The AACC CPOCT Division Presents

Promoting a Culture of Quality and Consistency in Critical and Point-of-Care Testing

24th International Symposium October 4-6, 2012
Hilton Prague Hotel Prague, The Czech Republic

This activity has not been approved for category 1 CME credit because the conflict indicated by the speaker could not be resolved according to guidelines from the Accreditation Council for Continuing Medical Education.

AACC EFCC ČESKÁ SPOLEČNOST KLINICKÉ BIOCHEMIE ifcc International Federation of Clinical Chemistry and Laboratory Medicine

AACC, 1850 K Street, Suite 625, Washington, DC 20006-2213 Email: custserv@aacc.org Web: www.aacc.org

Comparative Effectiveness of the VerifyNow® P2Y12 Test and Light Transmittance Aggregometry for Assessing the Antiplatelet Effect of Clopidogrel

Jeffrey R. Dahlen, PhD,[1] Lisa Swaim,[1] Merel Kuhbauch,[1] and Victor Serebruany, MD, PhD[2]

[1]*Accumetrics, Inc., San Diego, CA;* [2]*HeartDrug Research, Johns Hopkins University, Baltimore, MD*

Background

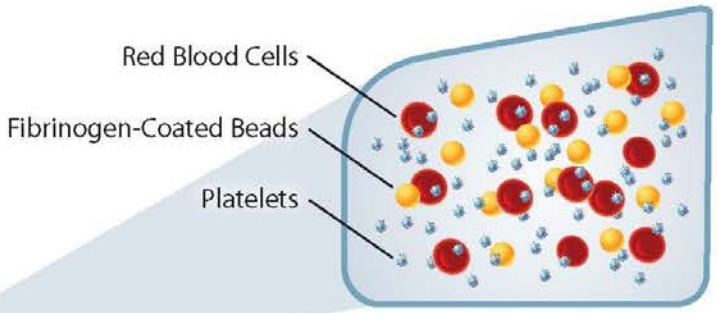
- Light transmittance aggregometry (LTA) and the VerifyNow P2Y12 Test (VN P2Y12) are the most frequently used methods for measuring the antiplatelet effect of platelet P2Y12 inhibitors
  - LTA is a historic laboratory method that uses adenosine diphosphate (ADP) to measure the effect of P2Y12 inhibitors
    - Labor intensive
    - Time-consuming
    - Operator-dependent
  - Simple and rapid alternatives to LTA were needed
- VN P2Y12 is a rapid, point-of-care method that uses ADP + prostaglandin E1 (PGE1) to measure the effect of P2Y12 inhibitors in P2Y12 Reaction Units (PRU)

VerifyNow Test Principle

Platelet response is measured as a function of an increase in light transmission through whole blood as platelets are activated by various agonists

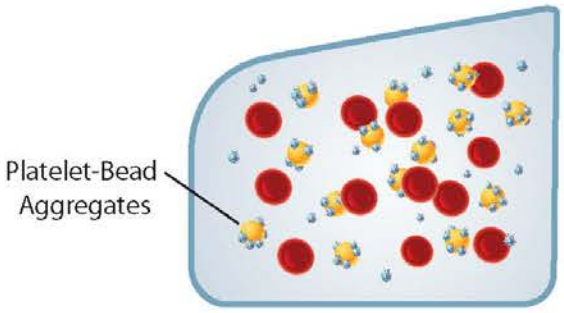
- If there is low residual platelet reactivity there is decreased light transmitted and detected.
- If there is high residual platelet reactivity there is increased light transmitted and detected.

Blood Sample Showing Inhibition of Platelet Function



Low Light Transmittance

Blood Sample Showing Normal Platelet Function



Increased Light Transmittance

VerifyNow Procedure

1. Open the cover and insert the test device

2. Insert the whole blood patient sample tube onto the assay device needle

3. Close the cover and read results, automatically displayed in < 5 minutes

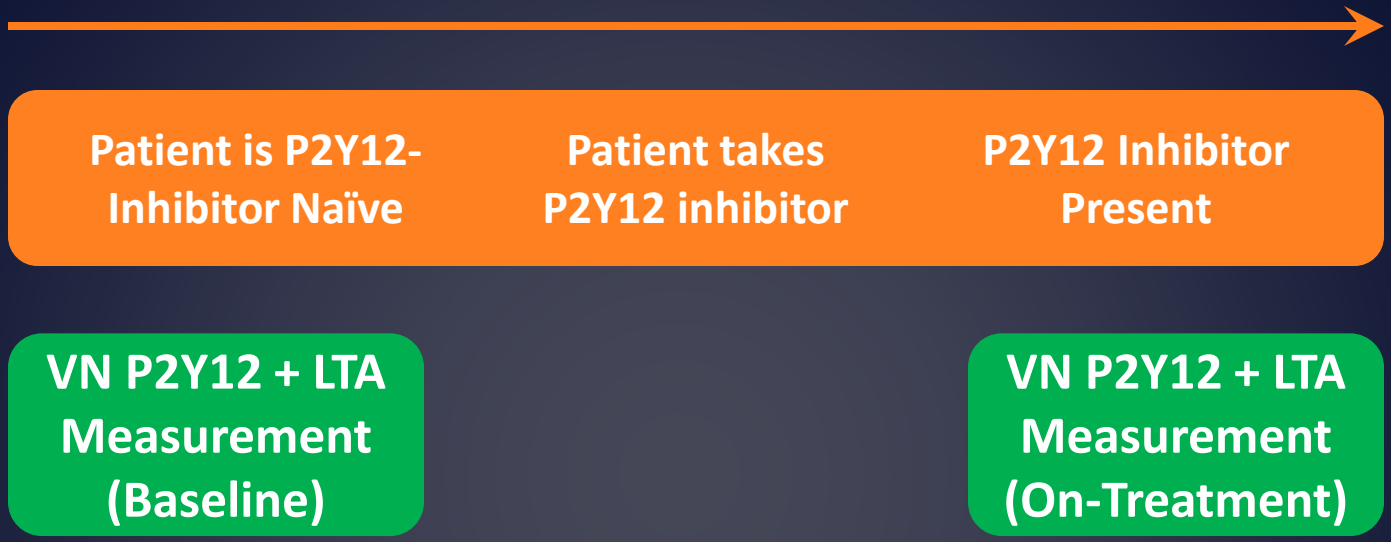
Clinical Value of PFT

- The use of a platelet P2Y12 inhibitor in addition to aspirin has been conclusively demonstrated to reduce the risk for thrombosis
- The efficacy benefit of platelet P2Y12 inhibitors is based on their pharmacodynamic effect
- Significant variability in patient response to P2Y12 inhibitor antiplatelet therapy has been well documented
- Several P2Y12 inhibitor treatment options are currently available:
  - Generic clopidogrel
  - Newer, more potent agents prasugrel and ticagrelor
- The ability to determine whether there is a measurable antiplatelet effect allows physicians to make informed treatment decisions
- The ideal test method should have a high degree of sensitivity and specificity for detecting the antiplatelet effect

Objective

- The objective of this study was to compare the diagnostic performance of VN P2Y12 and LTA for detecting the pharmacodynamic effect of a platelet P2Y12 inhibitor (clopidogrel)
  - The antiplatelet effect of platelet P2Y12 inhibitors is measured as reduced reactivity to ADP
  - The ability of the test to correctly identify samples collected when a P2Y12 inhibitor is present is identical to the test's ability to detect a significant pharmacodynamic effect

Study Design



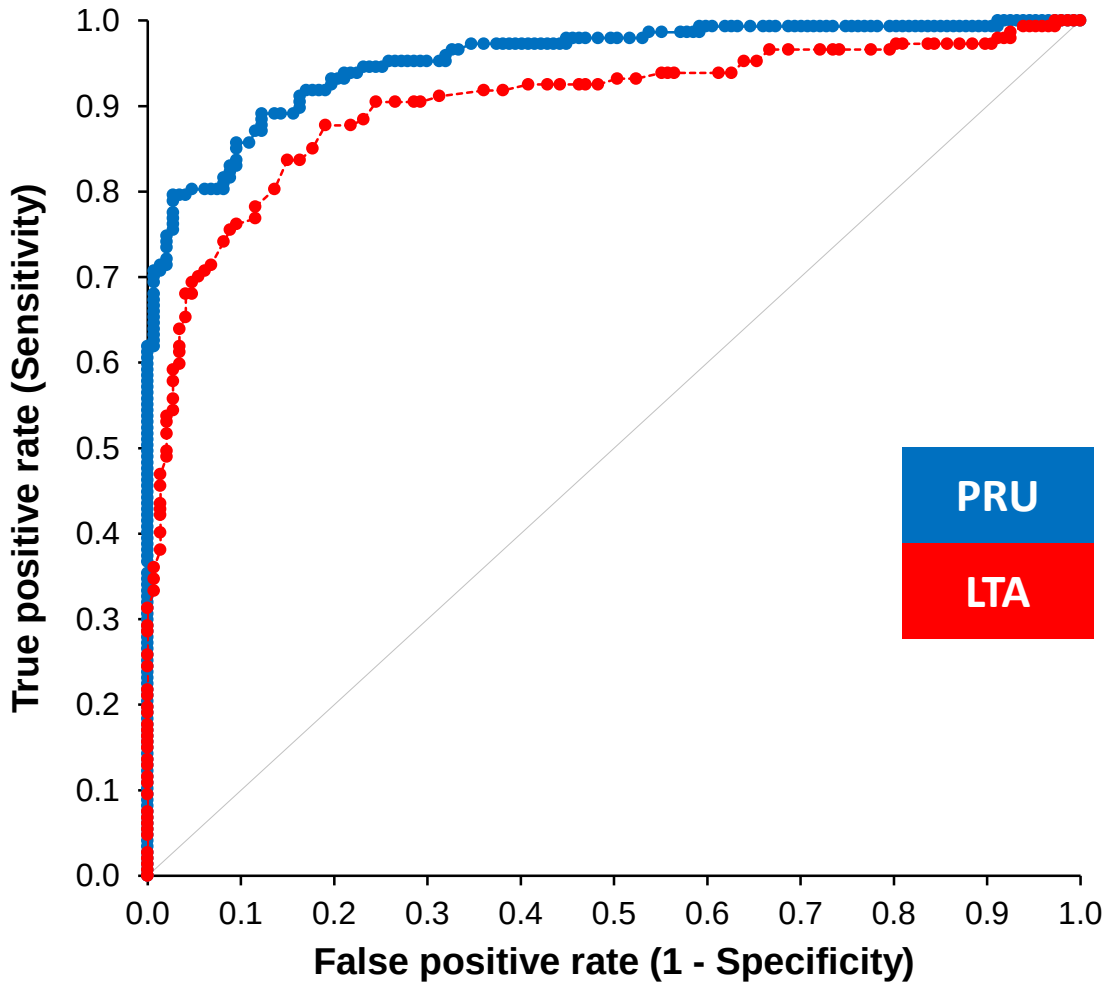
- 147 subjects with either a history of vascular disease or at least 2 risk factors for developing vascular disease.
- Subjects receiving planned treatment with clopidogrel.
  - 450 mg clopidogrel loading dose (110 patients)
  - 75 mg/day clopidogrel maintenance dose (37 patients)
- Light transmittance aggregometry (LTA) with 10 µM ADP also performed at the same time points.

Malinin A et al. *Thromb Res*. 2007; 119 (3):277-84.

Data Analysis

- Pre-drug baseline measurements and post-drug measurements were pooled for the analysis
- Because platelet P2Y12 inhibitors reduce reactivity to ADP, lower results were considered to be positive for the presence of a P2Y12 inhibitor
- Receiver operating characteristic (ROC) curve analysis was used to compare the ability of LTA and VN P2Y12 to discriminate between P2Y12 inhibitor (–) and P2Y12 inhibitor (+) samples
  - Discriminatory performance characterized by area under the curve (AUC)
  - The best cutoff was identified as the highest combination of sensitivity + specificity from the ROC analysis
- Sensitivity and specificity were calculated at relevant cutoffs
  - Sensitivity = true (+)/total P2Y12 inhibitor (+) samples
  - Specificity = true (–)/total P2Y12 inhibitor (–) samples

Results – ROC Curve Analysis



PRU AUC = 0.95

– 95% CI 0.93-0.98

– Cutoff = 208

LTA AUC = 0.90

– 95% CI 0.86-0.94

– Cutoff = 62%

Difference = 0.05

– 95% CI 0.01-0.09

– p = 0.0067

Sensitivity & Specificity for Detecting a P2Y12 Inhibitor Effect

PRU Cutoff	Sensitivity	Specificity
194 (Reference Range Limit)	72%	98%
208 (Best Cutoff by ROC Analysis)	79%	97%
230 (Cited in Literature)	86%	89%

LTA Cutoff	Sensitivity	Specificity
62% (Best Cutoff by ROC Analysis)	84%	84%
51% (Cutoff with 97% Specificity)*	59%	97%

* Same specificity as PRU = 208 cutoff

Brar SS et al. *J Am Coll Cardiol*. 2011 Nov 1; 58 (19):1945-54; Price MJ et al. *Am Heart J*. 2009 May; 157 (5):818-24.

Sensitivity & Specificity Observations

- Best cutoffs for VN P2Y12 and LTA from ROC curve analyses for detecting antiplatelet effect have been previously reported to be useful for identifying high on-treatment platelet reactivity
  - No measurable drug effect = not taking drug = increased risk
- VN P2Y12 PRU result is more specific than LTA for P2Y12 receptor blockade
  - ADP can activate platelets through both the P2Y1 and P2Y12 receptors
  - Presence of PGE1 in VN P2Y12 assay reagents improves specificity for P2Y12 receptor blockade
- VN P2Y12 PRU result is more sensitive than LTA for detecting the antiplatelet effect of P2Y12 inhibitors
  - PRU result showed 20% greater sensitivity than LTA when specificity was fixed at 97%

Gremmel et al. *Thromb Haemost* 2009;101:333–9; Campo G et al. *J Am Coll Cardiol*. 2010 Oct 26; 56 (18):1447-55; Price MJ et al. *Circulation*. 2011 Sep 6; 124 (10):1132-7; Malinin A et al. *Methods Find Exp Clin Pharmacol*. 2006 Jun; 28 (5):315-22.

Factors That Can Cause "False Negatives"

- Lack of specificity for P2Y12 receptor blockade (P2Y1 contribution)
- Time since last dose
  - Testing performed too soon after 1st dose
  - Testing performed too long after most recent dose
    - Non-compliance or physician-directed therapy interruption
- Potency of therapy
- Inter-individual variability in the response to antiplatelet therapy
  - Genetic factors
  - Concomitant medications
  - Comorbidities
  - Compliance

No measurable effect of the drug is the same as not taking the drug – is this really a "false negative"?

- No, this is described as high on-treatment platelet reactivity because there is no evidence of a pharmacodynamic effect

Conclusions

- The VerifyNow P2Y12 Test was superior to LTA for detecting the P2Y12 inhibitor effect, as evidenced by:
  - Significantly greater area under the ROC curve
  - Significantly greater sensitivity at constant specificity
  - Significantly greater specificity due to the presence of PGE1 in the VN P2Y12 assay

Conclusions (continued)

- The VerifyNow P2Y12 Test is the superior method for use in clinical settings where it is necessary to rapidly and reliably determine whether there is a measurable effect of a platelet P2Y12 inhibitor
  - Looking for confirmation of a measurable P2Y12 inhibitor effect (e.g. when treatment is being administered to reduce the risk for thrombosis)
  - Looking for the absence of a measurable P2Y12 inhibitor effect (e.g. when treatment is being interrupted to reduce the risk for antiplatelet therapy-mediated bleeding)