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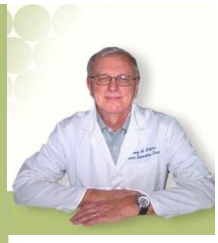

Holiday Inn By The Bay
NORTHEAST LABORATORY CONFERENCE
October 21-23
2009
PORTLAND, MAINE

Does Aspirin Work?

Monitoring Antiplatelet Response

George A. Fritsma, MS MT (ASCP)
The Fritsma Factor
Your Interactive Hemostasis Reference
www.fritsmafactor.com ~ george@fritsmafactor.com

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Participants…

- Provide the history and pharmacology of aspirin
- Review the history and pharmacology of thienopyridines clopidogrel (Plavix®) and prasugrel (Effient®)
- Outline current antiplatelet therapy regimens
- Review literature on prevalence and causes of antiplatelet therapy failure
- Perform antiplatelet therapy monitoring assays
- Interpret assay results
- Determine if antiplatelet therapy monitoring is effective

For a copy of this presentation, go to www.fritsmafactor.com.

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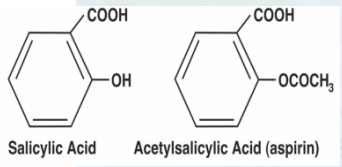

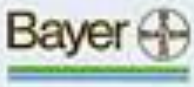

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Felix Hoffman; 1897

- August 10, 1897: Felix Hoffman synthesized pure, stable acetyl salicylic acid
  - Bayer laboratories in Leverkusen, Germany
- Aspirin: a = acetyl; spir = Spirea (plant)
- 1900: Bayer mixes aspirin with starch to make the first drug in tablet form
  - No prescription: 5 grains [~325 mg]
  - Uruguayan stamp shows Hoffman, willow branch, and his signature taken from the laboratory record

Mann CC, Plummer ML. The Aspirin Wars: Money, Medicine, and 100 Years of Rampant Competition. New York: Knopf 1991.

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Lawrence Craven MD: 1948

- 1948: Dr. Lawrence Craven, California GP noticed 400 men for whom he had prescribed aspirin had no heart attacks
  - Recommended aspirin a day to reduce risk of heart attacks
- 1971: JB Smith demonstrated aspirin's inhibition of platelet prostaglandins

Craven LL. Acetylsalicylic acid, possible preventive of coronary thrombosis. Ann Western Med 1950;4: 95–9.
Vane JN. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 1971;231:232–5.
Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nature 1971; 231: 235–7.

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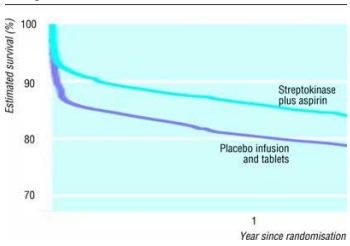
Aspirin Efficacy: ISIS-2

- 1988: ISIS-2 demonstrates 0.78 risk of death after MI using aspirin; US FDA approves aspirin for reducing risk of a second MI or a first MI in angina
- 1988: Based on the Physicians' Health Study, aspirin approved to prevent TIAs and strokes in healthy subjects



ISIS-2 Collaborative Group. Randomized trial of intravenous streptokinase, oral aspirin, both or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. Lancet 1988; 2: 349-60.

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Aspirin in Primary Prevention

- Physician's Health Study 1982–96
  - 1086 healthy male physicians age 40-84, 60 months
  - 325 mg aspirin on alternate days versus placebo
  - Ethical termination in 1988 when 44% reduction of fatal or nonfatal first AMIs was recorded
- Women's Health Study 1991–2000
  - 39,876 healthy women over 45 YO, 10 years
  - 100 mg aspirin on alternate days versus placebo
  - 25% reduction in fatal or non-fatal first MIs
  - 50% reduction in hypercholesterolemia, smokers, or hypertensives, greatest effect over 65 YO

Physician's health study: aspirin and primary prevention of coronary heart disease. N Engl J Med 1989; 321:129-35,183-5.

Gaziano JM, Skerrett PJ, Buring JE. Aspirin in the treatment and prevention of cardiovascular disease. Haemostasis 2000; 30:1-13S.

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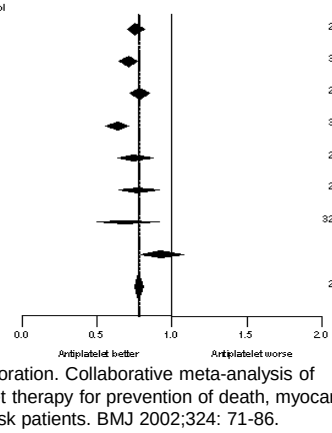
Antiplatelet Trialists' Collaboration

287 trials incorporating 100,000 subjects:

- Composite 32% decrease in death, MI, ischemic stroke in "high-risk" vascular patients on 75 to 150 mg aspirin daily:

Category of patient	No of trials with data	No of patients	Percent with vascular event: Allocated antiplatelet	Percent with vascular event: Adjusted control	Odds ratio (CI) Antiplatelet:Control	%Odds reduction (SE)
Previous myocardial infarction	12	20000	13.5	17.0		25(4)
Acute myocardial infarction	15	19300	10.4	14.2		30(4)
Previous stroke/TIA	21	12000	17.8	21.4		22(4)
Coronary artery disease	55	17500	6.2	8.9		37(5)
High risk of embolism	14	5200	13.5	16.8		26(7)
Peripheral arterial disease	42	9700	5.8	7.1		23(8)
Other vascular conditions	20	3400	4.2	6.4		32(13)
Diabetes mellitus	9	5100	15.7	16.7		7(8)
ALL TRIALS	195	144100	10.7	13.2		22(2)



Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324: 71-86.

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Cyclooxygenase-1 Acetylation

- Platelet COX-1 acetylated at ser_{529}
  - Blocks arachidonic acid's access to reactive "tunnel"
  - Active site amino acid tyr_{385} unaffected
- Platelet permanently loses COX-1 activation pathway
  - Recovery of function 10%/day as new platelets are produced
- Adhesion and shear-induced aggregation functions remain

Vane JR, Botting RM. Mechanism of action of aspirin-like drugs. Semin Arthr Rheum 1997; 25 Suppl I: 2-10.

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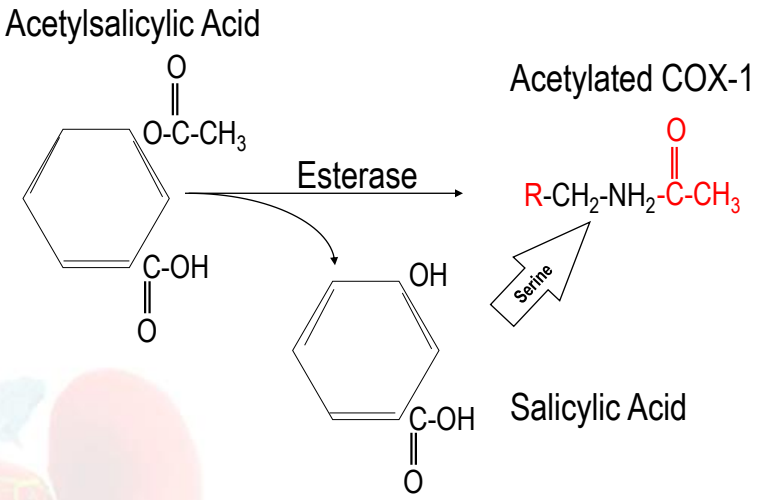
Acetylation of Cyclooxygenase-1



Pedersen AK, Fitzgerald GA. Dose-related kinetics of aspirin: Presystemic acetylation of platelet cyclooxygenase. New Engl J Med 1984; 311: 1206-11.

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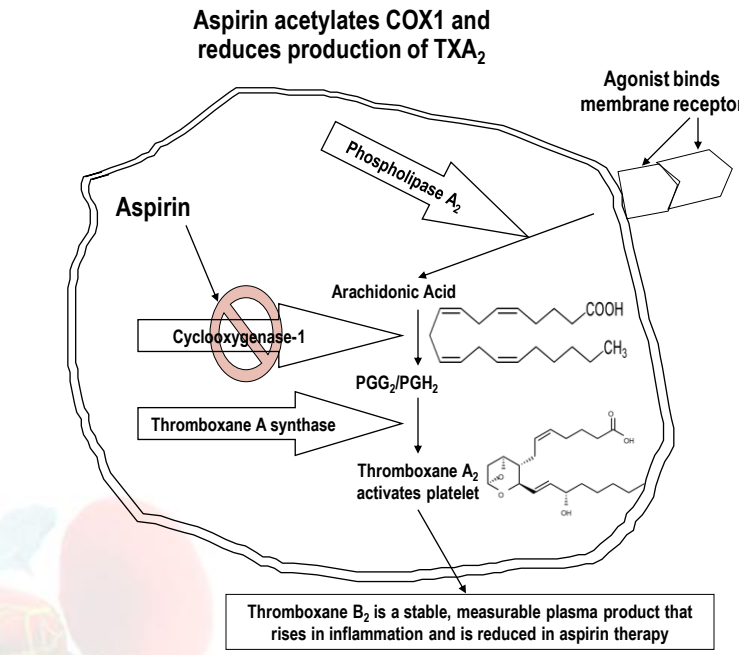

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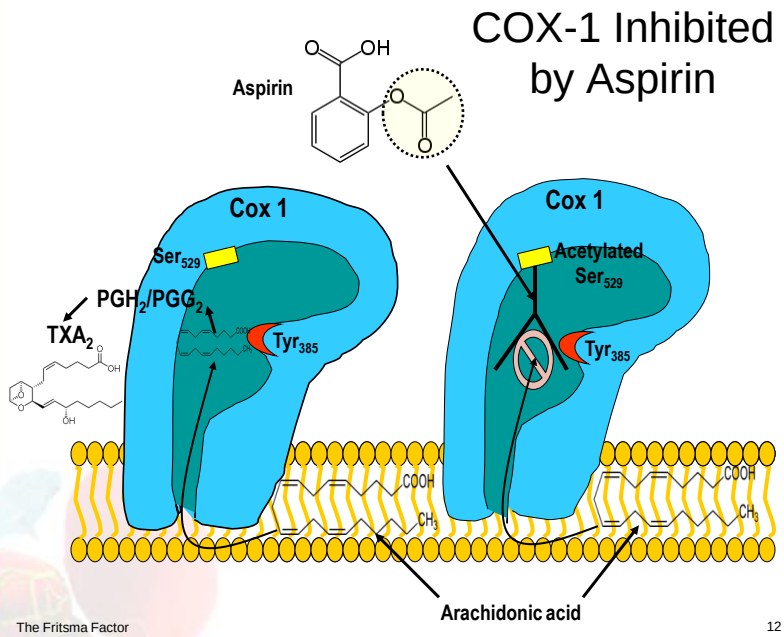

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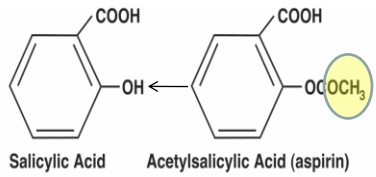
Aspirin Pharmacology

- 50% absorbed from stomach, duodenum
- Peak plasma levels at 40 m
- Acetyl group hydrolyzed to form salicylic acid (salicylate) in 20–30 m
  - Carboxyesterase in gut, liver and RBCs
  - Platelet COX-1 acetylation occurs in the presystemic (portal) circulation of gut and liver
- Reduces plasma TXB_2 within 5 m
  - Maximal reduction in 30 m
  - Salicylate remains measurable 6 h



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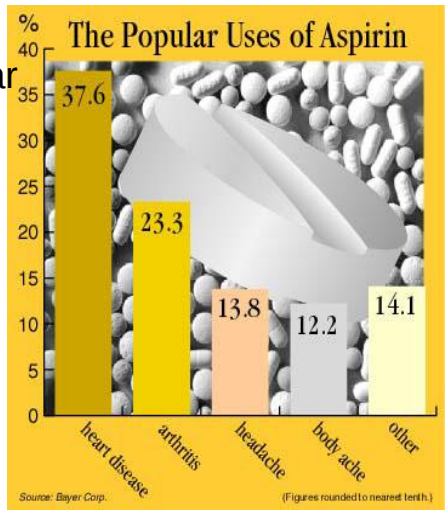
World Aspirin Usage

- 125,000,000,000 325 mg tablets (40,000 tons)/year
- But aspirin is not for everybody

Bayer market research



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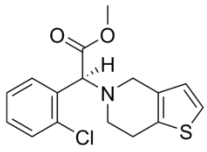
Clopidogrel (Plavix®)

- Thienopyridine pro-drug
  - Has largely replaced ticlopidine
  - (+)-(*S*)-methyl 2-(2-chlorophenyl)-2- (6,7-dihydrothieno[3,2-*c*] pyridin-5(4*H*)-yl) acetate



- Metabolite occupies platelet ADP receptor $P2Y_{12}$
  - Covalent cysteine binding, prevents aggregation
- Indications; *with aspirin*:
  - Prevents vascular ischemia in symptomatic atherosclerosis
  - Prevents secondary acute coronary syndrome
  - Prevents thrombosis after placement of drug-eluting coronary stent

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Clopidogrel (Plavix®)

- Oral administration: 75mg/d
  - Loading dose 600 mg
  - Continue with concurrent aspirin 1–2 years
  - Continue aspirin for life
- Peak plasma levels 1 hour
- Prodrug modified to active form in liver
  - Cytochrome P450 2C19 (CYP2C19)
  - 15% bioavailability
- Half-life 8 hours
- Excreted via kidneys and liver

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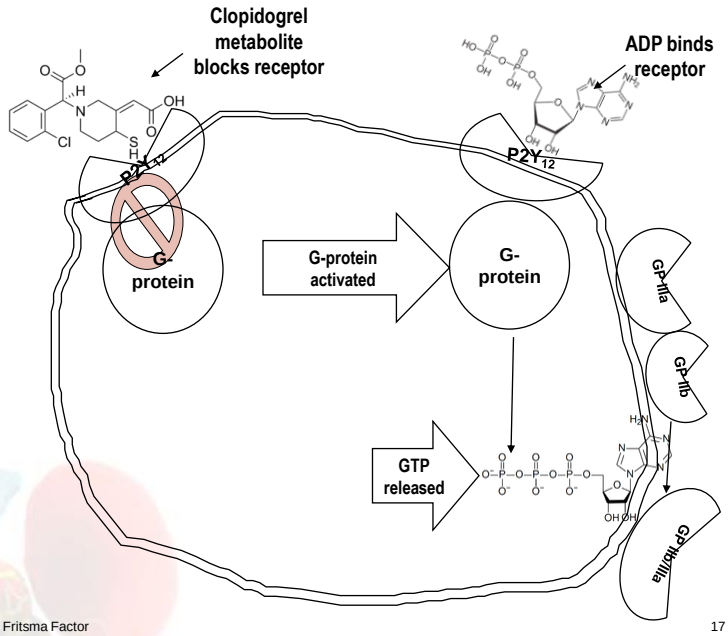

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Clopidogrel Efficacy

Condition	Study	Citation	Risk Reduction Vs. Aspirin Alone
Unstable angina	CURE	*NEJM 2001;345:494*	20%
Stent	CREDO	*JAMA 2002;288:2411*	26.9%
Myocardial infarction	CLARITY-TIMI 28	*NEJM 2005;352:1179*	20%
Low-risk	CHARISMA	*NEJM 2006;354:1706*	None

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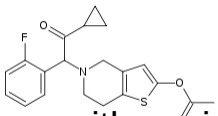
Prasugrel



- FDA-cleared July 10, 09 to use with aspirin
- Effient®: Daiichi Sankyo/Eli Lilly & Co
- TRITON-TIMI study of 13,608 patients
  - 60 mg loading dose, 10 mg/day
  - Prasugrel reduces the combined rate of death from CV causes; 12.1% for clopidogrel vs. 9.9% for prasugrel
  - However, 1.4%, vs. 0.9% serious bleeds and 0.4% vs. 0.1% fatal bleeds
  - Prasugrel generates net clinical harm in patients < 60 kg, > 75 YO or have a Hx of stroke or TIA
  - Overall mortality identical

Wiviott SD, Braunwald E, McCabe CH, *et al.* Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007;357: 2001–15.

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Antiplatelet Failure Vs Resistance

- Variable antiplatelet efficacy
  - Adverse events despite therapy: failure
- Laboratory testing for resistance
  - Bleeding time
  - Light transmittance aggregometry (LTA)
  - Whole blood impedance aggregometry (WBA)
  - AspirinWorks®: Urine 11-dehydrothromboxane B2 (UDHT) immunoassay
  - Accumetrics Ultegra VerifyNow® Aspirin
  - Siemens PFA-100® collagen-epinephrine cartridge (CEPI)
  - Helena PlateletWorks®
  - Thromboelastograph® (TEG)
  - Platelet vasodilator-stimulated phosphoprotein phosphorylation (VASP) flow cytometry

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Bleeding Time: Mielke Template

- Diagnose VWD and qualitative platelet disorders in controlled conditions
- 50% PPV for intraoperative bleeding 50%
- Over-diagnosis of aspirin resistance
- Affected by platelet count
- Leaves scars

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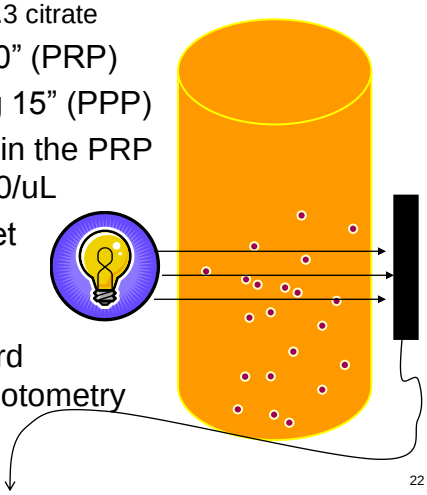
Light Transmittance Aggregometry (LTA) Specimen Preparation

- Collect 9–12 mL whole blood
  - 3–4 2.7 mL tubes + 0.3 citrate
- Centrifuge at 50×g 30” (PRP)
- Centrifuge at 1500×g 15” (PPP)
- Adjust platelet count in the PRP with PPP to ~200,000/uL
- Wait 30 m for “platelet shock”, dispense to cuvette
- Pipette agonist, record absorbance using photometry



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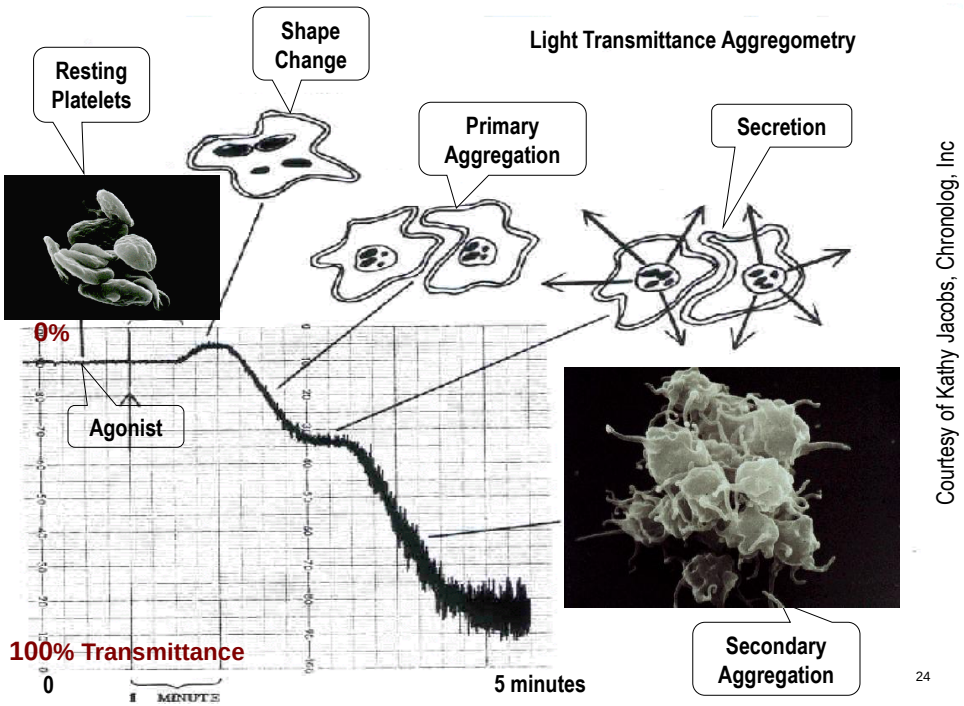
Antiplatelet Efficacy Agonists

- 0.5 mM arachidonic acid (AA)
  - Directly activates COX pathway to produce TXA_2
  - TXA_2 activates platelet by binding TPα or TPβ
  - Response reduced by aspirin
- 1 or 5 μg/mL collagen
  - Binds receptors GP Ia/IIa (integrin α2β1), GP IV, GP VI
  - 1 μg/mL response reduced by aspirin
  - 5 μg/mL may bypass aspirin effect, reduced in secretion (aspirin-like) disorder
- 5–10 μM ADP
  - ADP binds intact $P2Y_1$ & $P2Y_{12}$
  - $P2Y_{12}$ response reduced by thienopyridines
- Thrombin receptor activation peptide (TRAP)
  - Response reduced by GP IIb/IIIa inhibitor therapy



Courtesy of Kathy Jacobs, Chronolog, Inc

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LTA Limitations

- Reference method, however…
- Specimen volume
- Specimen preparation time and artifact
- Three-hour time limit
- Operator-dependent results
- Poor reproducibility
- Removes effect of RBCs and WBCs

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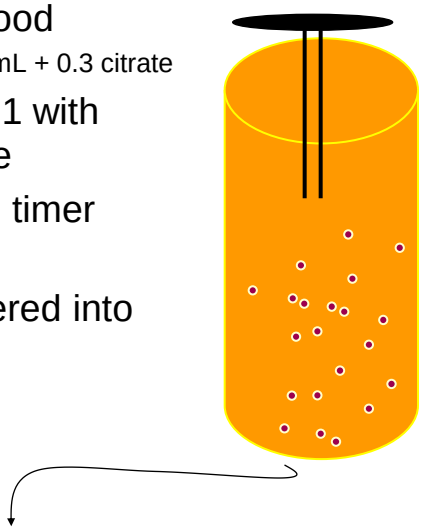
Impedance-based Whole Blood Platelet Aggregometry (WBA)

- Collect 9 mL blood
  - 3 tubes each 2.7 mL + 0.3 citrate
- Dilute aliquot 1:1 with saline in cuvette
- Pipette agonist, timer starts
- Electrodes lowered into suspension



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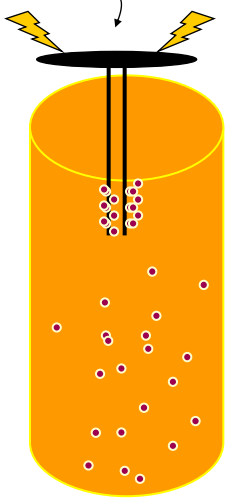
WBA: Impedance

- Aggregating platelets form layer on electrodes
- Current impeded by platelet layer
  - Resistance measured in ohms (Ω)
  - 0 Ω = no aggregation
  - Aggregation proportional to Ω



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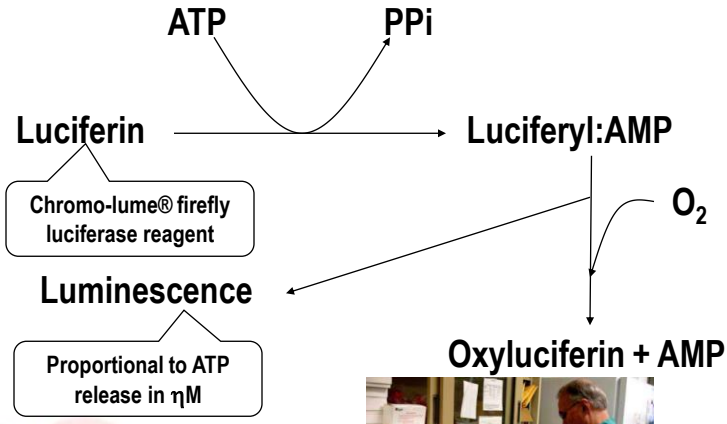
Secretion Assayed W/ "Firefly" Reaction



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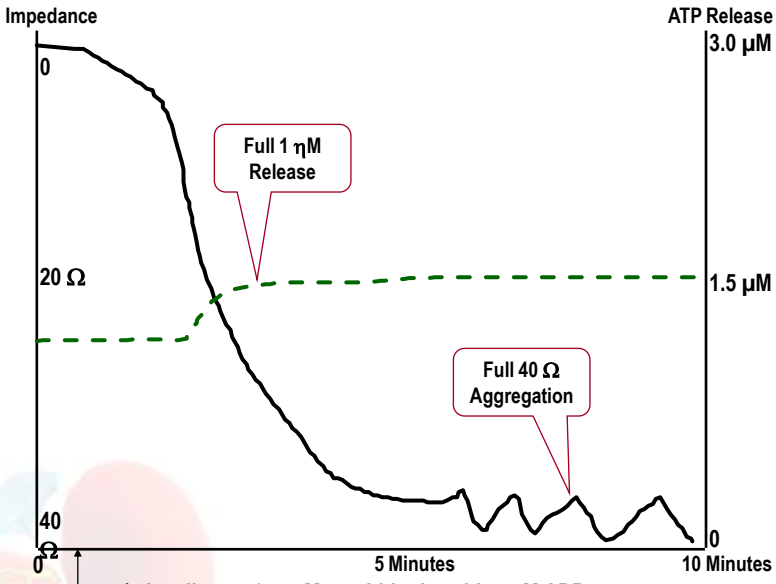
Normal Lumi-WBA



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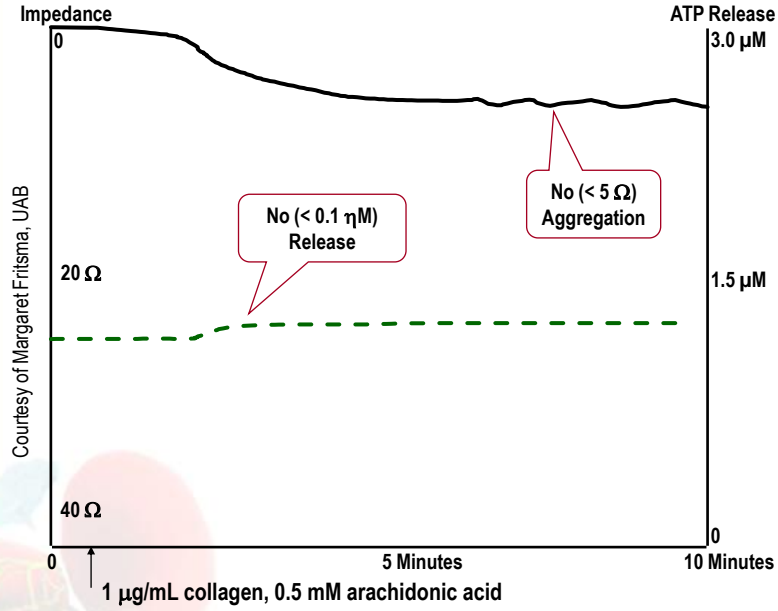
Aspirin, Aspirin-like Disorder (Secretion Defect)



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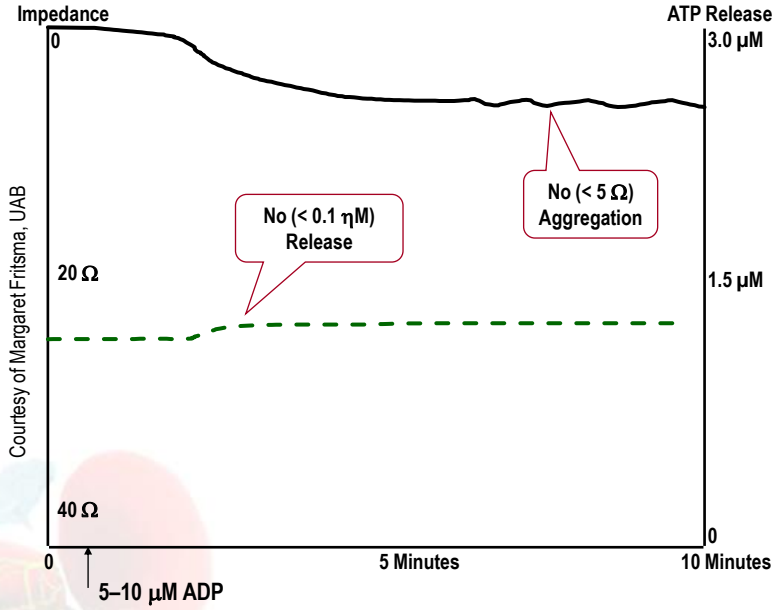
Clopidogrel, $P2Y_{12}$ Defect



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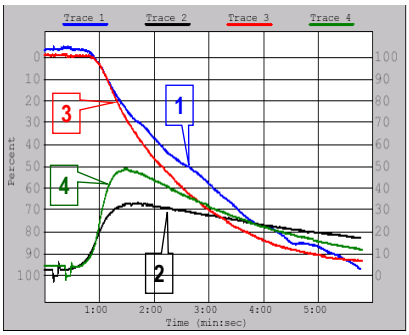
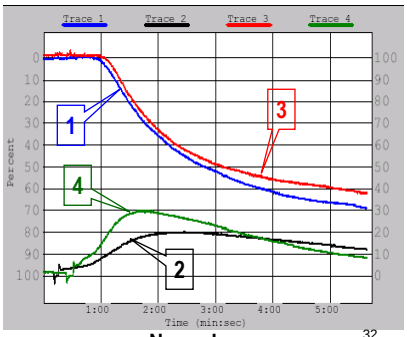
Agonist	Collagen 1 µg/mL	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	0.83ηm	0.81ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	38Ω	37Ω



Agonist	Arachidonic Acid 0.5 mM	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	0.45ηm	0.45ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	27Ω	25Ω

Courtesy of Patti Tichenor, UAB Coagulation; using AGGRO/LINK®



Normal

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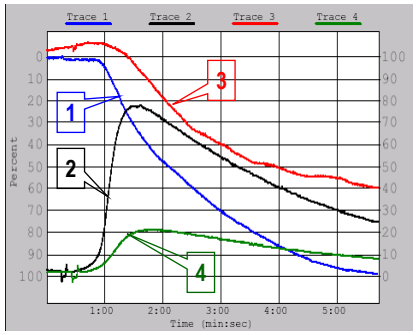
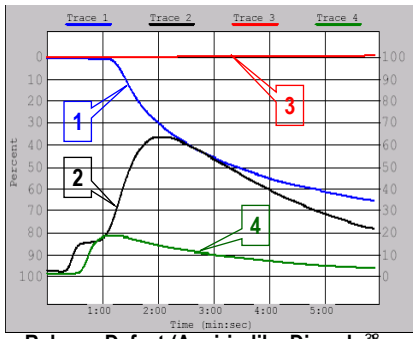
Agonist	Collagen 1-5 µg/mL	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	1.69ηm	0.57ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	39Ω	26Ω



Agonist	Arachidonic Acid 0.5 mM	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	1.42ηm	0.57ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	26Ω	0Ω



Courtesy of Laura Taylor, UAB
Coagulation; using AGGRO/LINK®

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Release Defect (Aspirin-like Disorder)

Aspirin Effect Using 5 µg/mL and 1 µg/mL Collagen

Test ID : Aspirin 2&5 Collagen

TRACE 1 Date: 10/8/2003 Time: 3:11:29 PM
Name: ,
ID : Lab :
Blood Draw Time :

TRACE 2 Date: 10/8/2003 Time: 3:11:29 PM
Name: ,
ID : Lab :
Blood Draw Time :

TRACE 3 Date: 10/8/2003 Time: 2:44:34 PM
Name: ,
ID : Lab :
Blood Draw Time :

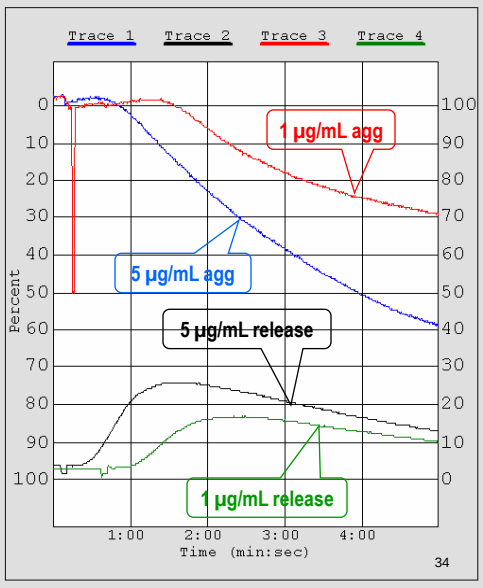
TRACE 4 Date: 10/8/2003 Time: 2:44:34 PM
Name: ,
ID : Lab :
Blood Draw Time :

TRACE	1	2	3	4
Instrument Reagent	IMP Collagen 5 ug/mL	LUM	IMP Collagen 1ug/mL	LUM
Stirrer	1200	1200	1200	1200
Gain	20/5	2/61	20/5	2/61
Amplitude	27 ohm	0.72 nm	12 ohm	0.46 nm
Slope	9	34	7	18
Lag Time	0:09	0:28	1:22	0:44
Area Under	89	2.6	30.1	1.4

Comments

1 µg/mL agg <50% 5/mL µg agg

Courtesy of Laura Taylor, UAB
Coagulation; using AGGRO/LINK®



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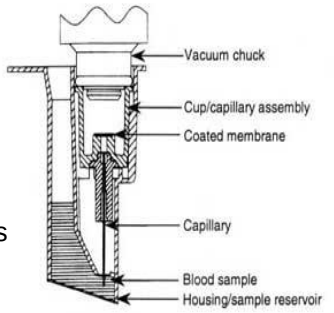
Siemens (Dade-Behring) PFA-100

- Transfer 800 μL citrated whole blood to each of two cartridges, load and run to closure time (CT)
  - Coated membrane: agonists
- Collagen and epinephrine (Col/Epi, CEPI)
  - "Weak:" normal CT 98–185 s
  - Initial screen cartridge
- Collagen and ADP (Col/ADP, CADP)
  - 50 μM ADP
  - "Strong:" normal CT 77–133 s
  - Confirmatory



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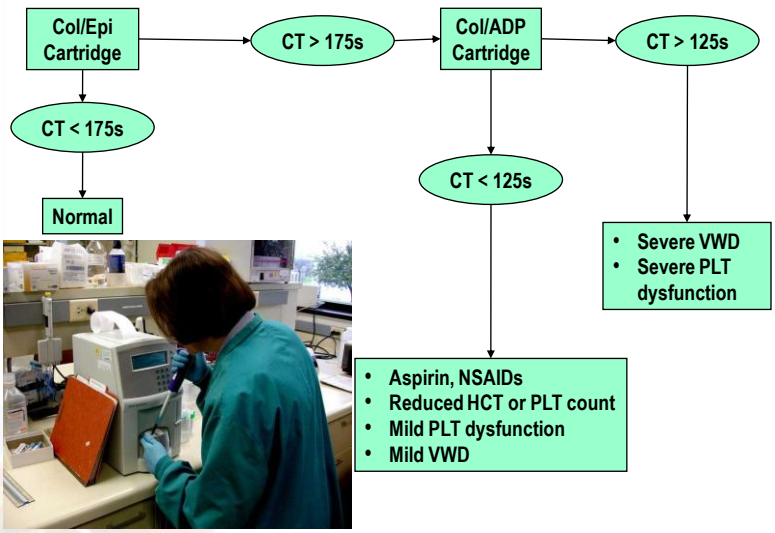
PLT Function Screen for Variable Aspirin Response



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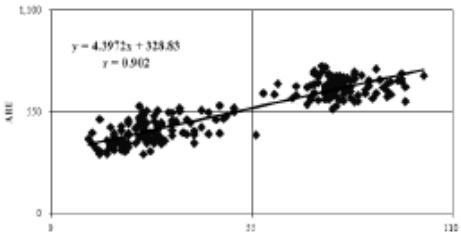
Accumetrics VerifyNow®

- δ-transmittance rise as platelets aggregate in response to agonists specific to aspirin, clopidogrel, or glycoprotein IIb/IIIa inhibitors
  - Whole blood
  - Low transmittance (ARU < 550): function inhibited
  - Normal transmittance (ARU > 550): function normal



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Accumetrics VerifyNow®

- VerifyNow Aspirin® uses AA to measure PLT eicosanoid synthesis pathway function
  - Aspirin acetylates cyclooxygenase
- VerifyNow $P2Y_{12}$® uses ADP to determine clopidogrel inhibition
  - Second channel uses TRAP to approximate baseline function without the need to discontinue medication
- VerifyNow IIb/IIIa® uses TRAP to determine response to GP IIb/IIIa inhibitors

VerifyNow™ P2Y12
Evaluating the Effect of Plavix® on Platelet Function

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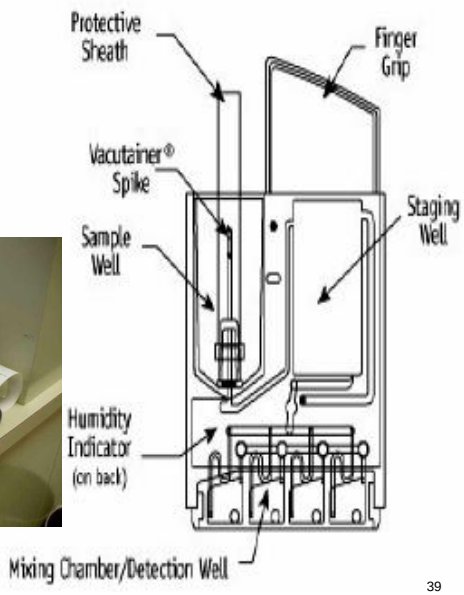
VerifyNow Reaction Chamber



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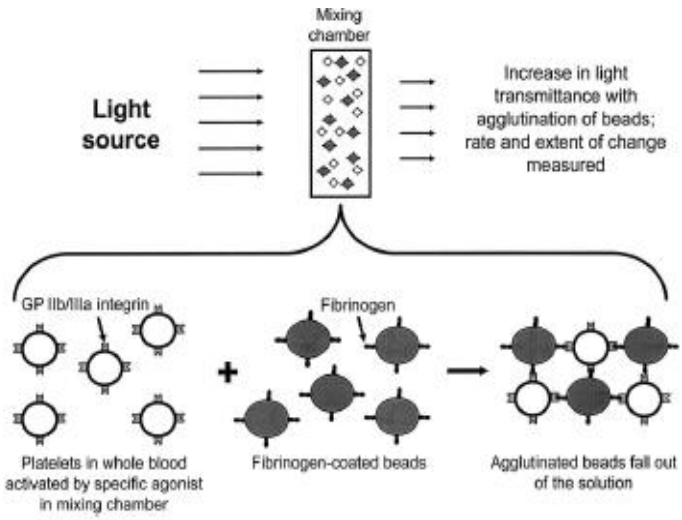
VerifyNow Principle



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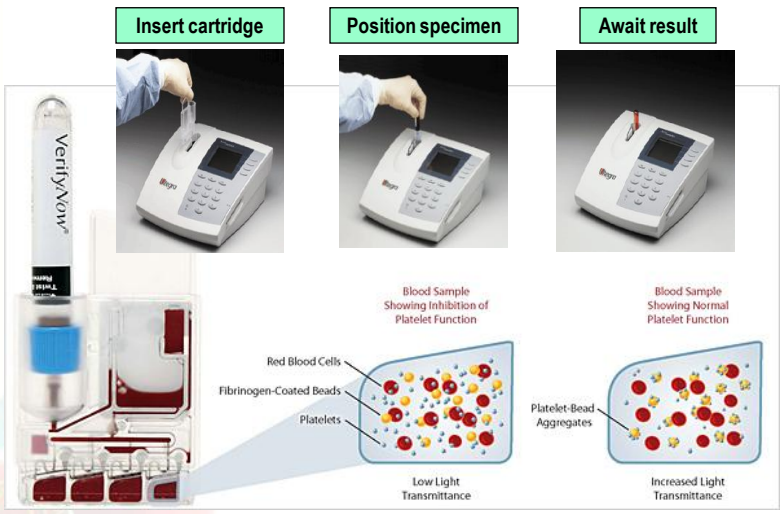
Accumetrics Methodology



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VerifyNow and PFA-100 Limitations

- Large specimen volume
- Must test within four hours
- Expensive cartridges
- Precision: CVs above 10%
- Variable effect of von Willebrand factor and hematocrit

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Aspirin Resistance Prevalence

Overall		**27.1%**
By definition	**PFA-100**	**29.0%**
	Ultegra VerifyNow	**26.2%**
	LTA	**21.3%**
By population	**CAD**	**22.9%**
	Stroke	**32.1%**
By dose	**< 100 mg/d**	**35.6%**
	101–299 mg/d	**28.2%**
	> 300 mg/d	**18.6%**

Hovens MMC, Snoep JD, Eikenboom CJ. Prevalence of persistent platelet reactivity despite use of aspirin: a systematic review. Am Heart J 2007;153:175–81.

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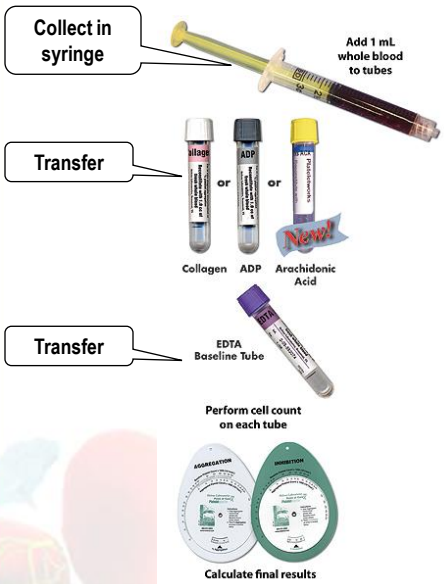
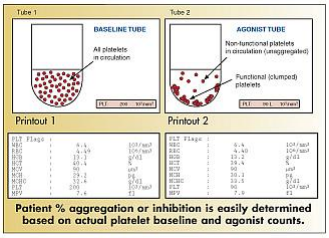
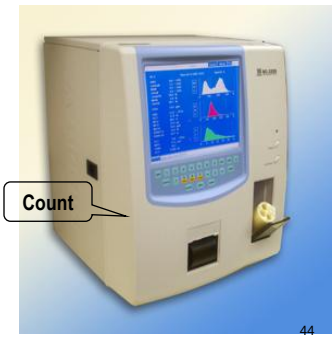
Helena PlateletWorks®







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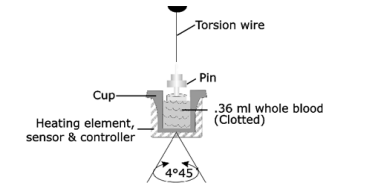
Thromboelastograph

- How it works



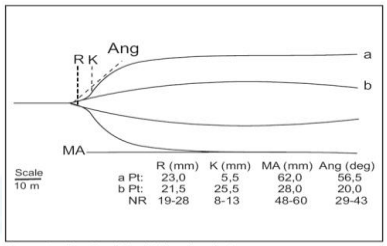


Figure 3 - Two Combined Trhomboelastograms
Parameters values are shown below. Plot a: contains parameter marks with mild hypercoagulation state; plot b: shows severe fibrinolysis.
MA: maximum amplitude; Pt: patient; NR: normal range

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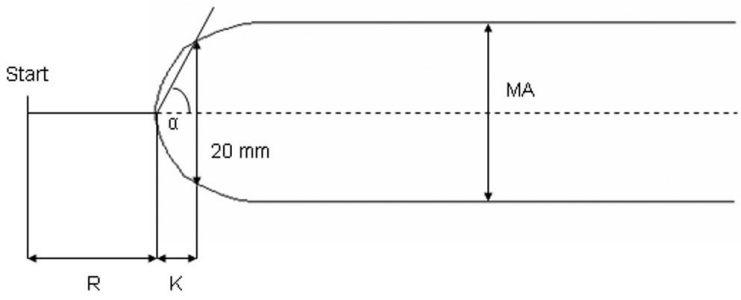
TEG Parameters

Clotting time	**R**	Interval from when the blood was placed in the analyzer until initial fibrin formation
Clot kinetics	**K**	Interval to reach a chosen clot strength
	α	Interval to fibrin buildup and cross-linking
Clot strength	**MA**	Maximum dynamic properties of fibrin and platelet bonding via GP IIb/IIIa
Hemostasis profile	**CI**	Linear combination of K, K, α, MA and G
Clot stability	**LY30**	Rate of amplitude reduction 30 m after MA



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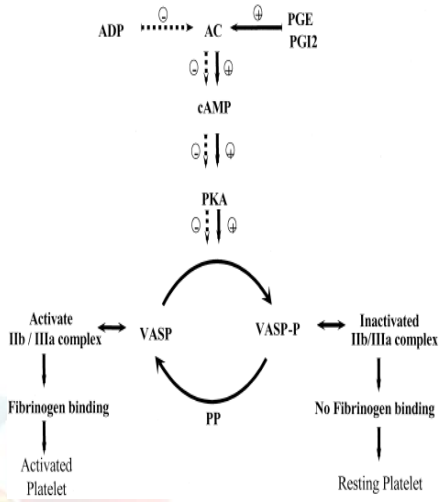
Platelet Vasodilator-stimulated Phosphoprotein Phosphorylation



Adenylate cyclase (AC) is inhibited by ADP but activated by the vasodilator PGI_2. Activated AC enhances cAMP synthesis, activating cAMP-dependent protein kinase (PKA). The kinase phophorylates VASP, detectable in flow cytometry. Dashed arrow denotes inhibition; solid arrow, activation.

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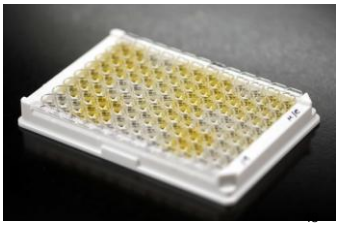
ASPIRINWORKS®
a division of Creative Clinical Concepts, Inc.

- Urinary 11-dehydrothromboxane B_2 (UDHT)
  - Urine metabolite of plasma thromboxane B_2
- Platelet is primary source
  - Also minor component renal endothelial cells, monocytes
  - Products of COX-1 and COX-2 detects chronic inflammation
- Random urine specimen
  - Normalized to urine creatinine
  - Pg UDHT/mg creatinine



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Platelet Function Testing
Platforms with Clinical Outcome Studies

Assay	ASA	Clopidogrel	GP IIb/IIIa Inhibitor
LTA	√	√	√
WBA	√	√	√
VerifyNow	√	√	√
UDHT	√		
PFA CEPI	√		
VASP			
TEG			
PlateletWorks			

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HOPE Study: Aspirin Resistance

- Nested retrospective case-control sample
  - 488 aspirin-treated vascular patients with MI, stroke, or CV death during 5 years of observation
  - 488 age- and sex-matched controls taking aspirin who did not have an MI, stroke, or CV death
- In aspirin-treated vascular patients, UDHT predicts risk of MI or CV death
- Fourth quartile UDHT = 3.5 CV death risk

Pg UDHT/ mg creatinine	Quartile	Odds Ratio		
		MI	CV Death	Stroke
<134	1	1.0	1.0	1.0
134–193	2	1.3	2.0	2.5
194–298	3	1.5	2.5	0.6
>298	4	2.0	3.5	0.6

Eikelboom JW, Hirsh J, Weitz JI, et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002; 105: 1650–55

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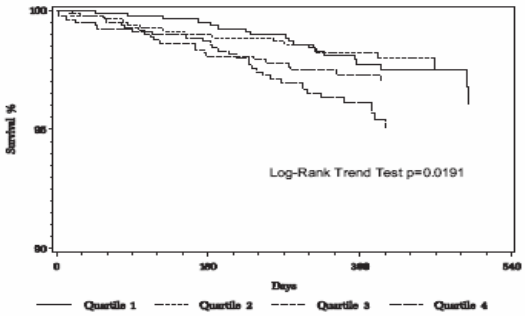
CHARISMA Trial

- Randomized double-blind prospective trial of 3261 clopidogrel Vs. placebo in patients on aspirin at high risk of atherothrombosis
  - Tested 1 month after starting clopidogrel
  - 144 with stroke, MI, or CV death
  - 3117 with no adverse event
- Fourth quartile UDHT composite RR=1.66

Kaplan–Meier curves for composite of stroke, MI, or CV death by UDHT quartiles



Eikelboom JW, Hankey GJ, Thom J, et al. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid. Determinants and effect on cardiovascular risk. Circulation 2008; 118: 1705–12

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CHARISMA Trial

- Fourth quartile UDHT levels
  - Increasing age, ♀ sex, Hx of PAD, smoking, oral hypoglycemic Rx, ACE-inhibitor Rx
- Low UDHT concentrations
  - Aspirin Rx >150 mg/d, NSAIDs, hypercholesterolemia, statin Rx
- Randomization to clopidogrel or placebo did not reduce risk ratio for CV events in patients in the fourth UDHT quartile
- UDHT is potentially modifiable

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Aspirin Resistance and Adverse Events

Type	Percutaneous Intervention (Cath)		Stable CAD
N	151	106	315
% AR	19.2		5.2
Method	VerifyNow	Light Transmittance Aggregometry	
Results	Elevated CK-MB and troponin I in AR	4th quartile ADP response associated with RR for CV events = 22.4	OR in AR • Composite: 3.12 • CV death: 2.98 • MI: 1.91 • CVA: 5.44
Ref	*Chen WH, JACC 2004;43:1122*	*Cuisset T, J Thromb Haemost 2006;4:542*	*Gum PA, JACC 2003;41:961*

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PFA-100 and Aspirin Resistance

Gum PA, JACC 2003;41:961	9.5% AR by CEPI CT, low correlation with LTA
Hézard N, Thromb Res 2002;108:43	Low AR correlation among LTA, CEPI CT, and flow
Sane DC. Thromb Haemost 2002;88:711	No CEPI CT difference between AR and aspirin sensitive
Ten Berg JM, Thromb Res 2002;105:385	CEPI CT did not distinguish low dose from high dose aspirin
Grundmann K, J Neurol 2003;250:63	53 patients on aspirin for stroke prevention: CEPI CT significantly shorter in 12/35 patients with recurrent stroke (p <0.01)
AR = aspirin resistance, CEPI CT = closure time using collagen-epinephrine cartridge, LTA = light transmittance aggregometry	

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Variation in Laboratory Detection of Aspirin Resistance

Assay	Aspirin Resistance %
Accumetrics VerifyNow Aspirin	17
Siemens PFA-100 CEPI	22
Arachidonic acid LTA	5 (COX-1 specific)
All tests abnormal per subject	2
Harrison P, Segal H, Blasbery K. Screening for aspirin responsiveness after transient ischemic attack and stroke: comparison of 2 point-of-care platelet function tests with optical aggregometry. Stroke 2005 36:1001–5.	
Tantry US, Mahla E, Gurbel PA. Aspirin resistance. Prog Cardiovasc Dis 2009; 52:141–52.	

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Seven-day Comparison to Whole Blood Aggregometry

Assay	Positive Predictive Value		Negative Predictive Value	
Dosage	81 mg	325 mg	81 mg	325 mg
AspirinWorks	74.3	82.1	40.2	0.0
PFA-100 CEPI	81.3	81.6	53.8	42.9
VerifyNow Aspirin	72.7	51.9	100	33.3
“Laboratory measures of PLT activity are suppressed by aspirin therapy, but are affected by the dosage and duration of therapy. Determinations of aspirin response should be made after at least 7 days of treatment. Laboratory test platform results do not closely reflect each other, thus application of laboratory platforms should be made consistently.”				
McGlasson DL, Fritsma GA. Comparison of four laboratory methods to assess aspirin sensitivity. Blood Coagulation Fibrinolysis 2008;9:20–3				

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Aspirin Resistance Study Limitations

- Inter-assay variation
- Biological variation over time
- Fail to adjust for race, age and sex
- Fail to confirm compliance using serum salicylate
  - Non-compliance and early withdrawal may account for most aspirin resistance
- Fail to separate confounding conditions
  - Hypertension, diabetes, peripheral vascular disease, smoking, and inflammation may contribute to aspirin resistance, while independently raising vascular risk

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Proposed Mechanisms of Aspirin Resistance

- Activation of alternate PLT pathways not blocked by aspirin
  - Diacylglycerol pathway activated through G-protein
  - Adhesion molecules: collagen (GP Ia/IIa) and von Willebrand factor receptors (GP Ib/V/IX)
  - Activation by shear stress in atherosclerosis
- Aspirin-mediated reduction of PLT-inhibiting prostacyclins from vascular endothelial cells
- Elevated von Willebrand factor levels
- Polypharmacy (> 4 drugs)

Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance. Int J Clin Pract 2007:61:826–34
Kilanowska J, Favaloro EJ, Lippi G. Aspirin “responsiveness,” “nonresponsiveness” or “resistance”: a putative role for von Willebrand factor? Blood Coagul Fibrinolysis 2008;19:823–4

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Proposed Mechanisms of Aspirin Resistance

- Induction of COX-2
  - Non-constitutive, induced by cytokines and inflammation
  - After bypass surgery, 16-fold increase of COX-2 causes transient aspirin resistance
  - Acetylation of COX-2 ser_{529} incompletely hinders arachidonic acid's access to reactive site
  - In megakaryocytes, monocytes, macrophages, vascular endothelial cells and newly released platelets
  - Smoking, diabetes, heart failure and hyperlipidemia
- NSAIDs compete for Ser_{529}

Weber AA, Zimmermann KC, Meyer-Kirchrath J, Schror K. Cyclooxygenase-2 in human platelets as a possible factor in aspirin resistance (letter). Lancet 1999; 353: 900.

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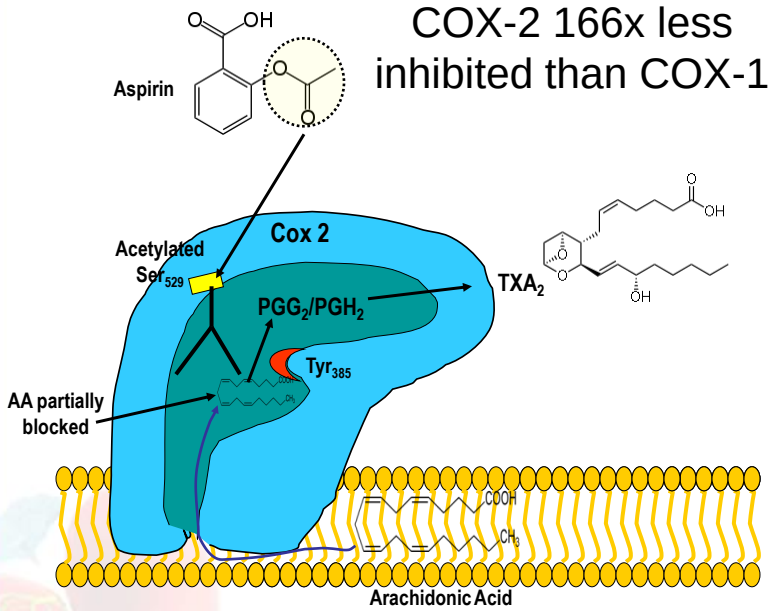
COX-2 166x less inhibited than COX-1



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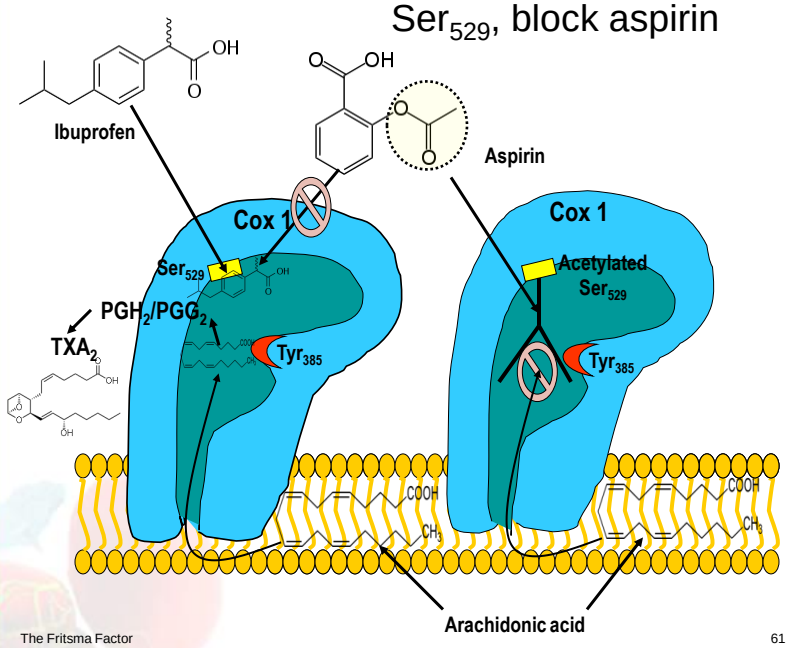
NSAIDs temporarily occupy Ser_{529}, block aspirin



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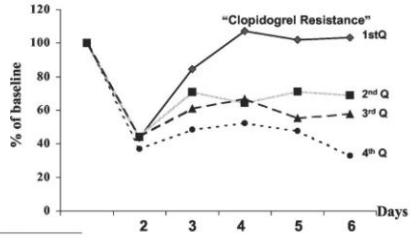
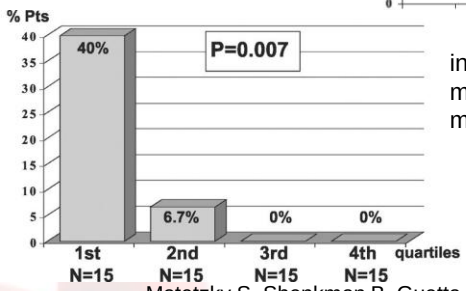
Plavix Resistance

changes in ADP-induced LTA expressed as % baseline





incidence of recurrent major CV events during 6-month follow-up

Matetzky S, Shenkman B, Guetta V. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004;109:3171-5.

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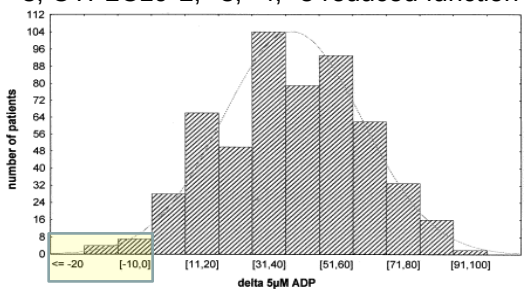
Clopidogrel Response Variation

- Platelet concentrate transfusions
- Polymorphisms
  - Cyp2C9*2 or *3, CYP2C19*2, *3, *4, *5 reduced function alleles
  - $P2Y_{12}$ receptor
  - HPA-2 phenotype



Distribution of reductions from baseline in 5 µmol ADP-induced PLT LTA in 544 patients after starting clopidogrel. Negative changes represent aggregation values < baseline .

Serebruany VL, Steinhubl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005; 45:246-51.

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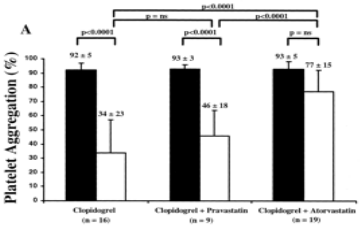
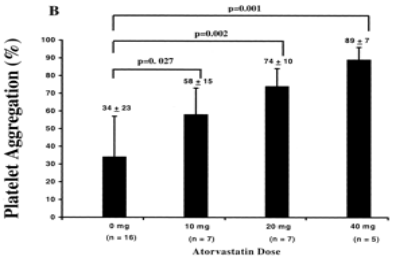
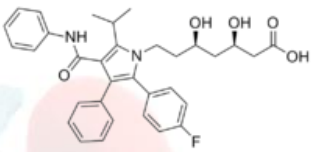
In Vivo Effect of Pravastatin and Atorvastatin on Antiplatelet Activity of Clopidogrel

A. PLT aggregation before (black) and 24 hours after (white columns) clopidogrel administration in patients treated with 40 mg pravastatin or 10–40 mg atorvastatin.

B. Antiplatelet activity of clopidogrel as a function of atorvastatin dose







Lau WC, Waskell LA, Watkins PB, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. Circulation 2003;107:32–7.

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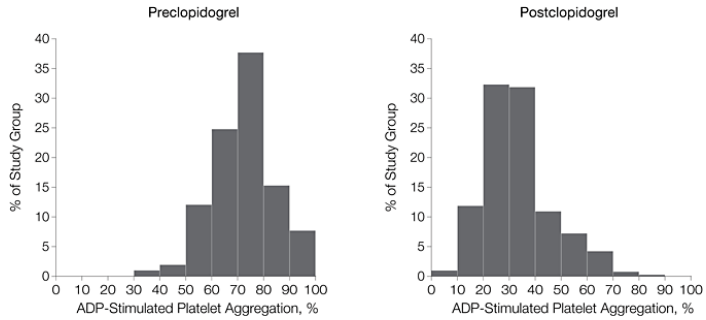
Distribution of 20 umol/L ADP-Stimulated Platelet Aggregation Before and After 7 Days of Clopidogrel Administration in 429 Members of the Amish Pharmacogenomics of Anti-Platelet Intervention (PAPI) Study



Shuldiner, A. R. et al. JAMA 2009;302:849-857.

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JAMA

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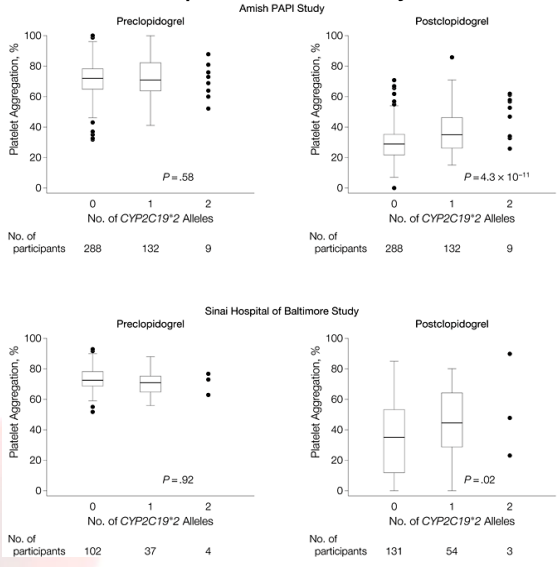
Association of CYP2C19*2 Loss-of-Function Variant With ADP-Stimulated Platelet Aggregation Before and After Clopidogrel Administration in Participants in the Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) Study and Sinai Hospital of Baltimore Study



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Shuldiner, A. R. et al. JAMA 2009;302:849-857.

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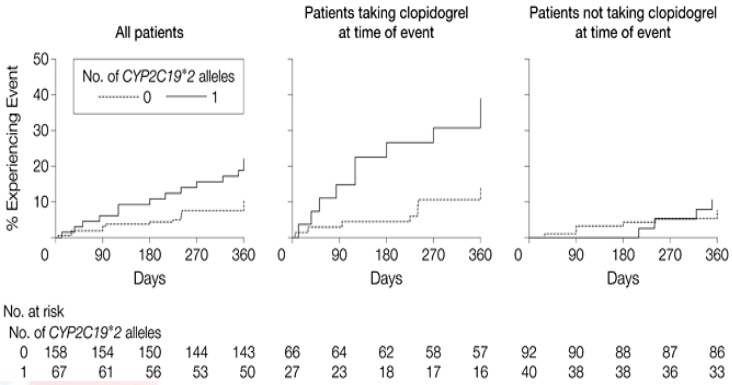
Event-Free Survival Over 1 Year of Follow-up in Sinai Hospital of Baltimore Patients Treated With Clopidogrel Following PCI, Stratified by CYP2C19*2 Genotype



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Shuldiner, A. R. et al. JAMA 2009;302:849-857.

JAMA

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Additional Clopidogrel Studies That Illustrate Resistance

Study	Date	N	Dx	Dose (mg)	Time	Resistance
Jaremo	2002	18	PCI	300/75	24 h	28%
Gurbel	2002	92	PCI	300/75	24 h	31-35%
Mueller	2003	105	PCI	66/75	4 h	5-11%
Kesmarkey	2003	226	CVD	75		31%

Gurbel PA, Lau WC, Bliden KP, Tantry US Clopidogrel resistance: implications for coronary stenting. Curr Pharm Des 2006; 12:1261-9.

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Prasugrel Response

- Prodrug that is metabolized to active form in the CYP450 pathway
  - Not affected by CYP2C9 or CYP2C19 loss of function genotypes
  - More efficient generation of active metabolite; 15” onset
- Higher peak plasma levels than clopidogrel
- Greater exposure of PLTs to metabolite
- Functions in clopidogrel resistant patients

Motovska Z, Widimsky P. Improving outcomes in patients undergoing percutaneous coronary intervention: role of prasugrel. Vasc Health Risk Manag 2009;5: 475–81

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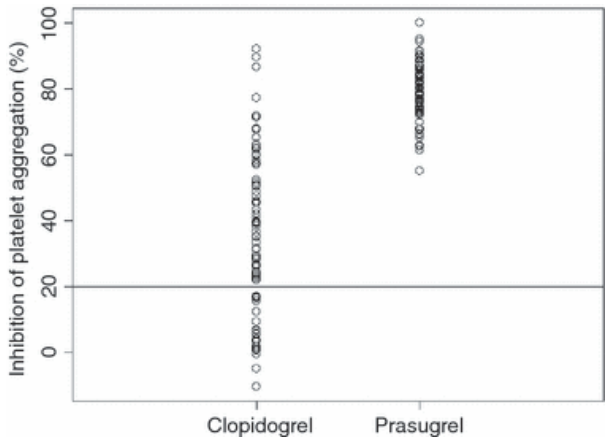

Inhibition of platelet LTA by 300 mg clopidogrel and 60 mg prasugrel,4 h post-dose. A value less than 20% (shown by line) is within the variability of the assay in the absence of drug administration.

Brandt JT, Close SL, Iturria SJ, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 2007;5:2429–36.

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So, do we screen for antiplatelet resistance?

W Check. Clot Knot; Unraveling Tests for Coag Disorders. CAP Today, December 2008.

Dr. Kristi Smock: “I think it is a problem of using different definitions for aspirin resistance and measuring it with tests that have different sensitivities and specificities.” “Moreover,” she adds, “testing for this condition is not generally recommended because it is not known what the treatment changes would be. “

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So, do we screen for aspirin resistance?

W Check. Clot Knot; Unraveling Tests for Coag Disorders. CAP Today, December 2008.

One might even wonder whether aspirin resistance actually exists. This entity was postulated on the basis of people having occlusive coronary events while taking cardioprotective doses of aspirin. However, Dr. Smock points out, aspirin reduces cardiovascular risk by only 25 percent. And cardiovascular disease is multifactorial. “It is simplistic to attribute coronary events entirely to aspirin resistance,” she says. The condition may exist but is probably rare, and prospective clinical studies are needed to document it. “True biochemical aspirin resistance may reflect a variant cyclooxygenase-1 [COX-1] enzyme that is not susceptible to inhibition by aspirin,” Dr. Smock says.

Response: Two Meta-Analyses

- *Snoep JD, Hovens MMC, Eikenboom JCJ, et al. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. Arch Intern Med 2007;167:1593-9:*

  Mean prevalence of laboratory aspirin resistance among all methods reviewed is 27%. Resistance predicts 3.8 OR for adverse cardiovascular outcomes. *No concordance among methods*.

- *Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ 2008; 336: 195-8.*

  20 studies totaling 2930 patients with cardiovascular disease. Classified 28% as aspirin resistant. Resistance confers a 3.85 OR for any adverse cardiovascular outcome including a 5.99 OR for death and a 2.96 OR for acute coronary syndrome. *No concordance among methods*.