UPDATE ON DIAGNOSTIC TESTING FOR PLATELET FUNCTION DISORDERS: WHAT IS PRACTICAL AND USEFUL?

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Objectives

Platelet function disorders (PFD) are important bleeding disorders that require validated and practical strategies for diagnosis

Attendees should be able to:
1. List the laboratory tests of platelet function that are validated to detect increased bleeding
2. Describe the variability in endpoints of commonly performed platelet function tests and how this effects diagnostic usefulness
3. List at least three reasons why many laboratories do not perform all recommended diagnostic tests for platelet function disorders
Bleeding Scores of the Patients We Typically Test for PFD

ISTH BAT data for a Hamilton cohort study

* Significantly different
Constitutional PFD

- Heterogeneity in features
  - Thrombocytopenia
  - Defective platelet function
  - Both of the above
  - Some: non-blood phenotypes
  - Some: syndromic disorders (e.g., Hermansky Pudlak syndrome)

- Molecular causes?
  - Distinguishing between pathogenic, likely pathogenic and variants of unknown significance (VUS) can be challenging
    - Genetic Med 2015;17:405-424
  - *Most without an obvious cause: FEW (<15%) have a probable cause found by exome sequencing*
    - Hayward et al, 2019 ISTH abstract
Genetics of inherited PFD: what is currently known

From: Lentaigne et al, Blood, 2016; 127(23):2814-2823

Note: The pathogenesis of many PFD that cause bleeding is presently unknown

*disorders with non-blood phenotypic features
Genes Implicated 5 Years Ago

- **Adhesion**
  - Platelet formation and numbers
    - **Transcription regulation**
      - FLI1: Paris-Trousseau Jacobsen syndrome
      - RUNX1
    - **GATA-1**
    - ankyrin repeat domain 26 (ANKRD26A)
    - **Cytoskeleton**
      - WAS: Wiskott-Aldrich syndrome
      - MASTL: microtubule associated serine/threonine like kinase
      - MYH9: Myosin heavy chain
      - FLNA: Filamin A
    - **RBM8A**: TAR exon junction complex
    - **CYCS**: Thrombocytopenia Cargeeg
    - **MPL**: Thrombopoietin receptor
- **Platelet formation and numbers**
  - **GP1BA**: Platelet type VWD
  - **GP1BxIXV**: Bernard-Soulier syndrome
  - **GP1BA, GP1BB, GP9**: Collagen receptor
  - **ITGA2**: Collagen receptor

- **Activation and signaling**
  - **Defective G-protein signalling**
    - GNAS1
    - GNAQ
    - RGS2
  - **GP6**: Collagen induced activation
  - **AN06**: Scott Syndrome
  - **TBXA2R**: Thromboxane receptor
  - **GP1BA, RAB27A, MLPH**: Griscelli syndrome

- **Storage granule secretion**
  - **VPS33B**: ARC syndrome
  - **NBEAL2**: Gray platelet syndrome

- **Aggregation**
  - **TGA2B, ITGB3**: Glanzmann thrombasthenia
  - **α2β1 GP1bIXV**
  - **1bα**

- **Degradation of α-granule proteins from increased uPA in α-granules**
  - **LYST**: Chediak-Higashi syndrome
  - **HPS1, HPS3-6, AP3B1, BLOC1S3, PLDN, DNTBP1**: Hermansky-Pudlak syndrome
  - **MYO5A, RAB27A, MLPH**: Griscelli syndrome

- **Genes Implicated 5 Years Ago**
  - **CD36**: Thrombospondin receptor
  - **GP1BA**: Platelet type VWD
  - **FLI1**: Paris-Trousseau Jacobsen syndrome
  - **RUNX1**
  - **GATA-1**
  - **ANKRD26A**

- **PLAU**: Quebec platelet disorder
  - Degradation of α-granule proteins from increased uPA in α-granules

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### Newly Implicated Genes

**Granulopoiesis**
- BLOC1S6
- AP3D1
- VIPAS39
- STXBP2
- NBEA

**Storage granule secretion**

**Aggregation**

**Activation and signaling**

**Defective signaling**
- PLA2G4A
- RASGRP2

**Thrombocytopenia/bone marrow failure**

**Thrombocytopenia and deafness**

### Platelet formation and numbers

**Transcription factors**
- ETV6
- GFI1B
- HOXA11
- MECOM

**Cytoskeleton**
- TPM4
- ACTN1
- ARPC1B
- CDC42
- TUBB1
- DIAPH1* (highlighted)
- FYB
- RNU4ATAC

**Incomplete**
- SRC
- SLFN14
- STIMI
- ABCG5
- ABCG8
- MPIG6B
- KDSR
- GNE

**Adhesion**

**THPO CAMT**
What is practical and useful for diagnosing constitutive PFD?

Consideration of:

- Evidence (laboratory diagnostic perspective)
- Experiences
- Research
- Literature review
- International survey of laboratories that test for PFD
<table>
<thead>
<tr>
<th>Strong Evidence</th>
<th>Weak Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clear cut criteria for deciding if findings are normal or abnormal</strong></td>
<td><strong>No well-established criteria to define an abnormal result</strong></td>
</tr>
<tr>
<td><strong>Performed with standardized operating procedures, quality controls, with ongoing surveillance of test performance including external quality assurance samples</strong></td>
<td><strong>Method is not procedure controlled and tests are done without quality controls and/or ongoing external quality assurance</strong></td>
</tr>
<tr>
<td><strong>RI established/validated by appropriate statistics (e.g., non-parametric) using data for a large number of male and female subjects</strong></td>
<td><strong>RI is either: unknown; determined for a very limited number of subjects; or did not consider that results are not normally distributed. Results are reported with comparison to a control sample, without a RI</strong></td>
</tr>
<tr>
<td><strong>Test has a within-subject CV &lt; 20%\nRepeat tests confirm original findings in most subjects</strong></td>
<td><strong>Test has a CV &gt;20% or is unknown. Reproducibility is unknown or poor</strong></td>
</tr>
<tr>
<td><strong>Test usefulness was evaluated by a large cohort study design, of patients typical of the population tested, with comparison of data for patients with and without the disorder(s)</strong></td>
<td><strong>Test was evaluated with a limited number of patients or a highly selective patient group. Evidence supporting the test use for diagnosis is limited (e.g., based on case studies only).</strong></td>
</tr>
<tr>
<td><strong>Abnormalities show significant association to clinically relevant outcomes</strong></td>
<td><strong>It is unknown if test abnormalities are associated with clinically relevant outcomes</strong></td>
</tr>
<tr>
<td><strong>Clear discrimination in the findings for those with and without the disorder</strong></td>
<td><strong>Significant overlap in findings for those with and without the disorder</strong></td>
</tr>
<tr>
<td><strong>Method is superior to alternatives, based on area under receiver operator curve analysis</strong></td>
<td><strong>Method has a lower CV than alternatives</strong></td>
</tr>
<tr>
<td><strong>Cross-over studies are done for reagent lot changes, with sufficient samples to detect altered performance</strong></td>
<td><strong>Procedure control is limited</strong></td>
</tr>
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</table>
Guidance on Laboratory Testing for Platelet Function Disorders

*note: evidence has emerged that differs from some consensus recommendations in these documents*


3. **Approved NASCOLA guideline:** Hayward CPM et al. North American guidelines on platelet function testing and test interpretation. AJCP, 2010;134:955-63*


* Only guideline that addresses interpretation
MOST RECENT GUIDANCE

Recommendations of an Expert Panel:

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

P. Gresele, for the Subcommittee on Platelet Physiology
Division of Internal and Cardiovascular Medicine, Department of Medicine, University of Perugia, Perugia, Italy

To cite this article: Gresele P, for the Subcommittee on Platelet Physiology. Diagnosis of inherited platelet function disorders: guidance from the SSC of the ISTH. J Thromb Haemost 2015; 13: 314–22.
“Upfront” testing for PFD – ISTH guidelines recommend doing it after excluding VWD
What if we test for both at the same time?
Hayward, Moffat, Liu. STH 2012;38(7):742-52

| Test                                           | Sensitivity | Specificity
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>APTT, PT/INR, Fibrinogen, Thrombin Time &amp; VWD Screen</td>
<td>8%</td>
<td>95%</td>
</tr>
<tr>
<td>APTT, PT/INR, Fibrinogen, Thrombin Time, VWD Screen &amp; LTA</td>
<td>29%</td>
<td>93%</td>
</tr>
</tbody>
</table>
ISTH RECOMMENDED INITIAL LABORATORY DIAGNOSTIC WORK-UP of PFD

**FIRST STEP TESTS**

**Blood smear**
- LTA (screening)
- Granules release
- Flow cytometry (FC)

**LTA (screening)**
- Platelet size
  - normal
  - altered
  - Scott

**Granules release**
- GPIIb/IIIa activation
- Normal +/- altered
- Scott

**Flow cytometry (FC)**
- Platelet GP
- Normal +/- altered

**EPI (**)**
- QPD/\(\alpha_2\) receptor
- P2Y\(_{12}\) defect
- \(\alpha_\beta\) GPF/SPD
- \(\alpha_\beta\) defect

**ADP**
- P2Y\(_{12}\) defect
- \(\alpha_\beta\) GPF/SPD

**COLLAGEN**
- \(\alpha_\beta\) GPF/SPD
- \(\alpha_\beta\) defect

**AA**
- COX-1/TP defect
- P2Y\(_{12}\) defect

**RISTOCETIN**
- PT-VWD/BSS/GATA1

**Total amount of blood required:** ~21-28 ml
SECOND STEP TESTS

**LTA (extension)**

- α-thr
  - GPS/GT/LADIII
- TRAP-6
  - GPS/GT/LADIII
- U46619
  - GPS/GT/LADIII
- CRP
  - TP defect/GT/LADIII
- CVX
  - GPVI/GT/LADIII
- PAR4-ap
  - GPVI/GT/LADIII/ Filaminopathy(±)
- PMA
  - PKC defect/GT/LADIII
- A23187
  - Ca2+ defects/GT/LADIII
- Inhibition by lloprost or PGE1
  - Gs platelet defect

**Flow cytometry (extension)**

- Platelet GP
  - GPⅠα/Ⅱa
  - αδ
  - GPⅣ
  - GPⅤ

- Platelet procoagulant activity
  - impaired
  - enhanced
  - Scott
  - Stormorken

**Mixing tests (LTA/Flow cytometry)**

- LTA ristocetin enhanced
  - A) Patient’s plasma + control plts enhanced
  - B) Patient’s plasma + control plts normal

**Granules content**

- GPS
- WAS(±)
- ARC
- Stormorken
- GATA1
- δ-SPD
- HPS
- CHS
- WAS(±)
- FPD
- AML
- MDS
- Filaminopathy(+)
- α-δ SPD

**Clot retraction**

- GT
- Stormorken
- WAS

**TEM**

- Granule content or morphology
- Structural abnormalities

**SECOND STEP TESTS**

- **LTA ristocetin enhanced**
  - A) Patient’s plasma + control plts enhanced
  - B) Patient’s plasma + control plts normal

**Total amount of blood required:** ~3-15 ml
So do clinical labs comply?

- 2015 Survey of North American Specialized Coagulation Laboratory Association (NASCOLA) and ECAT Foundation diagnostic laboratories
  - 26 question, on-line survey (with “yes/no” and comment box formats)
  - Goal: assess compliance with key recommendations for diagnosing inherited PFD in the latest International Society on Thrombosis and Haemostasis (ISTH) Scientific Subcommittee (SSC) document.
- NASCOLA and ECAT participants
  - received the identical survey, except around demographics
  - ≥ three weeks to respond.
- After removing duplicate entries, data was anonymized for analysis
Initial tests for PFD

**NASCOLA responses (n=47)**

- Blood smear: 64%
- LTA: 94%
- Platelet dense granule release: 32%
- Platelet alpha granule release: 6%
- Major platelet surface GP by flow: 9%
- Other: 23%

**ECAT responses (n=61)**

- Blood smear: 75%
- LTA: 80%
- Platelet dense granule release: 20%
- Platelet alpha granule release: 8%
- Major platelet surface GP by flow: 30%
- Other: 46%
Use of recommended agonists for LTA

**NASCOLA responses (n=46)**

- Epinephrine: 83%
- Adenosine diphosphate (ADP): 96%
- Collagen: 96%
- Arachidonic acid: 93%
- Ristocetin: 96%
- Not applicable (LTA not performed): 4%

**ECAT responses (n=58)**

- Epinephrine: 74%
- Adenosine diphosphate (ADP): 93%
- Collagen: 90%
- Arachidonic acid: 84%
- Ristocetin: 86%
- Not applicable (LTA not performed): 7%

**VERY FEW tested additional agonists**

- CRP: NASCOLA: 4%; ECAT: 0%
- TRAP: NASCOLA: 14%; ECAT: 31%
- Thromboxane analogue U46619: NASCOLA: 36%; ECAT: 33%
Additional Practices

• Less than half validate RI for PFD tests
  *if done, number of healthy volunteer samples used:*
    
    **median, range:** NASCOLA: 30, 10-73; ECAT: 30, 10-50

• More NASCOLA (51%) than ECAT participants (25%) performed crossover studies for new lots of agonists
  *But typically with only a few samples:*
  
  **median, range:** NASCOLA: 2, 1-20; ECAT: 5, 1-6
Why don’t labs comply with PFD testing recommendations?

• ISTH guidance document
  • Awesome roadmap to characterize platelet disorders for research purposes
  • BUT, not practical for many clinical labs
    • Fiscal and other constraints, including a lack of high quality evidence, limit offering an expanded test menu for PFD

• Flow
  • Labs typically only do flow if they suspect Bernard Soulier syndrome or Glanzmann thrombasthenia
Practical issue

Variability in endpoints: Aggregation vs. ATP Release
## Updated evidence on diagnostic usefulness of PFD tests (studies cited used well validated RI)

<table>
<thead>
<tr>
<th>Test</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Light transmission platelet aggregometry using platelet count adjusted PRP | Criteria:  
≥2 vs. no abnormalities: 41 (7-143)  
≥2 vs. 0-1 abnormality: 23 (5-105)  | Castilloux et al |
| Light transmission platelet aggregometry using native PRP            | Criteria:  
≥2 vs. no abnormalities: 32 (4-249)  
≥2 vs. 0-1 abnormality: 27 (4-214)  | Castilloux et al |
| Lumiaggregometry assessment of dense granule ATP release*           | Criteria:  
Abnormal findings with ≥2 agonists on two tests: 1.5 (0.6-3.9) | Badin et al                |
| Platelet whole mount EM to assess for dense granule deficiency:      | Criteria:  
Confirmed dense granule deficiency: 97 (5.4-1740) | Brunet et al               |
Hamilton Study of Individuals with Multiple Tests

Cohort I: all tested persons  Cohort II: participants in a platelet disorder study (ISTH-BAT scores assessed)

ATP release findings often inconsistent and not predictive of bleeding: Relates to high CV

Electron Microscopy Determination of Platelet Dense Granule Counts
Brunet et al, IJLH 2018; 40(4):400-407

• Evaluated 12 years of data
• 1115 unique patients, 126 unique controls (tested simultaneously)
  • many patients and controls tested multiple times
• dense granule deficiency (DGD)
  • more common in patients (6.3%) than controls (0.3%), p<0.01
• Key findings:
  • 1) test has an acceptable CV and
  • 2) abnormal findings are associated with bleeding problems
  
  For confirmed DGD, the OR for bleeding disorder are:
  97 (95%CI 5.4-1740, p<0.01)
Sensitivity of LTA and ATP release for detecting DGD: Data on patients with DGD
Brunet et al, IJLH 2018; 40(4):400-407
Respective Sensitivities for DGD: LTA: ~52% ATP release: ~70%
Errors in LTA Interpretation are a Concern

NASCOLA/ECAT Data, STH 2012:38;622-31

- **SCARY FINDINGS**
  - ~60% of real life cases were correctly interpreted by ≥75% of diagnostic labs

- **Common errors:**
  - Diagnosis too specific or incorrect
  - Normal variants or single agonist abnormalities reported as diagnostic of PFD

- **What can labs do to reduce interpretation errors:**
  - Use published guidance!
<table>
<thead>
<tr>
<th>LTA Finding (from AJCP 2010;134(6):955-63)</th>
<th>Recommended Interpretation</th>
<th>Follow-up Investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No or marked ↓ with AA, normal with U46619, ↓ with low dose collagen.</td>
<td>Aspirin-like defect (drug induced or inherited).</td>
<td>Repeat testing when subject not on NSAIDs.</td>
</tr>
<tr>
<td>Aggregation is present with only ristocetin.</td>
<td>Possible Glanzmann thrombasthenia (inherited or acquired).</td>
<td>Glycoprotein analysis of fibrinogen receptor αIIbβ3.</td>
</tr>
<tr>
<td>Aggregation absent with high conc. of ristocetin and patient has thrombocytopenia with very large platelets.</td>
<td>Possible Bernard Soulier Syndrome (inherited or acquired). VWD should be excluded.</td>
<td>Glycoprotein analysis to assess glycoprotein IbIXV,</td>
</tr>
<tr>
<td>Aggregation ↓ with high conc. of ristocetin, no thrombocytopenia.</td>
<td>Possible von Willebrand disease.</td>
<td>VWF levels.</td>
</tr>
<tr>
<td>Aggregation abnormally ↑ with low conc. of ristocetin.</td>
<td>Possible type 2B or platelet-type VWD.</td>
<td>VWF levels. Consider genetic testing.</td>
</tr>
<tr>
<td>Aggregation abnormally reduced with multiple agonist. Markedly ↓ with ADP with significant deaggregation.</td>
<td>Possible platelet ADP receptor defect (P2Y12). Drug induced defect should be excluded.</td>
<td>Repeat aggregation testing.</td>
</tr>
<tr>
<td>Other abnormalities with two or more agonists.</td>
<td>Suggest a platelet function disorder is present. Confirm on repeat testing.</td>
<td>Platelet ATP release and/or EM for dense granule deficiency (DGD).</td>
</tr>
<tr>
<td>Abnormalities with only one agonist (excluding collagen or ristocetin).</td>
<td>Nondiagnostic and could represent a false positive.</td>
<td>Repeat aggregation, ATP release and/or EM for DGD.</td>
</tr>
</tbody>
</table>
Acknowledgments:

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