Platelet function tests for monitoring antiplatelet agent therapy

Point-of-care Arizona
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Learning objectives

- Describe the principles of different tests used to monitor platelet function
- Define limitations in assessing response to antiplatelet agents using tests of platelet function
- Differentiate the ability of different platelet function tests to detect the effects of antiplatelet agent therapy
Platelet function testing

• Traditionally done to identify congenital and acquired platelet function defects
• Traditionally considered qualitative testing requiring interpretation in the context patient condition
• Multiple method exist
  • Each measures slightly different property of platelet activity or function
  • Each has advantages and disadvantages
Light Transmittance Aggregometry (LTA)

- Platelet-rich plasma added to cuvette
- Activator added
- Light transmits as plt clump
- Reported as % activity
Optical Aggregometry (LTA)

- Affected by:

  Age
  Gender
  Race
  Diet
  Hematocrit
  Sample collection and processing
  Type and concentration of agonist
  Operator variability
Optical Aggregometry (LTA)

- **Advantages**
  - One of few lab tests that have predicted outcome in patients on aspirin
  - Used to define expected clopidogrel effect (drug studies)
  - Often (probably incorrectly) considered gold standard

- **Disadvantages**
  - Difficult to standardize
  - No uniform definitions or methods (improving)
  - Labor intensive, not rapid
MultiPlate Whole blood impedance aggregometry

- Single cell (cuvette) whole blood aggregation
- Activated platelets coat wire, increase resistance
- Activators include ADP and AA
- Platelet activity reported in arbitrary units (AAU over 6 min)
MultiPlate Whole blood impedance aggregometry

• Advantages
  • Faster, less preanalytical work and variability (potentially) than LTA

• Disadvantages
  • Newer, not as much clinical data, doesn’t report % activity
  • FDA-approved version not available in U.S.
VerifyNow WB Aggregometry

- WB cartridge-based aggregation
- Fibrinogen-coated beads in multiple wells
- Each well has plt activator
- Platelets in WB form complex with beads upon activation
- Reports as arbitrary clotting units (ARU or PRU)
VerifyNow

• Advantages
  • Rapid, whole blood, point of care application
  • Most widely used and studied for clopidogrel effect

• Disadvantages
  • Concordance with LTA poor
  • Occasional channel failures
  • Doesn’t report % inhibition or activity
Thromboelastography Platelet Mapping (TEG-PM)

- Whole blood coagulation activated by kaolin (or other agonists) in cup
- Pin attached to torsion wire inserts into cup of whole blood
Thromboelastography (TEG)

- Multiple platelet agonists can be used to “isolate” ADP effect on platelet aggregation
- Platelet mapping:
  - Channel 1: Fibrin
  - Channel 2: ADP
  - Channel 3: thromboxane
  - Channel 4: Kaolin (max clot strength, thrombin)
- % Inhibition (ADP) = \((M_{ADP} - M_{Fibrin})/(M_{Kaolin} - M_{Fibrin})\)
Thromboelastography (TEG)

- **Advantages**
  - Whole blood, relatively rapid (MA in 20-30 min)
  - Gives information on clotting factors, fibrinogen

- **Disadvantages**
  - Requires calculation involving 3 relatively imprecise (CV ~ 20%) variables
  - Labor intensive
  - Artifacts in fibrin channel have been described that may impact % ADP or AA inhibition estimates
VASP flow cytometry

- Vasodilator-stimulated protein (VASP)
- Platelet membrane protein
- Phosphorylation stimulated by PE1 and inhibited by ADP
- Fix platelets, label with monoclonal Ab to phosphorylated form of VASP
- Measure VASP phosphorylation in presence of PE1 and presence and absence of ADP
- Reports as platelet reactivity index (0-100%)
  - \( \frac{\text{MFI}_{\text{PE1}} - \text{MFI}_{\text{ADP}}}{\text{MFI}_{\text{PE1}}} \times 100 \)
- Clopidogrel should decrease index
VASP flow cytometry

- **Advantages**
  - Flow cytometry may serve as better reference method for platelet function
  - Generally better able to separate “normal” from platelet inhibitor effect

- **Disadvantages**
  - Time-consuming and labor intensive
  - Requires specialized equipment and expertise
Preventing heart disease

- Heart disease #1 killer in US
- Aspirin reduces risk of cardiovascular events:
  - ~ 40% reduction in risk of first MI for middle age men
  - ~ 25% reduction in vascular death, MI and stroke in patients at high risk for vascular disease
- Clopidogrel (Plavix) reduces risk of events
  - ASA + Clopidogrel more effective than ASA alone in PCI and ACS
Aspirin mechanism of action

- For platelet function, important action is irreversible acetylation (for life of platelet) of serine-530 of cyclooxygenase 1 (COX 1)
- Inhibition of COX-1 leads to inhibition of thromboxane A2 production, one (of many) platelet agonists
- Platelets are therefore less reactive, and less likely to form clots
  - Decreased risk atherothrombosis
  - Increased risk of bleeding
Defining aspirin resistance

- Definition based on pharmacologic mechanism
  - Type I
    - Pharmacokinetic
    - COX-1 not inhibited
    - Lab test: Thromboxane
  - Type II
    - Pharmacodynamic
    - Platelet activation persists despite inhibition of COX-1
    - Lab test: Thromboxane (production despite adequate COX-1 inhibition)
  - Type III
    - Pseudoresistance
    - Thromboxane-independent platelet activation
    - Requires AA-induced platelet function test
Clopidogrel (Plavix) mechanism of action

• Metabolite irreversibly/covalently binds to P2Y$_{12}$ platelet receptor

  • P2Y$_{12}$ major receptor responsible for ADP-induced platelet aggregation
  • Single dose standard (300mg) dose leads to ~ 50% reduction in ADP-induced platelet aggregation
  • 3-7 days of 75 mg daily dosing will reach same level of inhibition
  • Time and dose dependence, not uniform
Clopidogrel mechanisms of resistance

- Extrinsic mechanisms
  - Non-compliance, under-dosing, drug-drug interactions
- Intrinsic mechanisms
  - Genetic variables
    - Polymorphisms of P2Y\textsubscript{12} receptor (pharmacodynamic)
    - Polymorphisms of CYP2C19 (pharmacokinetic)
  - Increased ADP release from platelet
  - Alternative mechanisms of platelet activation
    - Epinephrine, thrombin, thromboxane, collagen-mediated platelet aggregation
- Lab tests: Genetic tests or ADP-induced platelet function
PFT monitoring for aspirin/clopidogrel response

• Hot topic
  • “Aspirin resistance” research peaked 5-10 years ago, diminished since
• Desire to quantitate platelet response to clopidogrel
• Driven by variability in response
  • Role of pharmacogenomic testing
  • Role of platelet function testing (PFT)
• Several consensus statements on definition of “high on-treatment platelet reactivity” by PFT
  • No consideration of test performance at or near cut-offs
  • No recommended test or approach to testing
PFT monitoring for aspirin/clopidogrel response

• 2 large clinical trials examined whether treating based upon PFT improved outcome with clopidogrel therapy (GRAVITAS and ARCTIC)
  • Both studies negative
• One large study (TRILOGY-ACS) comparing clopidogrel to prasugrel therapy found no relationship between PFT and ischemic outcomes
• Trials done in a lower risk, “all comer” population patients put on antiplatelet agent before cardiac intervention
• Experts continue to advocate for testing for “high risk” patients
PFT monitoring for aspirin/clopidogrel response

- Who is “high risk” and needs testing
- What test to use?
- What cut-offs to apply?
- Does high on-treatment reactivity today mean high reactivity a week later, a month later, a year later?
PFT monitoring for aspirin/clopidogrel response

  116 patients from randomized trial of Plavix dosing  
  Patients pre-cath, on Plavix and ASA for 1 week  
  4 platelet function tests compared (LTA, WBA, PFA, Ultegra)  
  > 50% resistance found by all assays except WBA (47%)  
  Poor correlation between tests (κ 0.1-0.3, slight to fair agreement)

  Conclusion: Guidelines recommending measurement need to specify which test and under what conditions
PFT monitoring for aspirin/clopidogrel response

- Consensus definition of high on-treatment platelet reactivity (JACC 2010; 56:919-33)
  - > 50% PRI by VASP
  - 235-240 PRU by VerifyNow
  - >46% ADP-induced aggregation by LTA
  - > 468 AAU by Multiplate
- Based upon ROC analysis of outcome studies
- No consistency in methods or definitions used for testing or outcome determination
PFT monitoring for aspirin/clopidogrel response

- Madsen et al., Clin Chem 2010;56:839-47
  - 33 patients post cath, followed for one year
  - TEG, VerifyNow, LTA repeated 5 times over year
  - Both ASA and Plavix effects measured
  - VerifyNow and TEG showed highest intra-individual variability (20-40%)
  - By LTA only 2 patients defined Plavix non-response at all visits (3-5 at any one visit)
  - By VerifyNow and TEG no patients defined non-response at all visits (TEG 2-6 at any one visit)
  - For ASA non-response no patients defined non-response at > 1 visit by any method
  - Poor correlation between tests
Which platelet function methods are appropriate for titrating and monitoring antiplatelet therapy in the critical care setting?
Method Comparison Study

- Tests to detect/differentiate platelet function in patients on platelet inhibitors
  - AA and ADP-induced platelet function
- 5 platelet function tests (PFT)
  - TEG platelet mapping (TEG PM), VerifyNow, Light transmission aggregometry (LTA), Multiplate impedance aggregometry, Vasodilator-stimulated phosphoprotein (VASP) flow cytometry (ADP-induced only)
- Range of values in healthy volunteers and donors on aspirin and/or clopidogrel
  - Can the assay differentiate normal from inhibited platelet function?
- Analytic, pre-analytic (blood draw) variability
Study design

- 40 healthy donors (screened) donate blood for all 5 PFT
  - All 5 tests in duplicate
  - 24 healthy repeat blood draw within 24 hr
  - All 5 tests in duplicate
  - 64 duplicates used to calculate intra-assay CV
  - 24 quadruplicates (duplicate testing from each of two blood draws) used to calculate inter-assay CV
- 10-13 donors on daily aspirin and/or clopidogrel
  - All 5 tests in duplicate
  - All donors return within 24 hr
  - Intra and Inter-assay CV as above
Detecting effects of antiplatelet agents

- Scatter plot of all AA-induced and ADP-induced PLT activity values in healthy volunteers vs. donors on aspirin (AA) and/or clopidogrel (ADP)
- Mean (SD) platelet activity in healthy volunteers vs. donors on aspirin and/or clopidogrel
- ROC sensitivity analysis (average initial duplicate values) for distinguishing healthy volunteers from donors on aspirin and/or clopidogrel
- No reference method, no way to assess accuracy
Intra- and Inter-Assay Precision

- Intra-assay precision
  - Analytic precision, estimated from duplicate analysis of samples obtained from a single blood draw
- Inter-assay precision
  - Within person variability
    - Obtain 2 blood sample collected within 24 hr
    - Duplicate analysis each blood sample
    - WP variability = analytic + pre-analytic (blood draw and sample processing) + biologic variability
    - Minimize biologic variability (draws within 24 hr)
- Reliability coefficient
Acceptability criteria

- Precision
  - Healthy donors: Intra-assay CV < 10%
    - Inter-assay CV < 15%
  - Treatment (aspirin) donors: Intra-assay CV < 20%
    - Inter-assay CV < 30%
Distribution of results—AA aggregation

<table>
<thead>
<tr>
<th></th>
<th>TEG PM</th>
<th>LTA</th>
<th>VerifyNow</th>
<th>Multiplate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (●)</td>
<td>94 ± 12</td>
<td>72 ± 19</td>
<td>643 ± 28</td>
<td>925 ± 188</td>
</tr>
<tr>
<td>Treatment (x)</td>
<td>5 ± 10</td>
<td>9 ± 11</td>
<td>419 ± 63</td>
<td>148 ± 68</td>
</tr>
</tbody>
</table>
ROC analysis—AA aggregation

- AUC initial duplicate (average) values:
  - AUC TEG PM and Multiplate 1.00
    - Using average initial values, can distinguish healthy volunteers from aspirin-treated donors
  - AUC LTA 0.959
  - AUC VerifyNow 0.998
  - Chi-Square P value > 0.05
# Intra- and Inter-assay CV, AA-induced function

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-assay CV</td>
<td>N</td>
</tr>
<tr>
<td>VN</td>
<td>1.4%</td>
<td>120</td>
</tr>
<tr>
<td>MP</td>
<td>5.2%</td>
<td>128</td>
</tr>
<tr>
<td>LTA</td>
<td>2.7%</td>
<td>128</td>
</tr>
<tr>
<td>TEG PM</td>
<td>3.4%</td>
<td>124</td>
</tr>
</tbody>
</table>

- VerifyNow (VN) best precision
- LTA (Inter-assay) and TEG PM (Intra- and Inter-assay) failed criteria for treatment group (low absolute values)
Summary—AA induced platelet function

- TEG PM, Multiplate, LTA differentiate platelet function between healthy and aspirin-treated donors
  - Mean value healthy donors ~ 5 fold higher than mean treatment donors
  - VerifyNow less than 2 fold difference
- TEG PM and LTA had poorer precision treatment group
  - TEG PM CVs ~ 100%
  - VerifyNow best precision (< 5% all groups)
Distribution of results-- ADP

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<th>VASP</th>
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<tbody>
<tr>
<td>Normal</td>
<td>85 ± 14</td>
<td>74 ± 12</td>
<td>84 ± 10</td>
</tr>
<tr>
<td>Treatment</td>
<td>72 ± 23</td>
<td>38 ± 20</td>
<td>18 ± 16</td>
</tr>
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<tr>
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<th>Multiplate</th>
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<tbody>
<tr>
<td>Normal</td>
<td>265 ± 44</td>
<td>823 ± 144</td>
</tr>
<tr>
<td>Treatment</td>
<td>130 ± 79</td>
<td>420 ± 228</td>
</tr>
</tbody>
</table>
ROC analysis—ADP aggregation

- AUC initial duplicate (average) values:
  - VASP flow cytometry AUC 1.00
    - Using average initial values, can distinguish healthy volunteers from aspirin-treated donors
  - AUC LTA 0.892
  - AUC VerifyNow 0.950
  - AUC Multiplate 0.930
    - Chi-Square P value > 0.05
  - AUC TEG PM 0.589
    - Chi-Square P value < 0.05
# Intra- and Inter-assay precision, ADP-induced function

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<td>120</td>
</tr>
<tr>
<td>VN</td>
<td>4.4%</td>
<td>118</td>
</tr>
<tr>
<td>MP</td>
<td>4.3%</td>
<td>128</td>
</tr>
<tr>
<td>LTA</td>
<td>3.3%</td>
<td>128</td>
</tr>
<tr>
<td>TEG PM</td>
<td>6.7%</td>
<td>122</td>
</tr>
</tbody>
</table>

- All met precision criteria
Summary—ADP-induced platelet function

- TEG PM does not distinguish healthy donors from those on clopidogrel very well
- VASP best differentiates platelet function between healthy and treated donors

- Karon et al., Clin Chem 2014;60:1524-31
Study summary

• Multiplate whole blood impedance aggregometry only method that met acceptability criteria for precision and reliability coefficient for both AA- and ADP-induced platelet function among both healthy donor and those on antiplatelet therapy
  • Whole blood, rapid platelet function test
  • Less precise than VerifyNow, but better differentiates aspirin effect compared to healthy donors
• TEG PM least optimal (not appropriate) for measuring short-term impact of platelet inhibitors
Conclusions

• Multiple methods for testing platelet function exist
• Many based upon LTA principle, which was designed to be qualitative
• No good gold standard or reference method for quantitative assessment of platelet activity after antiplatelet agent therapy
• Assessing clopidogrel response likely to remain hot topic
Questions