Platelet Function Testing in the Clinic

Alan D. Michelson

Professor of Pediatrics and Professor of Medicine
Harvard Medical School
Director, Center for Platelet Research Studies
Director, Thrombosis and Anticoagulation Program
Boston Children’s Hospital, Dana-Farber Cancer Institute
Disclosures

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Platelet Function
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Antiplatelet Agents

Michelson *Nature Reviews Drug Discovery* 2010;9:154-169
Antiplatelet Agents

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Antiplatelet Agents
Congenital Disorders of Platelet Function

Congenital Disorders of Platelet Function


Receptors for Adhesive Proteins

Glanzmann thrombasthenia
Bernard-Soulier syndrome
Platelet-type VWD
GPVI defect
α2β1 defect

Congenital Disorders of Platelet Function

Modified from Michelson *Nature Reviews Drug Discovery* 2010;9:154-169

- **Receptors for Soluble Agonists**
  - P2Y12 defect
  - TXA2 receptor defect
Congenital Disorders of Platelet Function

α-Granules

α-storage pool deficiency (gray platelet syndrome)
Quebec platelet disorder
Paris-Trousseau syndrome/11-q terminal deletion disorder

Congenital Disorders of Platelet Function

δ-storage pool deficiency
Hermansky-Pudlak syndrome
Chediak-Higashi syndrome

Congenital Disorders of Platelet Function

α-Granules and Dense Granules

α,δ-storage pool deficiency

Congenital Disorders of Platelet Function

Procoagulant Phospholipids

Scott syndrome Stormorken

<table>
<thead>
<tr>
<th></th>
<th>Primary Hemostatic Disorder</th>
<th>Coagulation Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prototypic Disorders</td>
<td>Thrombocytopenia platelet function defect von Willebrand disease*</td>
<td>Hemophilia</td>
</tr>
<tr>
<td>Bleeding</td>
<td>Immediate</td>
<td>Delayed</td>
</tr>
<tr>
<td>Petechiae</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemarthroses</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Intramuscular hematomas</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Epistaxes</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
</tbody>
</table>

*In the uncommon type 3 von Willebrand disease, the factor VIII coagulant level is low enough for the clinical features to be those of a combined primary hemostatic and coagulation disorder.*
Bernard-Soulier Syndrome

MYH9-Related Disease

Gray platelet syndrome

Alan D. Michelson, Boston Children’s Hospital, Dana-Farber Cancer Institute, Harvard Medical School
Light Transmission Aggregometry

- Stirring PRP (baseline or 0% aggregation)
- Aggregate formation
- PPP (maximum or 100% aggregation)

Figure 26-1a

Jennings in PLATELETS (Michelson, 2nd ed, Elsevier/Academic Press, 2007)
Results of a worldwide survey on the assessment of platelet function by light transmission aggregometry: a report from the platelet physiology subcommittee of the SSC of the ISTH

M. CATTANEO,1* C. P. M. HAYWARD,1†‡§ K. A. MOFFAT, †§ M. T. PUGLIANO,* Y. LIU† and A. D. MICHELSON¶
*Unità di Medicina III, Ospedale San Paolo, Dipartimento di Medicina, Chirurgia e Odontoiatria, Università di Milano, Milan, Italy; †Department of Pathology and Molecular Medicine; ‡Department of Medicine, McMaster University, Hamilton, Canada; ¶Hamilton Regional Laboratory Medicine Program, Hamilton, Canada; and ¶Center for Platelet Research Studies, Division of Hematology/Oncology, Children’s Hospital Boston, Harvard Medical School, Boston, MA, USA
Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH

M. CATTANEO, * C. CERLETI, † P. HARRISON, ‡ C. P. M. HAYWARD, § D. KENNY, † D. NUGENT, ** P. NURDEN, † † A. K. RAO, † † † A. H. SCHMAIER, §§ S. P. WATSON, † † † F. LUSSANA, * M. T. PUGLIANO* and A. D. MICHELSON**
Measurement of Platelet ATP Release by Lumiaggregometry

\[ \text{D-Luciferin} + \text{ATP} \]

\[ \text{Firefly luciferase} \]

\[ \text{Mg}^{++} \]

\[ \text{Luciferyl adenylate} + \text{PPI} \]

\[ \text{O}_2 \]

\[ \text{Firefly luciferase} \]

\[ \text{Oxyluciferin} + \text{AMP} + \text{H}_2\text{O} + \text{CO}_2 + \text{light} \]

\text{PPI = inorganic pyrophosphate}

Hayward in PLATELETS (Michelson, 3\textsuperscript{rd} ed, Elsevier/Academic Press, 2013)
Lumiaggregometry

Dawood *Blood* 2012;120:5041
WHY HAVE GUIDELINES FOR THE DIAGNOSIS OF INHERITED PLATELET FUNCTION DISORDERS (IPFD)?

• Laboratory tests for platelet function disorders are not well standardized and not as widely available as blood clotting assays.

• A rational stepwise diagnostic approach, going from simple screening tests to highly specialized laboratory techniques, may be cost-effective.

• A stepwise diagnostic approach can also allow non-specialized centers with limited resources to formulate well-grounded diagnostic hypotheses for subsequent referral to specialized centers.

• Genetic diagnosis of IFPD, although desirable, is currently usually impractical because single candidate genes for conventional sequencing seldom exist and next generation sequencing is still not widely available, is relatively expensive and is prone to false-positives.

ISTH SSC Working Party JTH 2015;13:314
DIAGNOSTIC ALGORITHM

Flowchart

PROBAND

Clinical evaluation:

- Personal and family history and bleeding score: bleeding manifestations typical of IPFD
- Physical examination: bleeding manifestations typical of IPFD
- Syndromic forms: hearing loss; immunodeficiency; renal function; cardiac function; mental retardation; facial dysmorphism; eyes; bone; skin

Preliminary laboratory investigation

- Platelet count
- Routine coagulation tests
- VWF screening

DIAGNOSIS

- LOW: Thrombocytopenia
- ABNORMAL: VWD (Blood clotting defect); AFibrinogenemia

Potential platelet function disorder

- NORMAL/LOW
- NORMAL

Next generation sequencing

Platelet function studies

ISTH SSC Working Party JTH 2015;13:314
A standardized bleeding tool is to be encouraged to unify the assessment of bleeding severity.

The **quantitative ISTH VWD Bleeding Score** can be provisionally used but it is not validated for inherited platelet disorders.

A Bleeding Score Questionnaire should be specifically validated for IPFD.
Tests excluded from the diagnostic flow chart

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

- **Impedance aggregometry**: *not recommended* because inaccurate for milder platelet defects (although it has theoretical advantages like rapidity, lack of centrifugation, ease of execution).

- **Mepacrine uptake by flow cytometry** *not recommended* for the study of dense granules: lack of sensitivity/validation.

They may be used as additional tests in individual laboratories if adequately standardized.

ISTH SSC Working Party *JTH* 2015;13:314
**DIAGNOSTIC WORK-UP**

**SECOND STEP TESTS**

- **LTA (extension)**
  - \( \alpha \)-thr \( \rightarrow \) GPS/GT/LADIII
  - TRAP-6 \( \rightarrow \) GPS/GT/LADIII
  - U46619 \( \rightarrow \) TP defect/GT/LADIII
  - CRP \( \rightarrow \) GPVI/GT/LADIII
  - CVX \( \rightarrow \) GPVI/GT/LADIII/Filaminopathy(±)
  - PAR4-ap \( \rightarrow \) GPS/GT/LADIII
  - PMA \( \rightarrow \) PKC defect/GT/LADIII
  - A23187 \( \rightarrow \) Ca\(^{2+}\) defects/GT/LADIII
- **Flow cytometry (extension)**
  - Inhibition by iloprost or PGE\(_1\) \( \rightarrow \) Gs platelet defect
- **Mixing tests (LTA/Flow cytometry)**
  - \( c\text{PLA}_2 \) \( \rightarrow \) COX-1 \( \rightarrow \) Tx-synthase deficiency
  - 2B-VWD
  - PT-VWD
- **Granules content**
  - Granule content or morphology
  - Structural abnormalities
  - TEM
- **Clot retraction**
  - A) Patient’s plasma + control plts enhanced
  - B) Patient’s plasma + control plts normal
  - GT Stromorken WAS
  - Total amount of blood required: ~3-15 ml

**A)** Patient’s plasma + control plts enhanced
**B)** Patient’s plasma + control plts normal
Biochemical studies
Surface glycoproteins (WB) / Spreading assay/Adhesion and thrombus formation under flow conditions/Protein phosphorylation (WB, FC)/Second messengers (Ca\textsuperscript{2+}, IP3, cAMP)/Receptor binding studies/ Western Blotting for MYH10

Molecular genetic diagnosis

- ITGA2B, ITGB3 → GT
- GP1BA, GP1BB, GP9 → BSS
- GP1BA → PT-VWD
- WAS → WAS
- NBEAL2, GFI1B → GPS
- Del11q23 (FLI1) → PTS
- del22q11.2 → VCF
- CD36 → GPIV deficiency
- GP6 → GPVI deficiency
- TMEM16F → Scott Syndrome
- PLAU (duplication) → QPD
- TBXA2R → Thromboxane A2
- HPS1, AP32B1, HPS3, → HPS
- HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, PLDN →
- LYST → CHS
- P2RY12 → P2Y12 defect
- GNAS → Gs platelet defect
- VPS33B, VIPAS39 → ARC
- STIM1, ORAI1 → Stormorken
- FERMT3 → LADIII
- RUNX1 → FPD/AML/MDS
- GATA1 → GATA1
- FLNA → Filaminopathy
- TBXAS1 → Tx synthase deficiency
- PLA2G4A → cPLA\textsubscript{2}
- MYH9 → MYH9-RD

Total amount of blood required: ~3-50 ml
Platelet function tests, independent of platelet count, are associated with bleeding severity in ITP

Andrew L. Frelinger III, Rachael F. Grace, Anja J. Gerrits, Michelle A. Berny-Lang, Travis Brown, Sabrina L. Carmichael, Ellis J. Neufeld and Alan D. Michelson
Association of Explanatory Variables Identified as Significant in Univariate Analysis with Bleeding Score After Adjustment for the Platelet Count

These tests may be useful markers of future bleeding risk in ITP

Frelinger Blood 2015;126:873
Platelet Function Testing for Monitoring of Antiplatelet Therapy?
## Laboratory tests to monitor P2Y$_{12}$ inhibition

<table>
<thead>
<tr>
<th>Test</th>
<th>Principle</th>
<th>Readout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light transmission aggregation (LTA)</td>
<td>Decreased turbidity of aggregated vs. non-aggregated platelets</td>
<td>% aggregation</td>
</tr>
<tr>
<td>of platelet-rich plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VerifyNow P2Y$_{12}$</td>
<td>Co-aggregation of fibrinogen-coated beads with platelets in whole blood</td>
<td>PRU (Platelet Reactivity Units)</td>
</tr>
<tr>
<td>Multiplate MEA</td>
<td>Whole blood impedance aggregometry</td>
<td>AUC (area under the curve)</td>
</tr>
<tr>
<td>PLT VASP/P2Y12</td>
<td>Changes in VASP phosphorylation measured by flow cytometry</td>
<td>PRI (Platelet Reaction Index)</td>
</tr>
<tr>
<td>TEG PlateletMapping$^{(TM)}$</td>
<td>Platelet-dependent increase in clot strength</td>
<td>MA (Maximum Amplitude, mm)</td>
</tr>
</tbody>
</table>
Class IIb
Platelet function testing may be considered in patients at high risk for poor clinical outcomes. (Level of Evidence: C)
In patients treated with clopidogrel with high platelet reactivity, alternative agents, such as prasugrel or ticagrelor, might be considered. (Level of Evidence: C)

Class III: No Benefit
The routine clinical use of platelet function testing to screen patients treated with clopidogrel who are undergoing PCI is not recommended. (Level of Evidence: C)
Platelet Function Testing for Monitoring of Antiplatelet Therapy?

Not currently recommended in clinical practice, because randomized controlled trials of modification of antiplatelet therapy based on platelet function testing have not shown benefit:

– GRAVITAS (Price JAMA 2011;305;1097)
– TRIGGER-PCI (Trenk JACC 2012;59;2159)
– ARCTIC (Collet NEJM 2012;367;2100)