MAYO CLINIC

Platelet function tests for monitoring antiplatelet agent therapy

Brad S. Karon MD PhD
Professor of Laboratory Medicine and Pathology
Co-director, Point of Care Testing
Mayo Clinic Rochester

Learning objectives

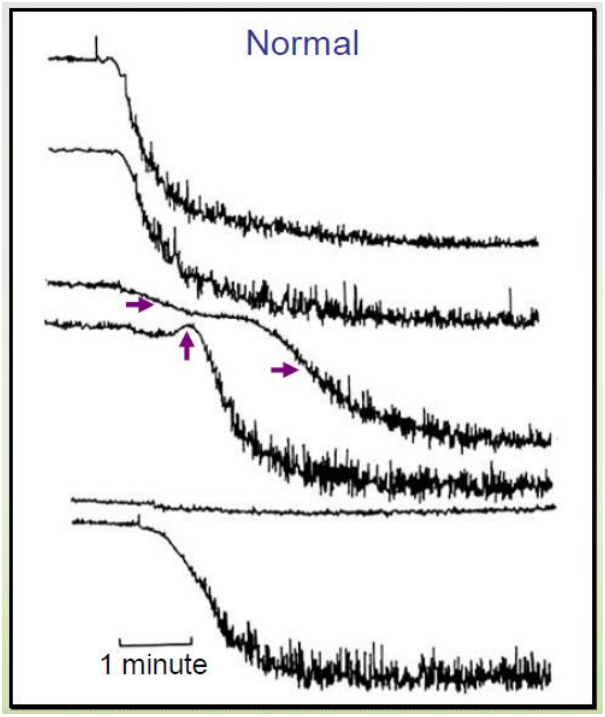
- Describe the principles of different tests used to monitor platelet function
- Define the use cases and guidelines for assessing antiplatelet agent response using functional tests
- Differentiate the ability of different platelet function tests to detect the effects of antiplatelet agent therapy

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) or AU
- FDA-approved in US but only commercially available outside US

MultiPlate Whole blood impedance aggregometry

- Advantages
  - Faster, less preanalytical work and variability (potentially) than LTA
- Disadvantages
  - Not as much clinical data but growing evidence outside US, doesn't report % activity
  - FDA-approved version not available in U.S.

VerifyNow WB Aggregometry

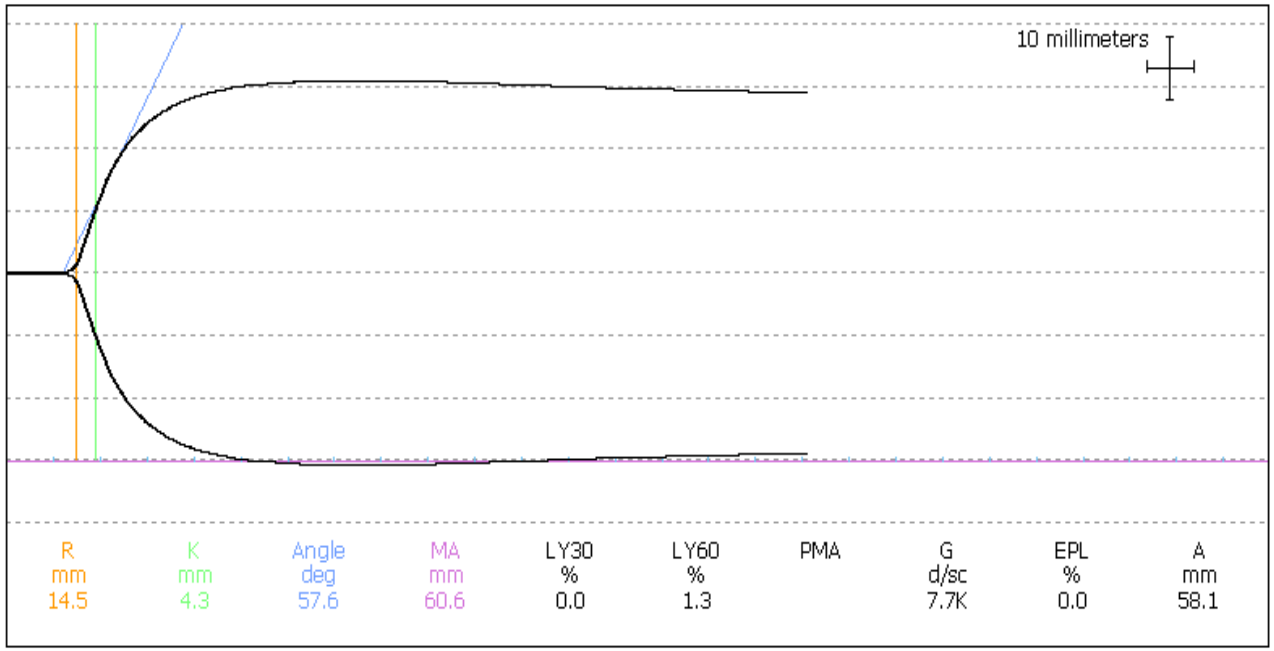
- WB cartridge-based aggregation
- Fibrinogen-coated beads in multiple wells
- Each well has plt activator
- Platelets in WB form complex with beads upon activation
- Reports as arbitrary clotting units (ARU or PRU)

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, arbitrary units
  - Where you start may define where you end up

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) = $(MA_{ADP} - MA_{Fibrin})/ (MA_{Kaolin} - MA_{Fibrin})$

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 (new 6S cartridge-based device has mitigated this issue)

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%)
  - (MFI $_{PE1}$ – MFI $_{ADP}$)/ MFI $_{PE1}$ x 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
  - Newer agents like prasugrel and ticagrelor common but clopidogrel still widely used

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 clopidogrel (300mg) 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
  - Newer $P2Y_{12}$ agents don't require metabolism (not given as pro-drug)

Clopidogrel mechanisms of resistance

- Extrinsic mechanisms
  - Non-compliance, under-dosing, drug-drug interactions
- Intrinsic mechanisms
  - Genetic variables

    Polymorphisms of $P2Y_{12}$ receptor (phamacodynamic)

    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

- "Aspirin resistance" research peaked >10 years ago, diminished since, little interest last 5-10 years
- Are we done with aspirin monitoring?
  - Not quite,
  - Berlin heart, pediatric LVAD (left ventricular assist device) protocols call for titration of aspirin in children getting these devices
  - Interest in assessing patients on aspirin prior to CV surgery for effect, greater interest in $P2Y_{12}$ agents but some data suggest aspirin causes worse bleeds

PFT monitoring for aspirin/clopidogrel response

- Desire to quantitate platelet response to clopidogrel diminishing due to negative trials and use of alternative agents, but…
  - Many still advocate escalation/de-escalation after PCI for high risk patients
  - Interest from CV surgery to assess antiplatelet agent effect prior to surgery
    - Society Thoracic Surgery recommends PFT monitoring over fixed days off $P2Y_{12}$ agents
  - Has become standard of care in neurointerventional procedures (pipeline embolization device)
    - VerifyNow therapeutic window 60-240 common

PFT monitoring for aspirin/clopidogrel response

- 5 large clinical trials examined whether treating based upon PFT improved outcome with $P2Y_{12}$ agent therapy (GRAVITAS, TRIGGER-PCI, ARCTIC, ANTARCTIC, TROPICAL-ACS)
  - No outcome advantages to monitoring identified
- One large study (TRILOGY-ACS) comparing clopidogrel to prasugrel therapy found no relationship between PFT and ischemic outcomes
- TAILOR-PCI randomized by genotype (loss of function) and found no difference in ischemic outcomes or death between LOF patients given ticagrelor vs clopidogrel
- Trials done in a lower risk, “all comer” population
- Experts continue to advocate for testing for “high risk”

PFT monitoring for $P2Y_{12}$ inhibitor response

- Updated Expert Consensus Statement on Platelet Function and Genetic Testing for Guiding P2Y12 Receptor Inhibitor Treatment in Percutaneous Coronary Intervention JACC Cardiovasc Interv 2019;26(12):1521-37

	Ischemic events	Bleeding risk
VerifyNow (PRU)	>208	<85
Multiplate (AU)	>46	<19
TEG PM (ADP MA in mm)	>47	<31
VASP (PRI)	>50%	<16%

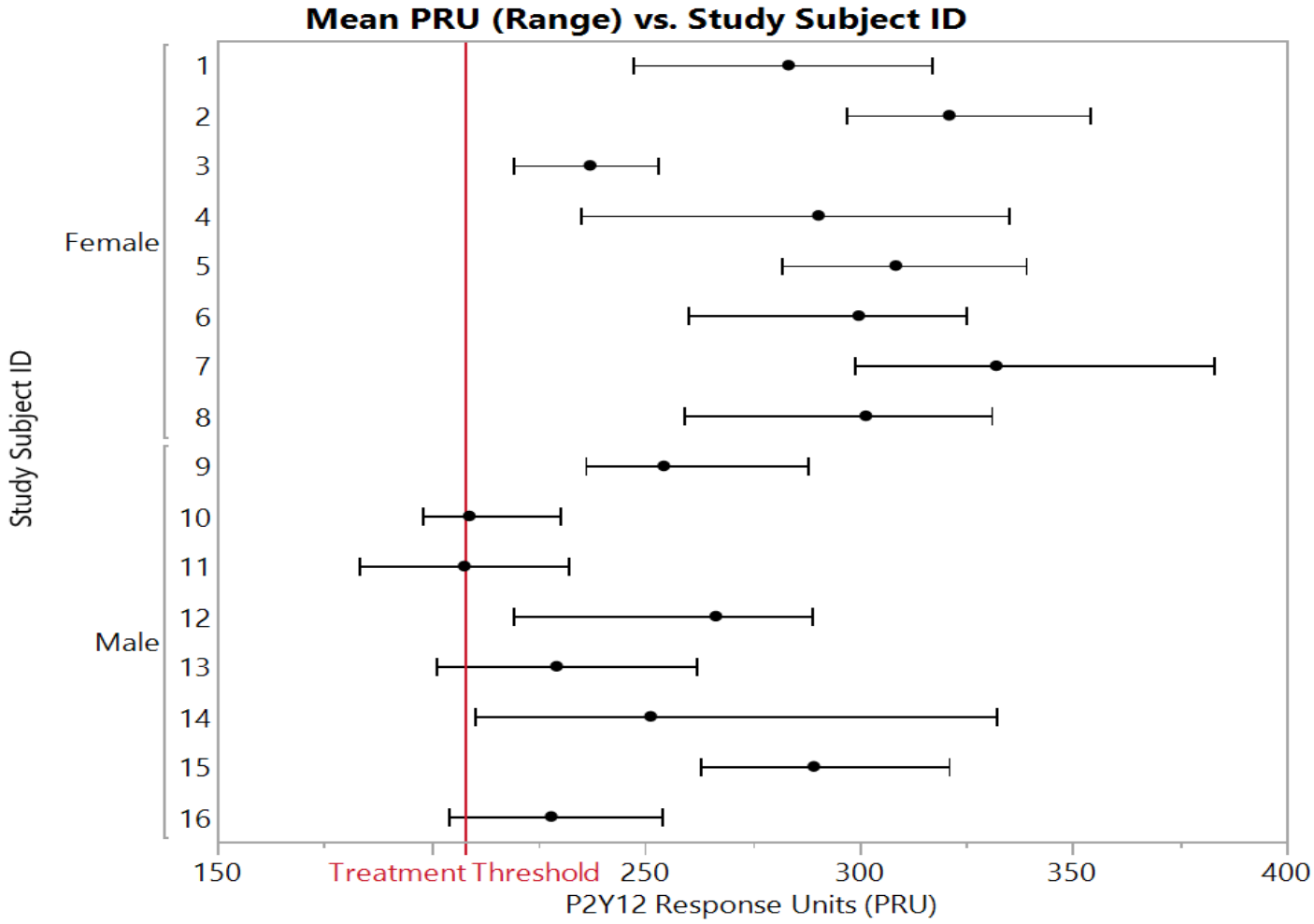
PFT monitoring for aspirin/clopidogrel response

- 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

- 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

Variability among healthy donors over time using VerifyNow PRU



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

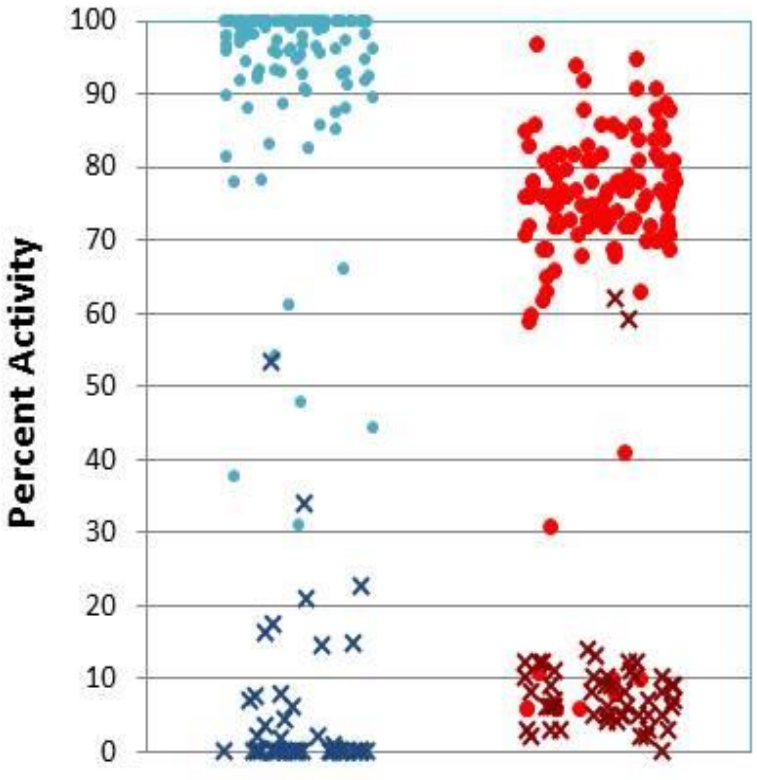
Intra- and Inter-Assay Precision

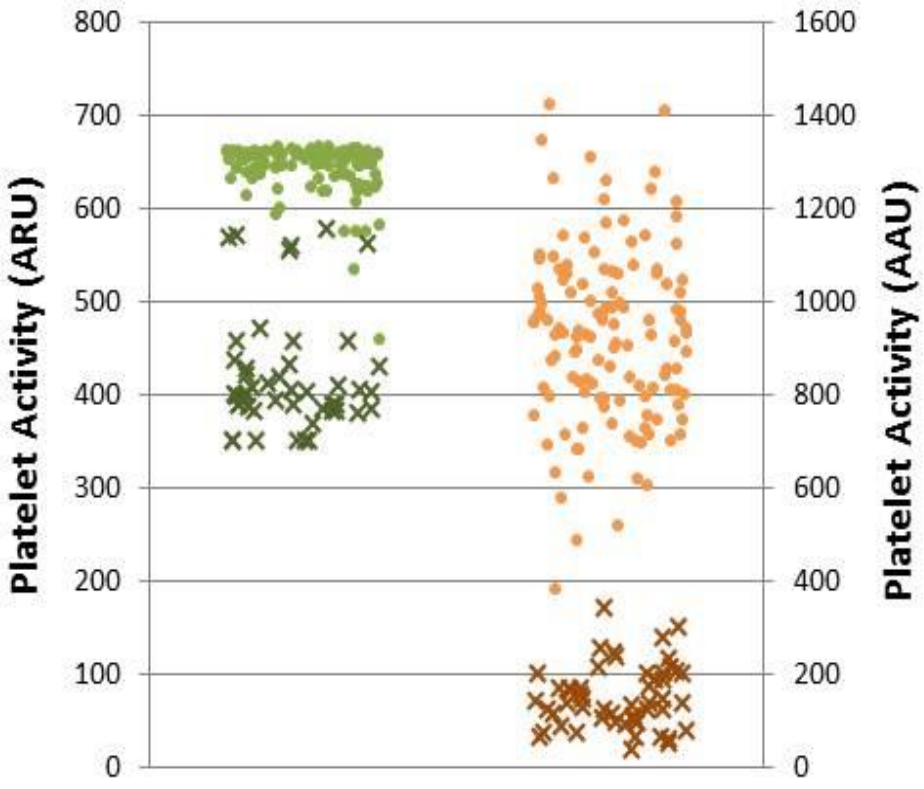
- Intra-assay precision
  - Analytic precision, estimated from duplicate analysis of samples obtained from single blood draw
- Inter-assay precision
  - Within person (between blood draw) variability
    - Obtain 2 blood sample collected within 24 hr
    - Duplicate analysis each blood sample

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





	TEG PM	LTA	VerifyNow	Multiplate
Normal (•)	94 ± 12	72 ± 19	643 ± 28	925 ± 188
Treatment (x)	5 ± 10	9 ± 11	419 ± 63	148 ± 68

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

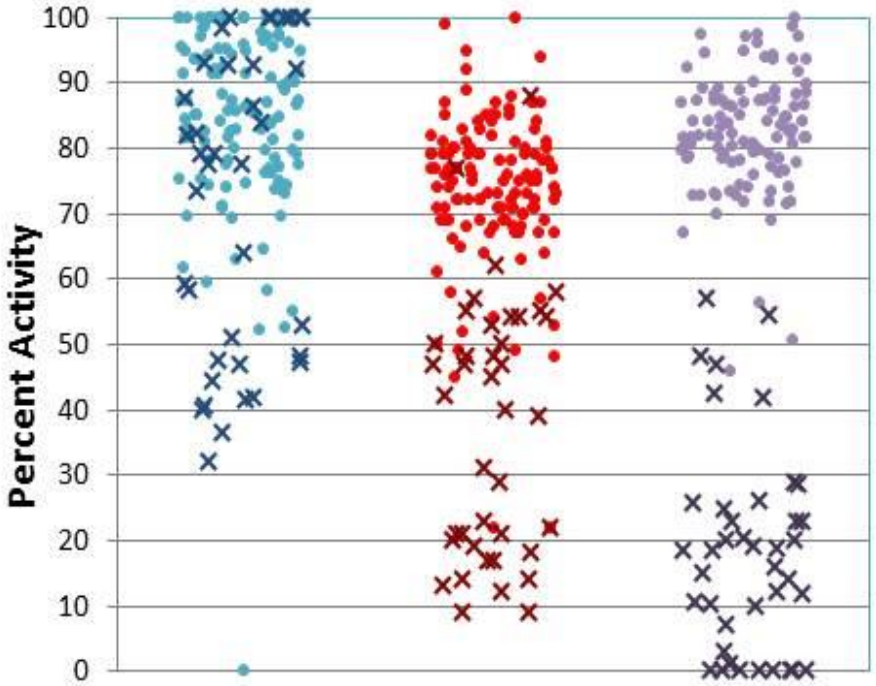
	Healthy volunteers				Treatment group			
	Intra-assay CV	N	Inter-assay CV	N	Intra-assay CV	N	Inter-assay CV	N
VN	1.4%	120	2.4%	22	3.7%	24	4.8%	10
MP	5.2%	128	9.7%	24	16.3%	26	24.7%	12
LTA	2.7%	128	7.2%	24	10.1%	26	**37.6%**	12
TEG PM	3.4%	124	6.4%	23	**95.3%**	26	**104%**	13

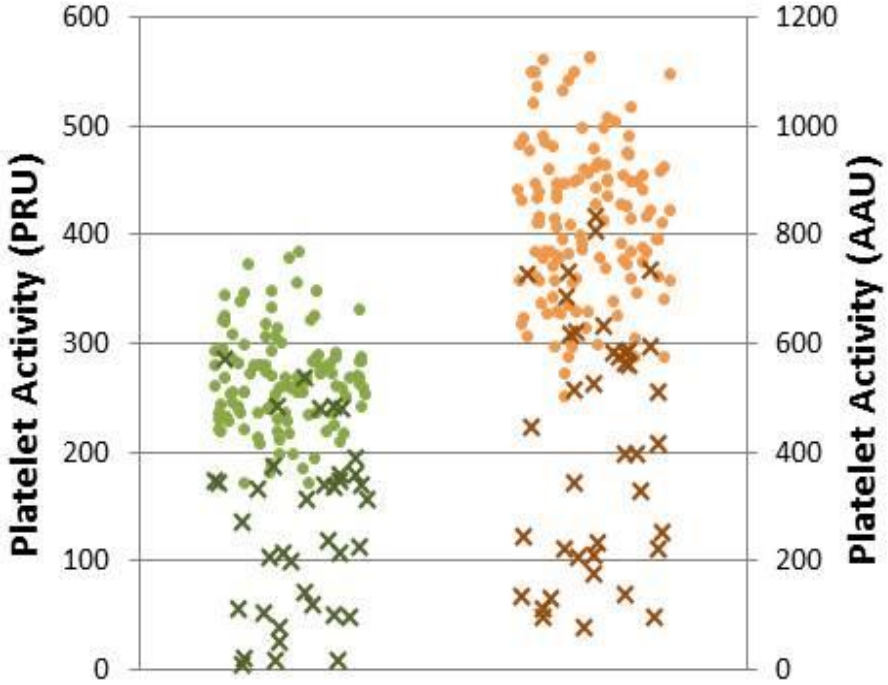
- VerifyNow (VN) best precision
- LTA (Inter-assay) and TEG PM (Intra- and Inter-assay) failed criteria for treatment group (low absolute values)

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





	TEG PM	LTA	VASP	VerifyNow	Multiplate
Normal (•)	85 ± 14	74 ± 12	84 ± 10	265 ± 44	823 ± 144
Treatment (x)	72 ± 23	38 ± 20	18 ± 16	130 ± 79	420 ± 228

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

	Healthy volunteers				Treatment group			
	Intra-assay CV	N	Inter-assay CV	N	Intra-assay CV	N	Inter-assay CV	N
VASP	1.9%	120	4.7%	21	5.0%	20	26.2%	10
VN	4.4%	118	5.2%	22	7.3%	19	12.9%	9
MP	4.3%	128	8.2%	24	8.2%	20	14.2%	10
LTA	3.3%	128	6.2%	24	5.7%	20	11.2%	10
TEG PM	6.7%	122	9.6%	23	5.5%	19	7.3%	9

- All met precision criteria

Summary—ADP-induced platelet function

- TEG PM does not distinguish healthy donors from those on clopidogrel very well (newer TEG 6S device may overcome these limitations)
- VASP best differentiates platelet function between healthy and treated donors

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

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
- Poor concordance between tests, variability within methods, but the major methods have data on cut-offs that increase risk for thrombosis or bleeding
- Little evidence to suggest that changing therapy based upon that risk is effective

28TH AACC INTERNATIONAL CPOCT SYMPOSIUM

Meeting Evolving Patient Needs Using Point-of-Care Testing

SEPTEMBER 21- 23, 2022
MONTREAL, QC, CANADA

Register Early & Save!

Deadline Ends: August 8

Join AACC to explore the latest science and cutting-edge technological advances in POCT.

Register & Save Today!
www.aacc.org/CPOCT

- Scientific Sessions
- Poster Presentations
- Exhibitor Hall
- Networking receptions

Questions