

Platelet reactivity and clinical application

The POPular-study

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The individual monitoring of antiplatelet therapy has gained much attention



"An aspirin a day is often prescribed for recovering heart attack patients or candidates for heart disease, but many people are resistant to the effects of aspirin. Now there's a new test to measure aspirin resistance... it's called the VerifyNow™ System."

March 17, 2004

The New York Times

"Recent studies have found that anywhere from 5 percent to more than 40 percent of aspirin users are "nonresponsive" or "resistant" to the medicine. That means that aspirin does not inhibit their blood from clotting, as it is supposed to."

"A small but growing number of doctors are starting to test patients."

July 20, 2004

PARADE

"For 20 years, Gary Burcham, a retired Navy pilot from Burbank, Calif., thought he was taking the right medication to protect his heart: a daily aspirin. But after he was diagnosed with a clogged artery, a new blood test, approved last year, revealed that he was "resistant" to aspirin's protective effects. "I had a false sense of security," says Burcham, 74, who now relies on another anti-clotting agent to prevent a heart attack."



"Twenty million Americans take a daily dose of aspirin to reduce their risk of heart disease and stroke. But now doctors are finding that aspirin, like most drugs, does not work the same in everybody."

July 21, 2004

THE WALL STREET JOURNAL

"Some labs already are offering tests that your doctor can order for you. The results may help you decide whether to increase your dose of aspirin or to consider another form of therapy, depending on your overall risk for heart problems."

Dec 28, 2004

Woman's Day

"Could you be aspirin resistant?"

"To find out if your daily aspirin isn't working, you can take a new blood test called VerifyNow™. Made by Accumetrics, the test costs about \$30 and produced results in 30 minutes."

November 1, 2004



Aspirin resistance may require a change in antiplatelet therapy

The VerifyNow test is proving its value as a quantitative device.

by Evan Young
Today in Cardiology Contributing Editor

May 2005

Clinical Laboratory News

THE AUTHORITATIVE
SOURCE FOR THE
CLINICAL LABORATORIAN

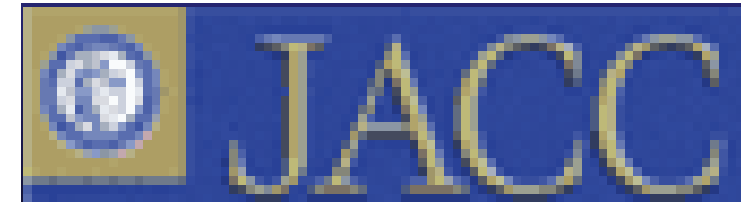
AACC

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Is Aspirin Resistance Testing Useful in Determining CVD Risk?

New POC Test Could Allow Tailored Therapy

BY PENNY ALLEN



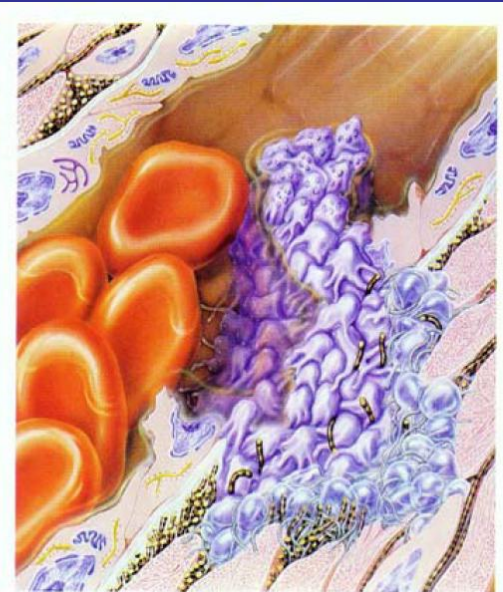
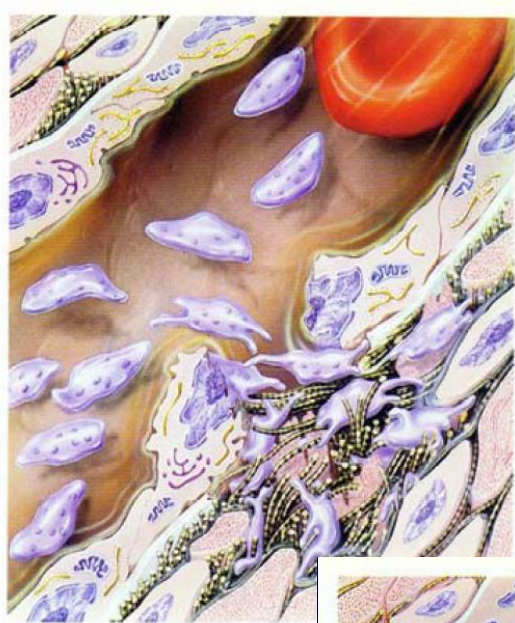
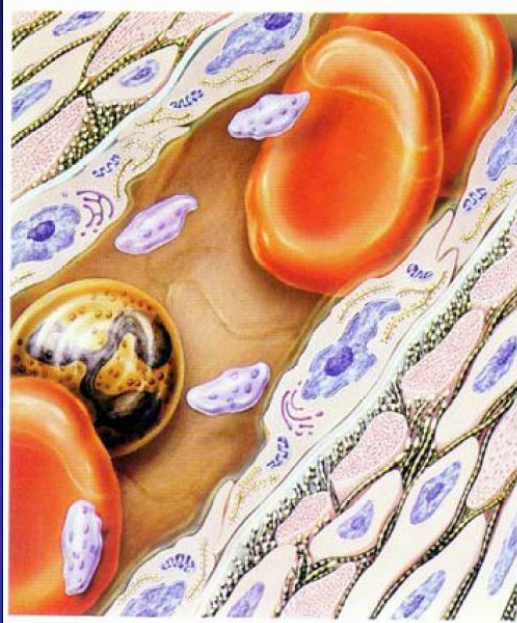
"Aspirin resistance is likely to be a major factor in stent thrombosis."

July 1, 2005

Platelets

- The key role of platelets in the pathophysiology of thrombus formation
- Variability in baseline (or intrinsic) platelet reactivity and association with atherothrombotic events

Background

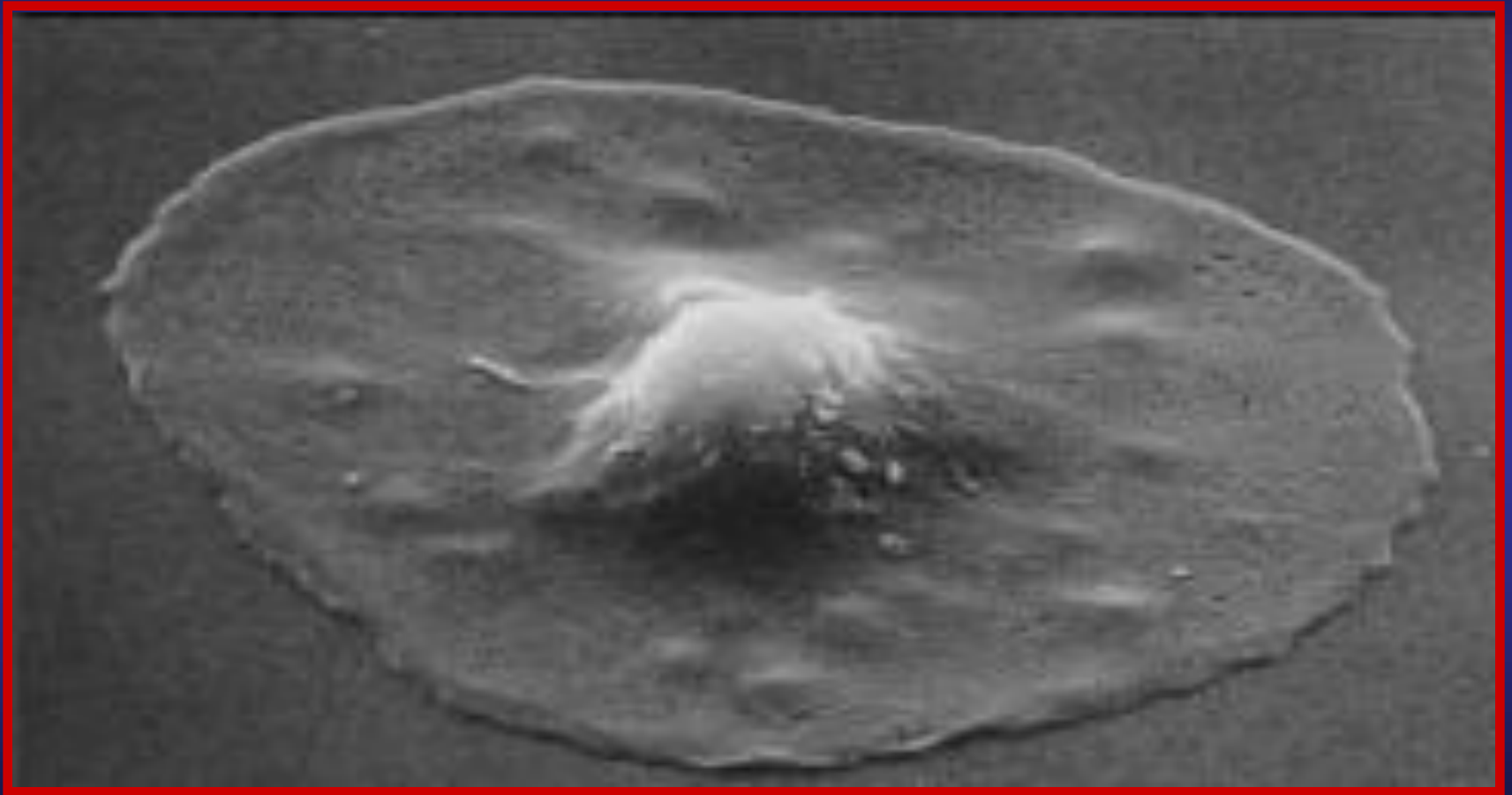


Platelet Adhesion, Activation & Aggregation

Normal Platelet



“Activating” Platelet

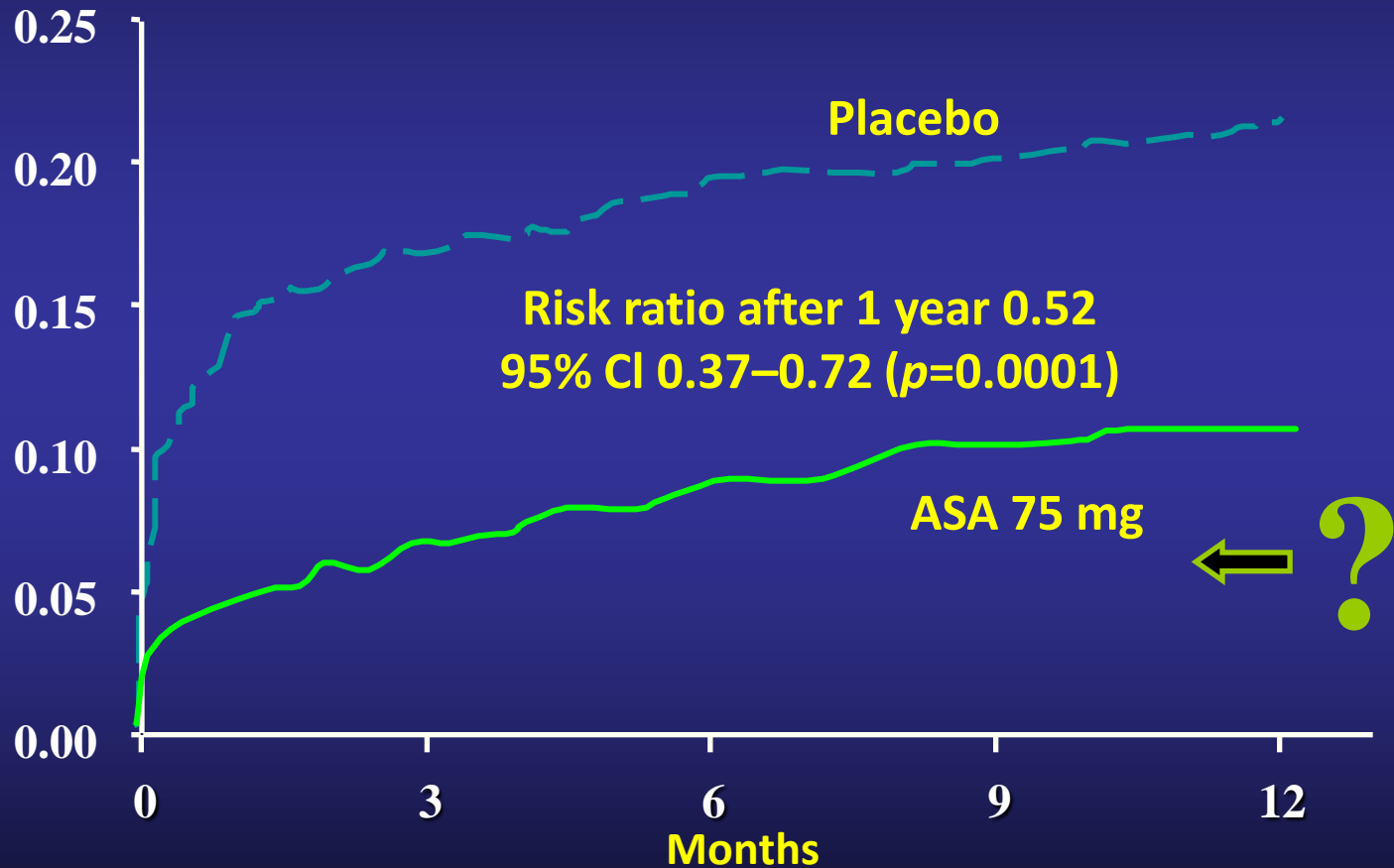


Activated Platelet

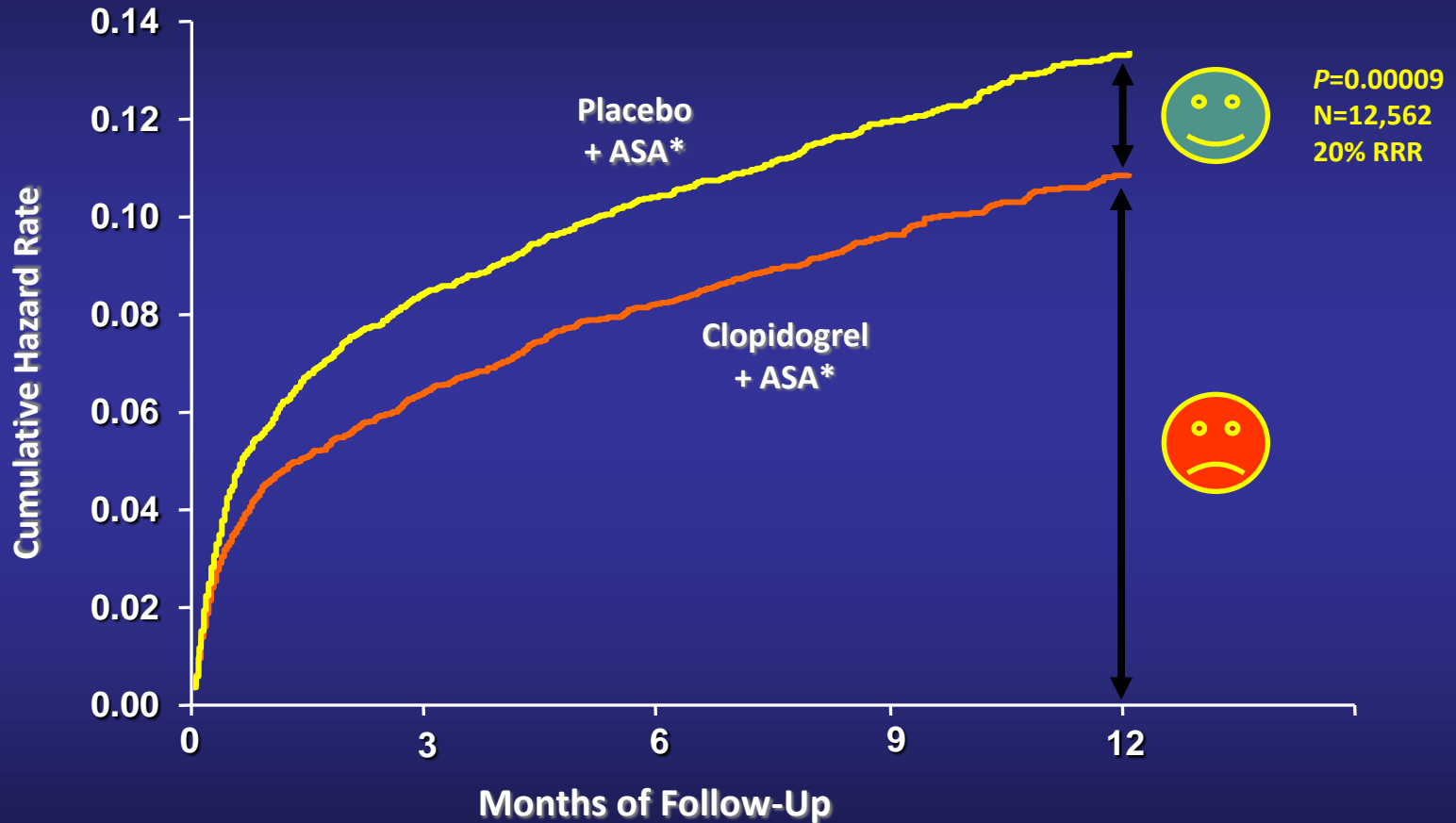


Long-term Efficacy of ASA in Reducing Death or MI in Patients with Unstable Angina

Probability
of death or MI



Primary Endpoint—MI/Stroke/CV Death



Background

- Dual antiplatelet therapy is treatment of choice in patients undergoing PCI with stent implantation^{1,2}
- Up to 36% of patients is less responsive to clopidogrel³
- High on-treatment platelet reactivity (**HPR**) is associated with thrombo-ischemic events^{4,5}
- Platelet function test that best predicts increased risk for thrombotic events unknown

¹ Steinhubl, JAMA 2002

² Mehta, Lancet 2001

³ Gurbel, J Thromb Haemost 2003

⁴ Gurbel, J Am Coll Cardiol 2005

⁵ Marcucci, Circulation 2009

Test platelet function

- Should we measure platelet function in patients undergoing PCI?
- What should we measure?

On-Treatment Reactivity & Outcome

Study	Instrument	Reagent	Setting	N	Clinical endpoint	Cut-off	Low-response rate	Hazard ratio
Hochholzer (JACC 2006)	LTA (PAP4)	5 µM ADP	Elective PCI	802	MACE (death, MI, target lesion revascularisation)	aggregation > median	50%	6.7
Geisler (EHJ 2006)	LTA (Chronolog)	20 µM ADP	PCI	379	MACE (death, MI, stroke)	aggregation > 70%	5.80%	4.9
Buonamici (JACC 2007)	LTA (APACT 4)	10 µM ADP	DES implantation	804	definite/probable stent thrombosis	aggregation > 70%	13%	3.1
Marcucci (Circulation 2009)	VerifyNow	P2Y12 assay	PCI/ACS	683	CV death / nonfatal MI	>240 PRU (ROC analysis)	32%	2.55/3.36
Price (EHJ 2008)	VerifyNow	P2Y12 assay	DES implantation	380	stent thrombosis (definite, probable, possible), CV death, nonfatal MI	> 235 PRU (ROC analysis)	32%	ND
Patti (JACC 2008)	VerifyNow	P2Y12 assay	PCI	160	MACE (death, MI, target lesion revascularisation)	PRU in upper quartile	25%	6.1
Bonello (JTH 2007)	VASP	P2Y12 assay	PCI	144	MACE (death, stroke, revascularization)	PRI >50%	80%	ND

→ Association of high on-treatment reactivity and clinical outcome

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- Small sample size
- Different cut-off values (upper quartile) and not using Receiver Operating Characteristics curve analysis
- Only one test per study

Do Platelet Function Assays Predict Clinical Outcomes in clopidogrel pretreated patients undergoing elective PCI

The POPular-study

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Henk T Ruven, Egbert T Bal,
Vera H Deneer, Ankie M Harmsze, Benno JWM Rensing,
Maarten J Suttorp, Jan AS van der Heyden,
Christian M Hackeng, Jurriën M ten Berg,

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Comparison of Platelet Function Tests in Predicting Clinical Outcome in Patients Undergoing Coronary Stent Implantation

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Context High on-treatment platelet reactivity is associated with atherothrombotic events following coronary stent implantation.

Objective To evaluate the capability of multiple platelet function tests to predict clinical outcome.

Design, Setting, and Patients Prospective, observational, single-center cohort study of 1069 consecutive patients taking clopidogrel undergoing elective coronary stent implantation between December 2005 and December 2007. On-treatment platelet reactivity was measured in parallel by light transmittance aggregometry, VerifyNow P2Y12 and Plateletworks assays, and the IMPACT-R and the platelet function analysis system (PFA-100) (with the Dade PFA collagen/adenosine diphosphate [ADP] cartridge and Innovance PFA P2Y). Cut-off values for high on-treatment platelet reactivity were established by receiver operating characteristic curve analysis.

Main Outcome Measurement The primary end point was defined as a composite of all-cause death, nonfatal acute myocardial infarction, stent thrombosis, and ischemic stroke. The primary safety end point included TIMI (Thrombolysis in Myocardial Infarction) criteria major and minor bleeding.

POPular Study

- Aim
 - To identify the platelet function tests that predict clinical outcome
- Which tests?
 - Light transmittance aggregometry (LTA) (5 & 20 $\mu\text{mol/L}$ ADP)
 - VerifyNow[®] P2Y12
 - Plateletworks[®]
 - IMPACT-R
 - IMPACT-R ADP
 - PFA-100 COL/ADP
 - INNOVANCE[®] PFA P2Y*

Platelet Function Tests

Light transmittance aggregometry (LTA)

Aggregation based, platelet rich plasma (PRP)
5 & 20 $\mu\text{mol/L}$ ADP peak platelet aggregation
Laboratory, trained technicians, time consuming



VerifyNow[®] P2Y12 assay

Aggregation based, whole blood
Bedside test, fully automated



Plateletworks[®]

Single platelet count, whole blood
Stimulation with ADP
Bedside test, semi-automated
Highly time dependent, performing < 10 minutes



Tests more physiological shear-dependent

IMPACT-R

Adhesion and shear-stress based,
whole blood
Extensive sample handling
Both with and without ADP-
stimulation



PFA-100[®] system

Shear-stress based, whole blood
Bedside test
Collagen/ADP cartrich

INNOVANCE[®] PFA P2Y*
ADP, PGE₁ and calciumchloride



Patient Characteristics

n=1069

Age - yr	64 ± 10.6
BMI – kg/m ²	27.2 ±4.0
Male - no (%)	790 (73.9%)
Diabetes Mellitus	200 (18.7%)
Hypercholesterolemia	859 (80.4%)
Hypertension	824 (77.1%)
Family Hx of CAD	654 (61.2%)
Current smoking	121 (11.3%)
Prior MI	487 (45.6%)
Prior PCI	347 (32.5%)
Prior CABG	120 (11.2%)
BMS only	388 (36.3%)
DES only	612 (57.2%)
BMS + DES	69 (6.5%)
Bifurcation	33 (3.1%)
LAD	514 (48.1%)

POPular

Consecutive patients on clopidogrel treatment undergoing elective PCI with stent implantation

n=1069

Measurements of on-treatment platelet reactivity in parallel

LTA 5 $\mu\text{mol/L}$ ADP
n=1049

LTA 20 $\mu\text{mol/L}$ ADP
n=1051

VerifyNow[®] P2Y12
n=1052

Plateletworks[®]
n=606

IMPACT-R
n=910

IMPACT-R ADP
n=905

PFA-100 COL/ADP
n=812

INNOVANCE PFA[®]P2Y*
n=588

POWER CALCULATION
n=800 per test

Primary Endpoint at one-year

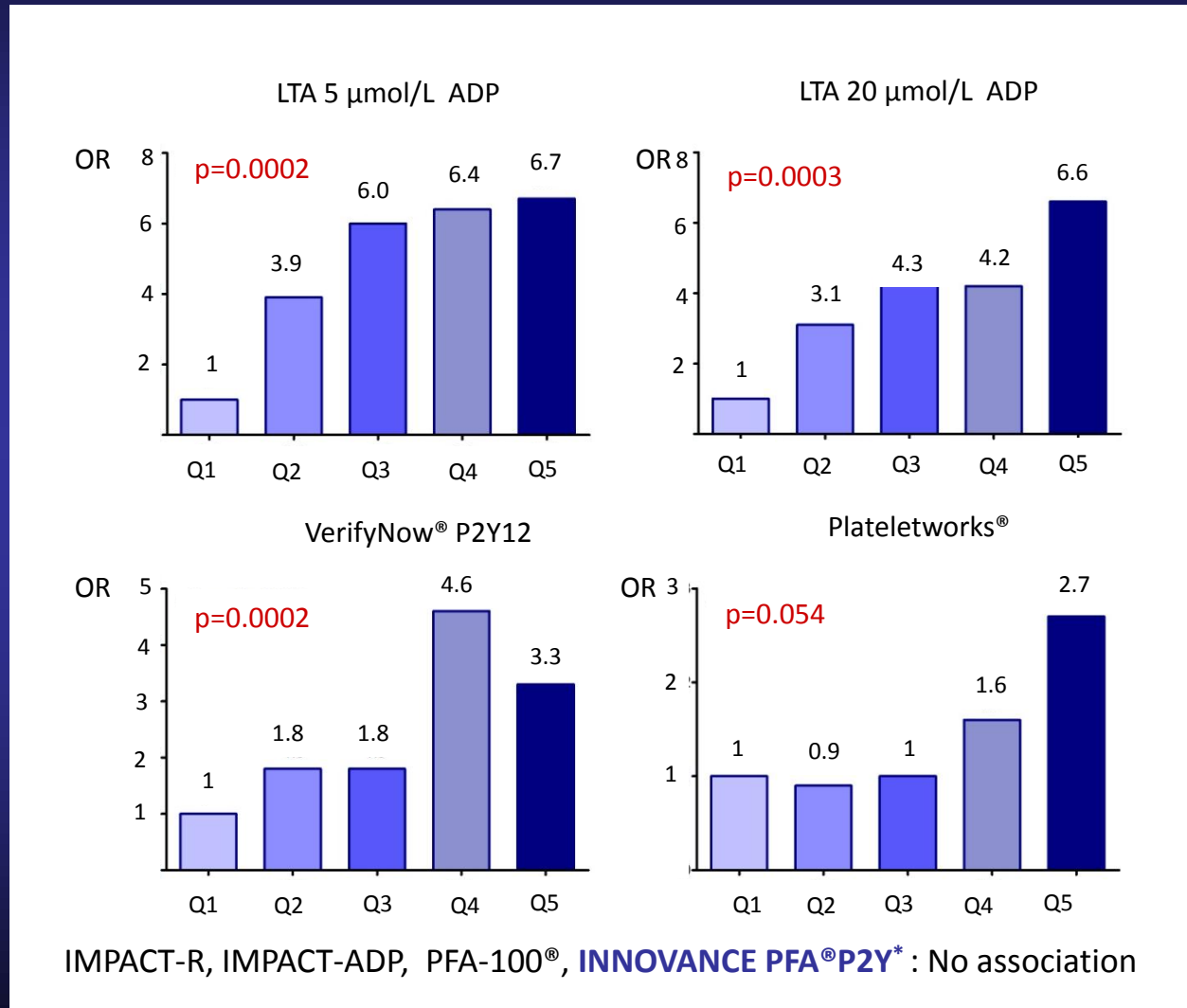
Composite of death, myocardial infarction, stent thrombosis and stroke

Primary Safety Endpoint at one year

TIMI major and minor bleeding

Results: On-Treatment Platelet Reactivity

When divided into quintiles there appeared to be a dose-response relationship



Composite of death, non-fatal myocardial infarction, definite stent thrombosis and stroke

Results

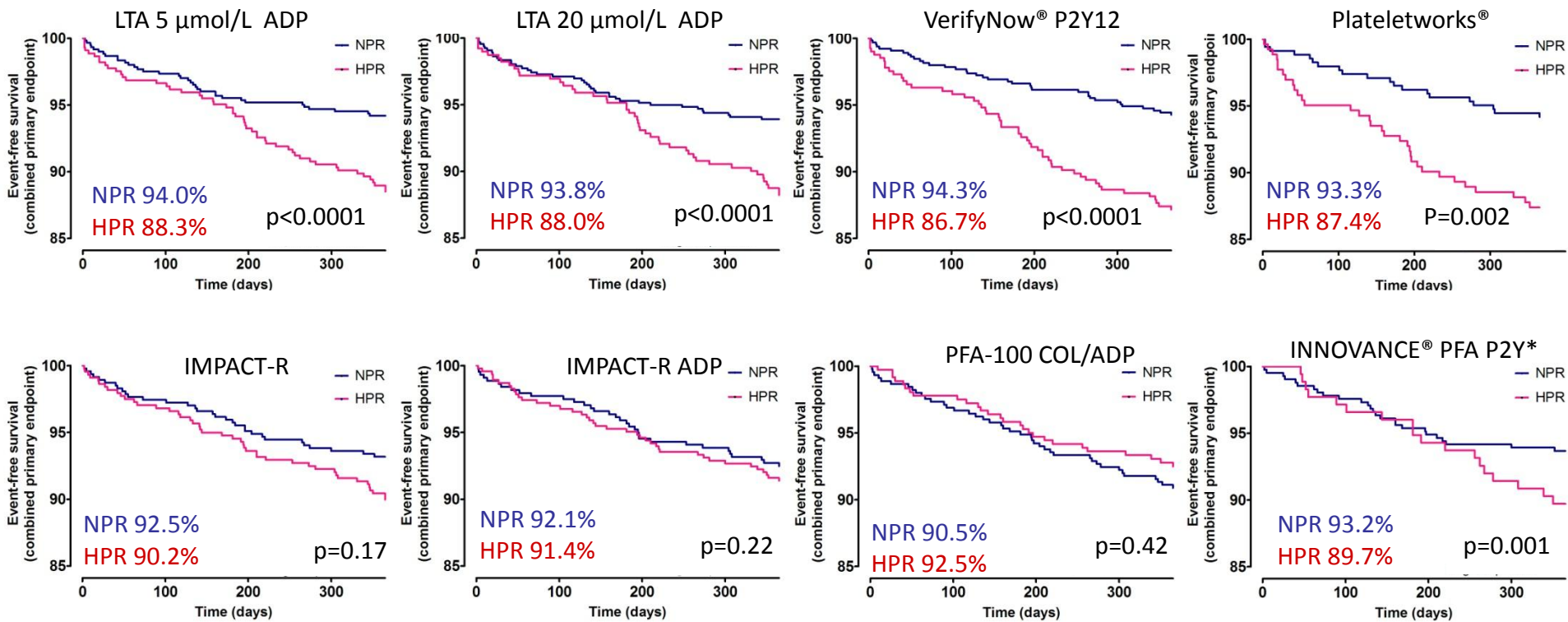
- Receiver Operator Characteristic (ROC) Curve analysis
 - Based on one-year primary endpoint
 - To determine cut-off for high-on treatment platelet reactivity (HPR) for every test

	AUC	Cut-off
LTA 5 μ mol/L ADP	0.63	42.9%
LTA 20 μ mol/L ADP	0.62	64.5%
VerifyNow [®] P2Y12	0.62	236 PRU
Plateletworks [®]	0.61	80.5%
IMPACT-R	0.56	8.4% SC
IMPACT-R ADP	0.53	3.0% SC
PFA-100 COL/ADP	0.50	116 s
INNOVANCE [®] PFA P2Y*	0.56	299 s

Results

Survival free from Primary Endpoint

Composite of death, non-fatal myocardial infarction, definite stent thrombosis and stroke



HPR = high-on treatment platelet reactivity

NPR = non-HPR

Results

Composite of death, non-fatal myocardial infarction, definite stent thrombosis and stroke

Platelet function tests	OR (95% CI)	p
LTA 5 μ mol/L ADP	2.09 (1.34 – 3.25)	0.0009
LTA 20 μ mol/L ADP	2.05 (1.32 - 3.19)	0.001
VerifyNow [®] P2Y12	2.53 (1.63 – 3.91)	<0.0001
Plateletworks [®]	2.22 (1.25 – 3.93)	0.005
IMPACT-R	1.34 (0.84 – 2.14)	0.21
IMPACT-R ADP	1.11 (0.69 - 1.78)	0.68
PFA-100 [®] COL/ADP	0.77 (0.47 – 1.28)	0.31
INNOVANCE [®] PFA P2Y*	1.59 (0.85 – 2.94)	0.15

Clinical risk factors

LVEF<45%	1.99 (1.20-3.30)	0.008
Renal Failure	2.29 (1.23-4.24)	0.009
Prior CABG	2.17 (1.22-3.81)	0.008
Age per 10 yrs increase	1.38 (1.12-1.71)	0.003

Predictive model

- Logistic regression modelling
 - Determine predictive value of addition novel risk factor: **HPR**
 - Identify independent correlates primary endpoint
 - Model predicting the primary endpoint

Model 1

Classic Risk factors

Age, Gender, Hypertension, Hypercholesterolemia, Diabetes Mellitus, Current smoking, Family Hx of CAD, LVEF<45%, Renal failure, Prior CABG

AUC = 0.64

Model 2

Model 1 + Procedural Risk Factors

Total stent length, no.of lesions, no.of stents, LAD-stenting, graft-stenting, bifurcation lesion

AUC=0.64 → AUC= 0.72, p=0.004

Model 3

Model 2 + HPR

AUC=0.72 → + HPR?

Results

Model 2

AUC = 0.72

Classic Risk Factors

Age, Gender, Hypertension, Hypercholesterolemia, Diabetes Mellitus, Current smoking, LVEF<45%, Renal failure

Procedural Risk Factors

Total stent length, no.of lesions, no.of stents, LAD-stenting, graft-stenting, bifurcation lesion

Model 2 + HPR

AUC

p-value

- LTA 5 µmol/L ADP	0.74	0.004
- LTA 20 µmol/L ADP	0.73	<0.0001
- VerifyNow® P2Y12	0.74	<0.0001
- Plateletworks®	0.78	0.001
- IMPACT-R	0.72	0.17
- IMPACT-R ADP	0.72	0.92
- PFA-100® COL/ADP	0.73	0.15
- INNOVANCE® PFA P2Y*	0.73	0.24

Results

Primary Safety Endpoint

- Receiver Operator Characteristic (ROC) Curve analysis
 - Based on one-year primary safety endpoint
 - To determine cut-off for every test

None of the tests able to discriminate between patients with and without TIMI major and minor bleeding

Conclusion

- POPular first study with multiple platelet function tests
- Provides cut-off values for LTA, VerifyNow and Plateletworks based on ROC-curve analysis
- LTA, VerifyNow and Plateletworks are able to identify patients at higher risk
- IMPACT-R, IMPACT-ADP, PFA-100 COL/ADP and INNOVANCE® PFA P2Y* are unable to identify higher-risk patients
- None of the tests were able to identify high-risk patients for bleeding

Conclusion

- The predictive accuracy of the 4 tests was only modest in this low-risk population undergoing elective stenting
- The negative predictive value of the 4 tests was high

How to use platelet function tests in patients undergoing elective PCI?

Table 2. Area Under the Receiver Operating Characteristic Curve for Prediction of Composite Outcome

Values by Test	Light Transmittance Aggregometry		VerityNow P2Y12	Plateletworks	IMPACT-R		PFA-100 Collagen/ADP	Innovance PFA P2Y
	5 µmol/L	20 µmol/L			Spontaneous	ADP Stimulated		
AUC, % (95% CI)	0.63 (0.58-0.68)	0.62 (0.56-0.67)	0.62 (0.57-0.67)	0.61 (0.53-0.69)	0.56 (0.50-0.62)	0.53 (0.48-0.59)	0.50 (0.46-0.55)	0.56 (0.48-0.63)
Sensitivity, % (95% CI)	60.2 (49.8-69.8)	54.6 (44.2-64.5)	60.4 (50.2-69.9)	63.0 (49.8-74.6)	56.4 (45.4-66.8)	44.0 (33.3-55.3)	62.9 (51.2-73.2)	60.9 (48.5-73.6)
Specificity, % (95% CI)	59.1 (56.0-62.2)	63.9 (60.8-66.8)	63.1 (60.0-66.1)	58.5 (54.4-62.6)	52.5 (49.1-55.9)	53.5 (50.1-56.9)	44.3 (40.8-47.8)	29.0 (25.3-32.8)
Optimal cut-off, % ^a	42.9	64.5	236 ^b	80.5	8.4 ^b	3.0 ^b	115 ^b	233 ^b
NPV, %	94	93.8	94.3	93.9	90	91.2	92.5	89.7
PPV, %	11.7	12	13.3	12.6	7.2	7.7	5.3	4.6
Values for Backward Regression Models^c								
AUC, %	0.73	0.73	0.74	0.77	0.72	0.72	0.72	0.72
P-value for addition to model ^d	.004	.001	<.001	.001	.2	.83	.15	.27

Abbreviations: ADP, adenosine diphosphate; AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

^aCalculated by determining smallest distance between receiver operating characteristic curve and upper left corner of the graph.

^bCut-off % units are P2Y12 reaction units for VerityNow, % surface coverage for IMPACT-R spontaneous and IMPACT-R ADP stimulated tests, and closure time in seconds for the PFA-100 system.

Negative Predictive Value > 93%

Table 1. ROC Analysis for Selected Platelet Function Tests

	LTA (5 μ mol)	LTA (20 μ mol)	VerifyNo w	Plateletwor ks
AUC, % (95% CI)	0.63	0.62	0.62	0.61
Sensitivity	60.2	54.6	60.4	63.0
Specificity	59.1	63.9	63.1	58.5
Optimal cut- off	42.9	64.5	236	80.5
NPV, %	94	93.8	94.3	93.9
PPV, %	11.7	12	13.3	12.6
AUC for regression	0.73	0.73	0.74	0.77
p value for addition to model	0.004	0.001	<0.001	0.001

How to use platelet function tests in patients undergoing elective PCI?

- 75 % of the patients tested have a residual platelet reactivity below cut-of value
 - <42.9% aggregation; 64.5% aggregation
 - <236 PRU
- Those patients are at **very low risk** of thrombotic events (tests have high >90% NPV. Thus clopidogrel (generic low cost) works fine in this low-risk group
-
- If you treat those patients with Prasugrel the absolute **risk reduction** would be relatively small, at the cost of an **increased bleeding** hazard

How to use platelet function tests in patients undergoing elective PCI?

- Thus, use aggregation based platelet function tests with well defined (ROC analysis based) cut-off values to define patients at low risk for thrombotic events and treat those with safe low cost clopidogrel
- Treat remaining 25% of the patients with stronger anti-platelet agents

Successful PCI with DES without major complication or GPIIb/IIIa use

N ~ 6600

Post-PCI VerifyNow Assays 12-24 hours post-PCI

Yes

PRU \geq 230?

No

Responder

Non-Responder

Random Selection

A

N = 1100

B

N = 1100

C

N = 583

“Tailored Therapy”
clopidogrel 150-mg/day

“Standard Therapy”
clopidogrel 75mg +placebo/day

“Standard Therapy”
clopidogrel 75mg +placebo/day

Clinical Follow-up And VerifyNow Assessment at 30days, 6M

Primary Endpoint: 6 month CV Death, Non-Fatal MI, ARC definite/prob ST

Flow-chart **TRIGGER-PCI** study

Successful **PCI** with DES without major complication and NO GPIIb/IIIa use

N ~6,500

Post-**PCI** VerifyNow P2Y12 Assay (PRU)
2 - 4 hours after 1st MD of clopidogrel 75 mg at day 1 post-**PCI**

Non-Responder

Yes

PRU > 208

No

Responder

A N = 1075

"Prasugrel arm"
Prasugrel 60 mg LD
Prasugrel 10 mg MD
+ Clopidogrel placebo

B N = 1075

"Clopidogrel arm"
Placebo LD
Clopidogrel 75 mg MD
+ Prasugrel placebo

C N = 4,350

"Standard Therapy"
Clopidogrel 75 mg

Non-interventional
study (Registry)

N = 2,150 → 33%

Clinical Follow-up and blinded VerifyNow Assessment at 90 days, 180 days

Primary Endpoint: 6 month CV Death and MI