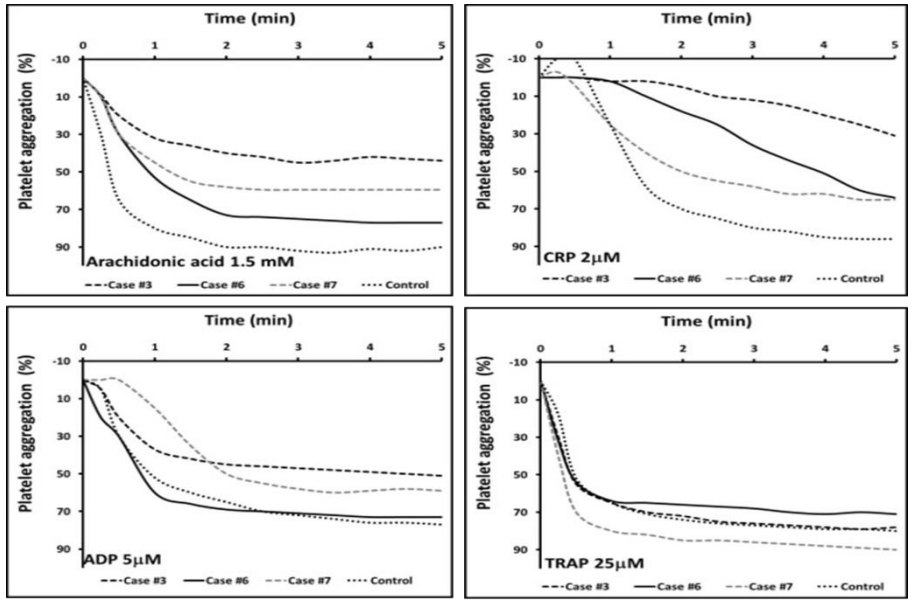




LTA findings in moderate IPFDs: SRC Gain of Function-RT



38-139 x 10⁹/L MTP
Immune defects & recurrent infections — Psoriasis; uvular angioedema; IgM/IgA deficiency, Crohn´s disease; Aasthma, dermatitis
Recurrent tonsillitis, Pityriasis versicolor, upper respiratory, (>1/year); otitis
Neurological complications
Thrombosis — portal thrombosis, pulmonary embolism, and jugular vein thrombosis
Epilepsy; Speech and language impairment
Anxiety and behavior abnormalities



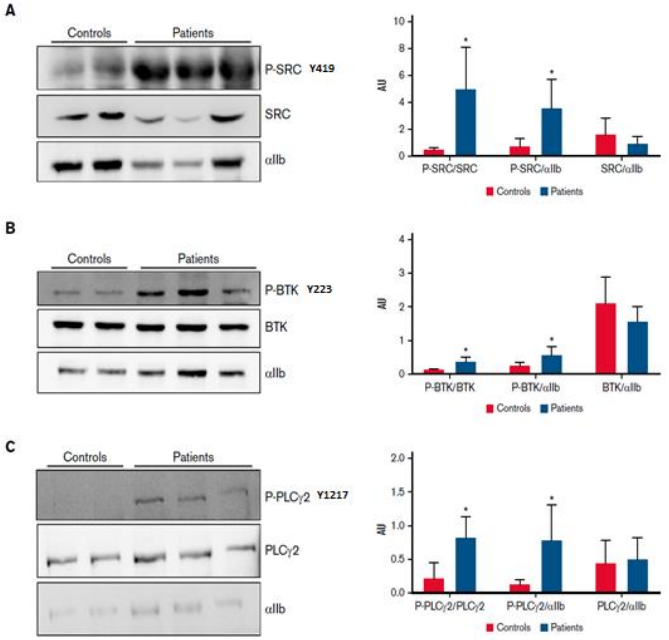
Mildly impaired platelet aggregation

Palma-Barqueros V et al., Blood Advancers 2022

Gene Panel

SRC
c.1579G>A [p.Glu527Lys]

Gain of Fucntion variant



Resting platelets

A dominant gain-of-function mutation in universal tyrosine kinase *SRC* causes thrombocytopenia, myelofibrosis, bleeding, and bone pathologies

Ernest Turro,[1,2,3,4] Daniel Greene,[1,3,4] Anouck Wijgaerts,[5] Chantal Thys,[5] Claire Lentaigne,[6,7]

Sci Transl Med. 2016

Large family presenting with thrombocytopenia. Affected patients showed juvenile myelofibrosis, splenomegaly, and bone diseases including mild facial dysmorphia and premature edentulism

De novo variant in tyrosine kinase SRC causes thrombocytopenia: case report of a second family

Lore De Kock[1], Chantal Thys[1], Kate Downes[2,3], Daniel Duarte[2,3], Karyn Megy[2,3], Chris Van Geet[1], & Kathleen Freson[1]

Platelets. 2019

Two unrelated children with very different clinical features. One with nonsyndromic thrombocytopenia, the second thrombocytopenia and facial dysmorphism, severe osteoporosis, such autism and delayed language bocytopenia.23

2025 ISTH Meeting OC 42.1 - Automated Platelet Function Tests Analysis: Insights from the Autoplate Study Group

Dr. Panagiotis Christoforou, NHS, U. Cambriged, UK

Study Rationale

Limitations of Traditional Platelet Function Testing

- Traditional platelet function testing faces challenges such as **subjective interpretation** and **time-consuming procedures.**
- These limitations **hinder** the **efficiency** and **accuracy of diagnostic processes.**

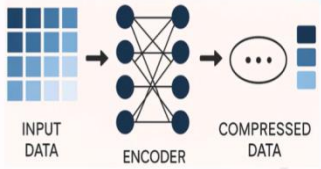
Objective of the Study

- The study aims to a**utomate platelet function analysis using Machine Learning (ML) techniques**.
- This approach seeks to enhance **diagnostic accuracy** and **streamline the testing process**.

Data Compression Technique: Variational Autoencoder (VAE)

Simon Deltadahl, PhD
Department of Applied Mathematics and Theoretical Physics

- Use of **Variational Autoencoder (VAE)**
  - A 'smart filter' that **compresses complex curves into simpler numbers**.
  - It **learns to represent each LTA curve** (platelet aggregation response) **with just a few key values** (called latent dimensions)
- Took each LTA curve and **reduced it to 6 numbers that capture its core shape/pattern**.
- This made the **data easier to visualize and analyze** while **still preserving essential differences** between **normal** and **abnormal** tests.

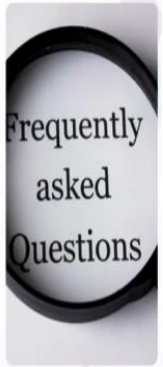


Results: Diagnostic Metrics

Methodology Overview

Sample Collection and Analysis

- A total of 1,021 samples were collected from 771 individuals with confirmed diagnoses between June 2021 and 2024
- These samples were analyzed using Hyphen Biomed reagents on Sysmex CN-series analyzers.



LTA Curve Generation

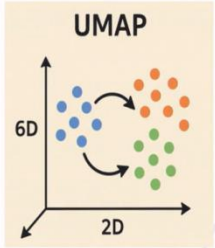
- LTA curves were generated using various agonists to study platelet function.
- The agonists included AA 500µg/mL, ADP 10µM, ADP 5µM, Col 1.25µg/mL, Col 2.5µg/mL, Epi 10µM, Epi 5µM, Ris 0.5 mg/mL, Ris 1.25 mg/mL, and U46619 1nM.

Methodology Overview

- The study utilized **advanced analytical techniques** to ensure accurate results.
- The methodology was designed to provide **comprehensive insights** into the samples' characteristics.

Visualization with Uniform Manifold Approximation and Projection (UMAP)

- UMAP is a tool that **helps visualize complex data in 2D or 3D**
- **Took the 6-dimensional output from the VAE and plotted it into 2D**, so we could see **clusters of samples that behaved similarly.**
- Helped identify patterns like:
  - **A group of samples with normal responses**
  - **A separate cluster with abnormal platelet function**



Conclusions

Enhanced Diagnostic Accuracy

- **Automation improves diagnostic accuracy and visualization.**
- This advancement **supports better clinical decision-making and patient outcomes.**

Clinical Metrics and Clustering

- **Clinical relevance demonstrated through metrics and clustering.**
- These methods provide **insights into data patterns** and **enhance understanding.**

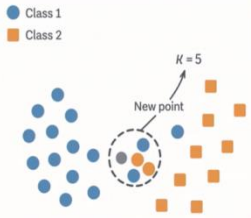
Classification Model: K-Nearest Neighbors (KNN)

Overview of K-Nearest Neighbors (KNN)

KNN is a **classification model** that **determines the category of new data points based on the proximity to existing examples.**

Application in Predicting Test Outcomes

We tested samples using UMAP where each sample landed on the 2D map and for others **KNN was used (6D) to decide whether a new sample** looked more like a "normal" or "abnormal" test based on nearby examples



Strengths

- Scalability & Standardization
- Reduced Human Variability
- Rapid reporting/Clustering (UMAP) - Clinician's aid tool

Limitations

- Need for Balanced & Labeled Datasets
- Underperformance in Borderline Cases
- Prospective Validation Required

Thank you

Grupo Español de Alteraciones Plaquetarias Congénitas

Murcia Platelet Group

European Reference Network
for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

Cues for LTA intrepretation in moderate IPFDs

Evaluation of participants with suspected heritable platelet function disorders including recommendation and validation of a streamlined agonist panel

Ban B. Dawood,[1] Gillian C. Lowe,[1] Marie Lordkipanidzé,[1] Danai Bem,[1] Martina E. Daly,[2] Mike Makris,[2] Andrew Mumford,[3] Jonathan T. Wilde,[4] and Steve P. Watson[1]

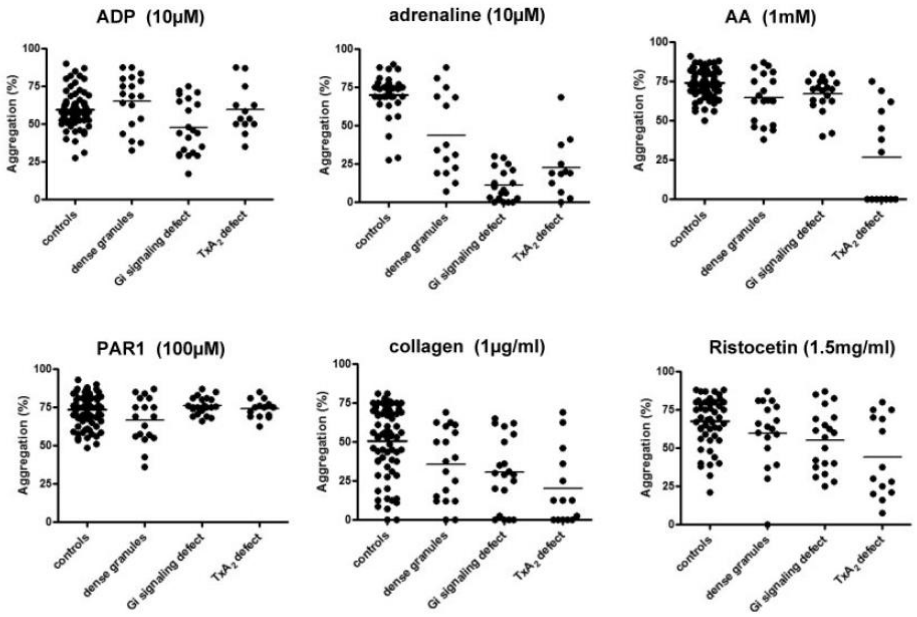


Figure 6. Maximal aggregation response in participants with platelet function defects and healthy volunteers. The percentage of maximal aggregation was measured in a Born lumi-aggregometer in PRP in response to the shown concentrations of the following agonists: ADP, adrenaline, arachidonic acid, collagen, PAR-1–specific peptide, and ristocetin. The results are shown as the percentage increase in light transmission relative to platelet-poor plasma.

Table 2. Streamlined panel of agonists with interpretative notes for diagnosing platelet function defects

Agonist	Concentration	% of maximal aggregation, mean ± SD	Expected	Abnormal pattern and further testing
ADP*	10μM	59.8% ± 12.5%	Maximal, sustained aggregation and secretion	Reduced or transient aggregation and absent secretion: use 30μM
Adrenaline*	10μM	70.3% ± 13.7%	Biphasic aggregation with secretion coincident with second phase	Reduced or absent primary wave and absent secretion: use 30μM
Arachidonic acid	1mM	71.0% ± 8.5%	Maximal, sustained aggregation and secretion	Absent or delayed/reduced aggregation and secretion: use U46619 (3μM)
PAR-1 receptor–specific peptide (SFLLRN)	100μM	73.8% ± 11.1%	Maximal, sustained aggregation and marked secretion	Transient aggregation and reduced secretion: use PAR-4 receptor–specific peptide (AYPGKF; 500μM).
Collagen*	1 μg/mL	50.4% ± 22.5%	Sustained aggregation and secretion	Reversible aggregation and absent secretion: use 3 μg/mL and collagen-related peptide (CRP; 3 μg/mL) or convulxin
Ristocetin	1.5 mg/mL	70.8% ± 16.1%	Maximal sustained aggregation (often biphasic) and secretion	Reduced or absent aggregation and secretion

Different concentrations of agonists with the percentage of maximal aggregation ± SD are shown.
*ATP secretion from dense granules should be measured for the following agonist concentrations: ADP (30μM), adrenaline (30μM), arachidonic acid (1mM), PAR-1–specific peptide (100μM), and collagen (3 μg/mL).

Table 3. Comparison of the expanded agonist panel and a streamlined agonist panel in diagnosing platelet function defect

	Expanded agonist panel positive	Expanded agonist panel negative	
Streamlined agonist panel positive	45	6	Positive predictive value 88%
Streamlined agonist panel negative	7	36	Negative predictive value 84%
	Sensitivity (87%)	Specificity (86%)	

Table shows a comparison between diagnoses of platelet function defect using the expanded agonist panel and a streamlined agonist panel. The sensitivity (87%), specificity (86%), negative predictive value (84%), and positive predictive value (88%) are shown. The kappa statistic was 0.721 ($P < .001$).

Most PFDs can be diagnosed using a streamlined panel of key platelet agonists and specified concentrations suitable for testing in most clinical diagnostic laboratories

Dawood BB et al. Blood. 2012

OC 42.2 - Evaluation of national guidelines for light transmission aggregometry in adults.

Mr. Sean Platton. Royal London Haemophilia Center, UK

National Guidelines

British Society for Haematology guidelines, published 2021[1]

bjh guideline

Table I. Recommended final concentrations of agonists for initial, full and minimal aggregometry panels.

Agonist	Suggested initial panel (for use with eight-channel analyser)	Full panel	Minimum panel (when sample volume is limited and aim is to exclude a severe disorder)
Adenine diphosphate (ADP)	5 and 10 μM	5 and 10 μM	10 μM
Arachidonic acid (AA)	1 mM	1 mM	1 mM
Collagen (type I, tendon)	1·00 or 1·25 μg/ml	1·00 or 1·25 μg/ml and 2·00 or 2·50 μg/ml	2·00 or 2·50 μg/ml
Epinephrine	5 and 10 μM	5 and 10 μM	10 μM
Ristocetin	0·5 and 1·25 g/l	0·5 and 1·25 g/l	0·5 g/l and 1·25 g/l
U46619 (thromboxane A2 receptor agonist)	–	1 μM	–
Thrombin receptor agonist peptide (TRAP) (PAR-1)	–	5 and 10 μM	–
Normal saline	–	0·9% w/v	–
Approximate volume of whole blood required*	7–12 ml	12–20 ml	5–9 ml

*Dependent on technology used for aggregation assays.

AUTOPLATE Study

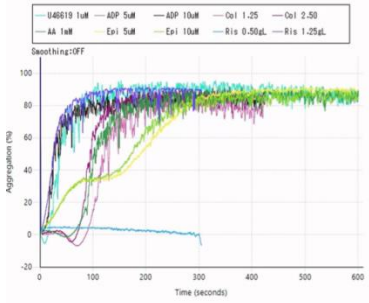
- Collaborative study between seven laboratories across England, using Sysmex CN-series analysers
- 1021 samples from 771 individuals
- 738 samples from adults

Aim

- To validate whether the full agonist panel recommended by BSH is suitable for diagnosis of inherited platelet disorders in adults

Reagents

- Sysmex Revohem reagents:
  - 10 & 5 μM ADP; and
  - 10 & 5 μM epinephrine; and
  - 1 mM arachidonic acid (AA); and
  - 1.25 & 0.50 g/L ristocetin; and
  - collagen at:
    - 2.50 & 1.25 μg/mL; or
    - 2.00 & 1.00 μg/mL.
- Existing reagent suppliers for:
  - U46619 (and TRAP [one site])
- Interpretation by local MDT

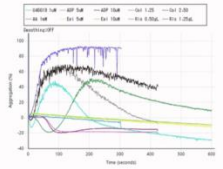


Normal platelet aggregation

- Immediate response to ADP (black and grey)
- Immediate response to high dose ristocetin (dark blue)
- Immediate response to U46619 (turquoise).
- Longer lag phase with collagen (purple/pink) and AA (dark green)
- Biphasic response to epinephrine (yellow and green)
- No response to ristocetin (pale blue) or saline (not shown)

LTA response as expected in 27 GT *cases & 8* BSS patients

Storage Pool Disorders



Impaired response to AA and U46619, with or without abnormal response to collagen

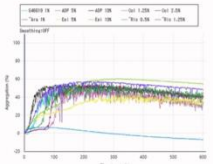
11 patients

10 gave expected results

- Homozygous *HPS3* (n=2)
- Homozygous *HPS6* (n=2)
- Homozygous *BLOC1S3* (n=1)
- Heterozygous *RUNX1* (n=4)
- Heterozygous *TBXA2/PTGS1* (n=1)
- Historical diagnosis (n=1)

Grey Platelet Syndrome

α-granule abnormalities

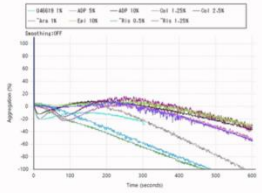


Variable results. Most commonly impaired response to collagen and/or thrombin

2 samples – both normal

- Compound heterozygous *NBEAL2* (n=1)
- Homozygous *NBEAL2* (n=1)

Autosomal dominant thrombocytopenias



Reduced maximum aggregation. Normal shaped curves

24 samples tested (PRP PLT 16-168 x10⁹/

19 with interpretable results

- Homozygous *GFI1B* (n=2)
- Heterozygous *GFI1B* (n=9)
- Heterozygous *MYH9* (n=8)
- Heterozygous *ACTN1* (n=1)
- Heterozygous *ETV6* (n=1)
- Heterozygous *GBA* (n=1)
- Heterozygous *RASGRP1* (n=1)
- Heterozygous *THPO* (n=1)

PRP prepared by centrifugation (n=15)

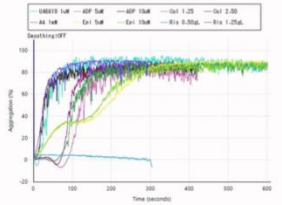
- Range tested 27-168 x10⁹/L
- Lowest interpretable result at 63 x10⁹/L

PRP prepared by sedimentation (n=16)

(including some *GP1BB* samples)

- Range tested 16-136 x10⁹/L
- Lowest interpretable result at 16 x10⁹/L

Normals



Normal aggregation expected

373 samples tested

330 with normal results

- Normal controls
- Historical normals
- Normal ISTH BAT score
- R90: no putative pathogenic variant detected
- Bleeding disorders not associated with platelets:
  - *F11*, *F5* (x2), *F7*, *F8*, *FGA*, *VWF* [type 2M]

Conclusion

Table I. Recommended final concentrations of agonists for initial, full and minimal aggregometry panels.

Agonist	Suggested initial panel (for use with eight-channel analyser)	Full panel	Minimum panel (when sample volume is limited and aim is to exclude a severe disorder)
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Ristocetin	0·5 and 1·25 g/l	0·5 and 1·25 g/l	0·5 g/l and 1·25 g/l
U46619 (thromboxane A2 receptor agonist)	–	1 μM	–
Thrombin receptor agonist peptide (TRAP) (PAR-1)	–	~~5 and 10 μM~~	–
Normal saline	–	0·9% w/v	–
Approximate volume of whole blood required*	7–12 ml	12–20 ml	5–9 ml

*Dependent on technology used for aggregation assays.

Sensitivity for inherited platelet disorders: 86.6%

Specificity for inherited platelet disorders: 94.4%

TRAP (PAR-1) is of little diagnostic value

Gomez et al. Br J Haematol, 195: 46-72. doi:10.1111/bjh.17690