

# 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 methods 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

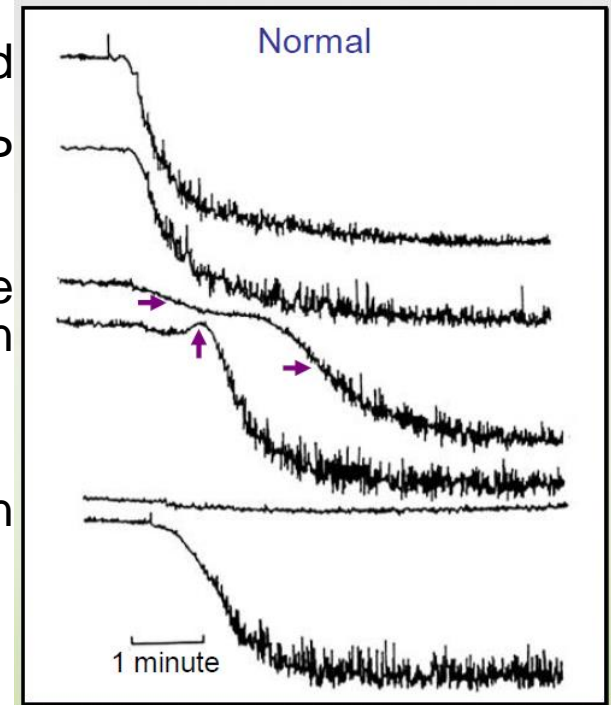
Arachidonic Acid

ADP

Epinephrine

Collagen

Ristocetin



# 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

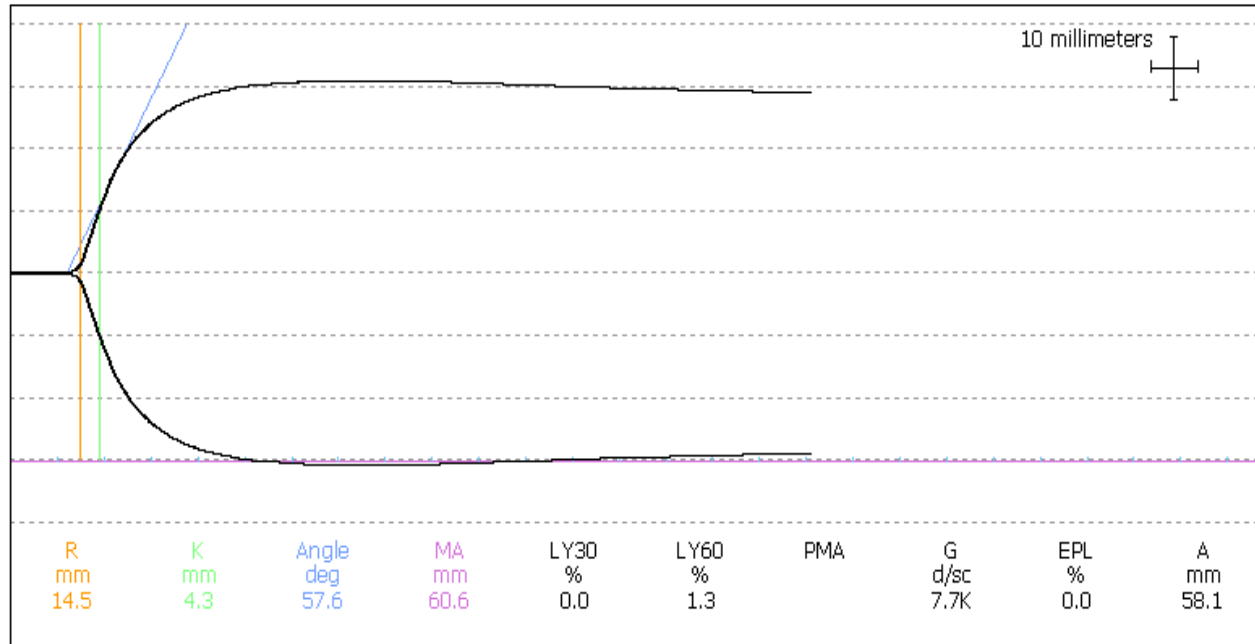
- WB cartridge-based aggregation
- Fibrinogen-coated beads in multiple wells
- Each well has plt activator
- Platelets in WB form complex with beads upon activation
- Reports as arbitrary clotting units (ARU or PRU)



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

# 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

# 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%)
  - $(\text{MFI}_{\text{PE1}} - \text{MFI}_{\text{ADP}}) / \text{MFI}_{\text{PE1}} \times 100$
  - 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<sub>12</sub> platelet receptor
  - P2Y<sub>12</sub> 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<sub>12</sub> 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

- Lordkipanidze et al., Eur Heart J 2008;29:2877-85.  
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 ( $\kappa$  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

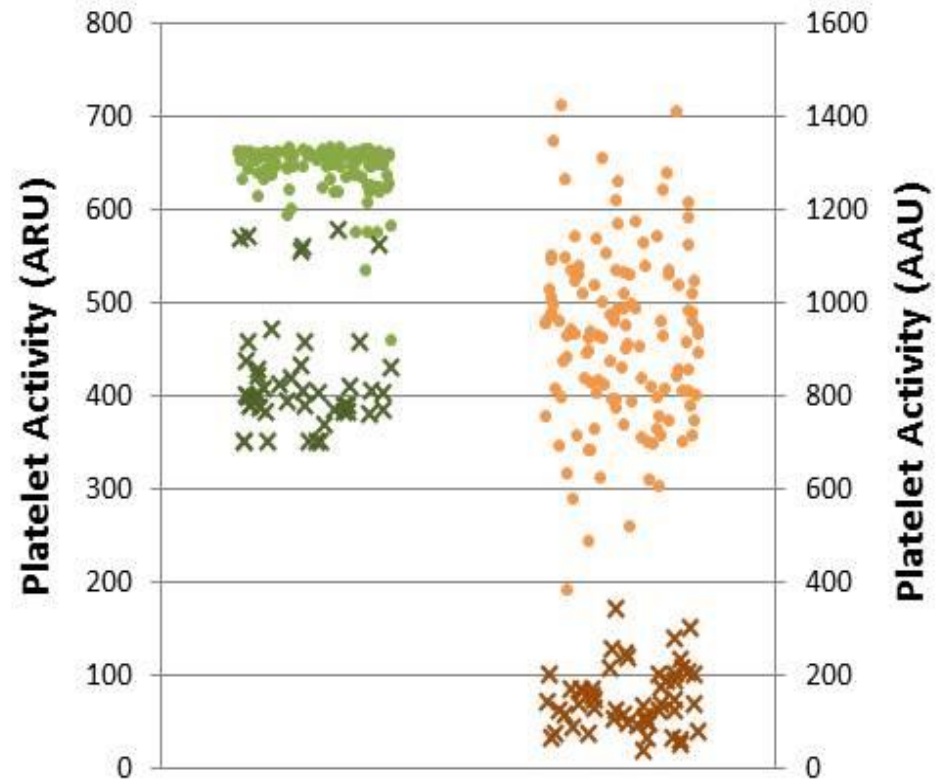
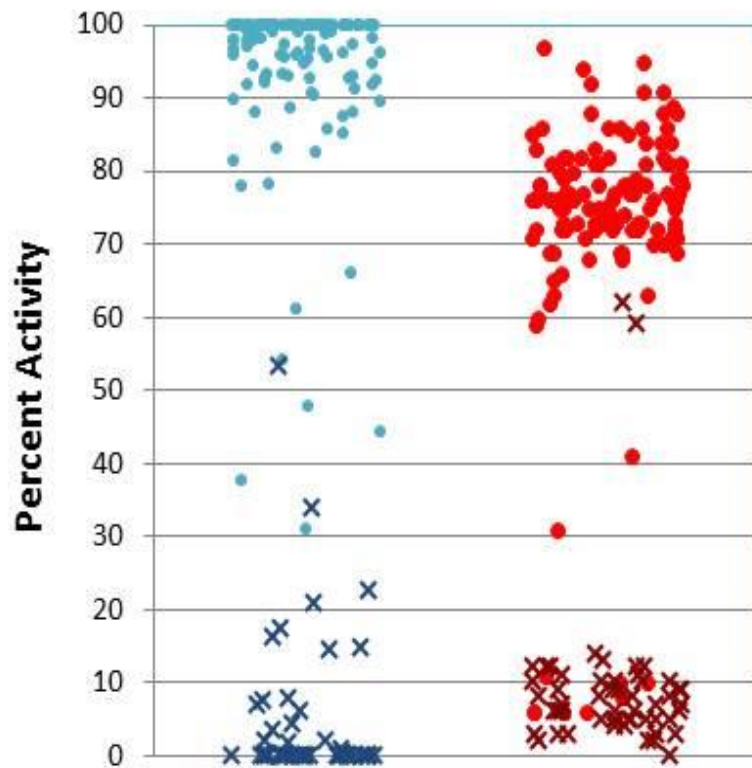
- Intra-assay precision
  - Analytic precision, estimated from duplicate analysis of samples obtained from single blood draw
- Inter-assay precision
  - Within person variability
    - Obtain 2 blood sample collected within 24 hr
    - Duplicate analysis each blood sample
    - WP variability = analytic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



	TEG PM	LTA
Normal (●)	94 ± 12	72 ± 19
Treatment (x)	5 ± 10	9 ± 11

	VerifyNow	Multiplate
Normal (●)	643 ± 28	925 ± 188
Treatment (x)	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

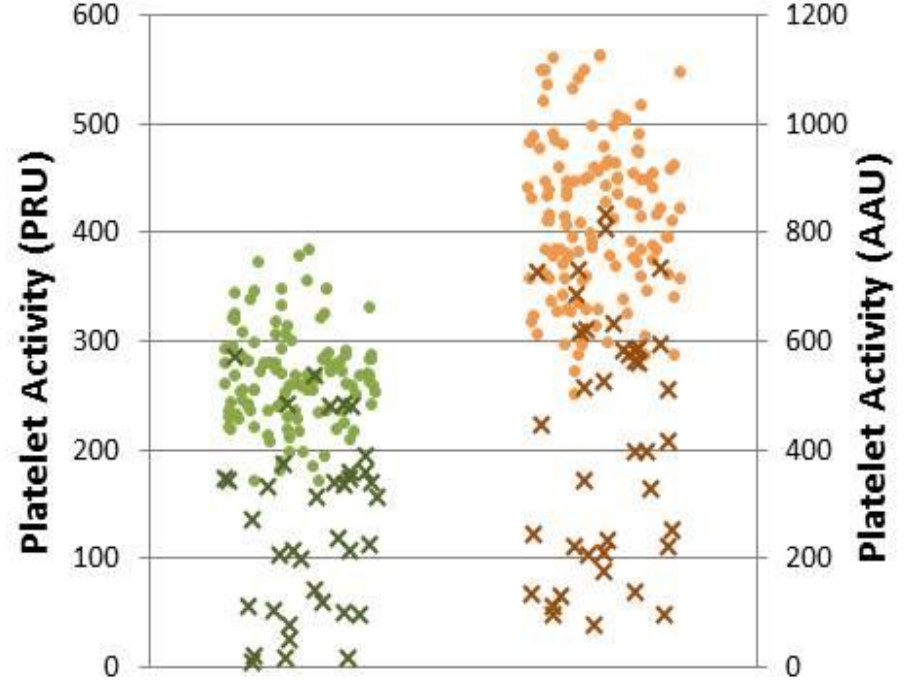
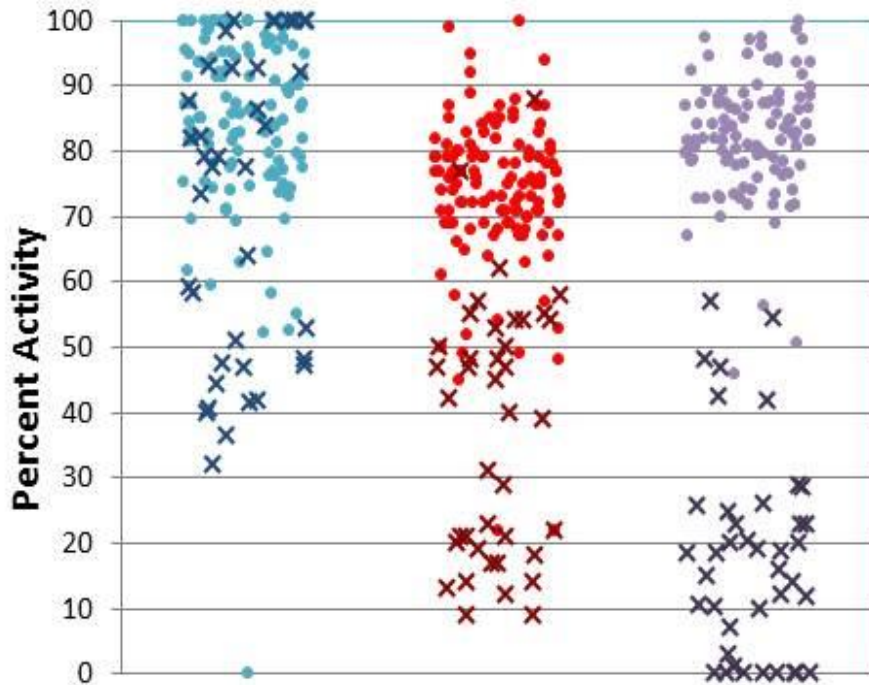
	Healthy volunteers				Treatment group			
	Intra-assay CV	N	Inter-assay CV	N	Intra-assay CV	N	Inter-assay CV	N
VN	1.4%	120	2.4%	22	3.7%	24	4.8%	10
MP	5.2%	128	9.7%	24	16.3%	26	24.7%	12
LTA	2.7%	128	7.2%	24	10.1%	26	<b>37.6%</b>	12
TEG PM	3.4%	124	6.4%	23	<b>95.3%</b>	26	<b>104%</b>	13

- 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
- 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