

Managing Antiplatelet Therapy

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LEARNING OBJECTIVES

1. List antiplatelet drugs that are used in dual antiplatelet therapy.
2. Describe the basis for aspirin and thienopyridine antiplatelet property.
3. Define aspirin and clopidogrel low response.
4. List and describe platelet function testing that is applied to detecting aspirin and clopidogrel low response.

ABBREVIATIONS: AA-arachidonic acid, ACS-acute coronary syndrome, ADP-adenosine diphosphate, ALR-aspirin low responder, AMI-acute myocardial infarction, ARU-aspirin reaction (or resistance) units, ASA-acetylsalicylic acid, CABG-coronary artery bypass graft, CLR-clopidogrel low responder, COX-cyclooxygenase, CT-closure time, DAPT-dual antiplatelet therapy, CLIA-clinical laboratory improvements amendment, FDA-US Food and Drug Administration, LTA-light transmittance aggregometry, MI-myocardial infarction, NSAID-non-steroidal anti-inflammatory drug, CYP-cytochrome oxidase pathway, PCI-percutaneous intervention, PGE₁-prostaglandin E₁, PGG₂-prostaglandin G₂, PGH₂-prostaglandin H₂, POC-point of care, PRU-P2Y₁₂ reaction units, PT/INR-prothrombin time with international normalized ratio, TEG-thromboelastography, TEM-thromboelastometry, TXA₂-thromboxane A₂, WBA-whole blood aggregometry, VASP-vasodilator-stimulated phosphoprotein

INDEX TERMS: Aspirin, thienopyridine, clopidogrel, prasugrel, ticagrelor, platelet function, platelet function testing, aggregometry, thromboelastography, thromboelastometry, antiplatelet therapy

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Dual Antiplatelet Therapy

In 1955, President Dwight Eisenhower, recovering from an acute myocardial infarction (MI), was treated with warfarin (Coumadin®) to prevent a repeat MI (Figures 1 and 2).¹ Coumadin, FDA-cleared in 1954, had become an essential part of acute coronary syndrome treatment; it was intended to reduce the risk of a secondary MI, peripheral artery disease, ischemic stroke, and venous thromboembolic disease subsequent to an MI. Coumadin continues today as the most-prescribed anticoagulant in North America, though now its most frequent indication is prophylaxis to reduce the risk of ischemic stroke in non-valvular atrial fibrillation. Because of its narrow therapeutic and safety range, Coumadin requires monthly laboratory monitoring using the prothrombin time assay, which, beginning in 1987, was enhanced through computation of the international normalized ratio (PT/INR).² The accepted therapeutic range worldwide is an INR of 2–3. An INR below 2 signals increased risk of thrombosis, above 3, risk of hemorrhage.³

In 1992, thienopyridine, a nucleic acid derivative distributed as ticlopidine (Ticlid®), was added to post-MI Coumadin therapy (Figure 3).⁴ Ticlopidine is an antiplatelet drug that reversibly occupies the platelet membrane ADP receptor, P2Y₁₂ (Figure 3). Ticlopidine competes with ADP for P2Y₁₂ receptor sites, suppressing ADP's platelet activation property. From 1992 to 2000, Coumadin and Ticlid administered together were the most commonly used post-MI antithrombotic regimen. This regimen was typically discontinued six months to two years after the event. Regrettably, 1 in 3–5000 ticlopidine patients developed life-threatening thrombotic thrombocytopenic purpura or aplastic anemia, creating a negative public perception.⁵ This plus the 1997 release of the

biochemically similar but seemingly safer thienopyridine clopidogrel (Plavix®) caused Ticlid to be withdrawn from the North American market in 2010, though generic ticlopidine preparations remain available (Figure 3). Popular knowledge to the contrary, thrombotic thrombocytopenic purpura continues to be associated with all forms of thienopyridine.

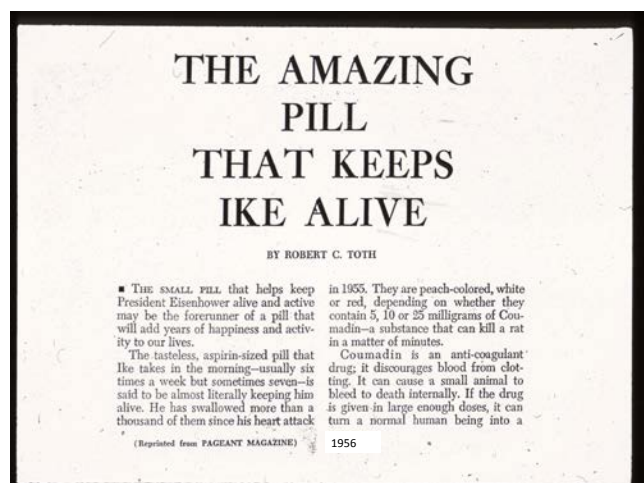


Figure 1. 1956 newspaper article describing President Dwight D. Eisenhower's Coumadin therapy. He began using the drug subsequent to an MI in 1955, just one year after it was cleared by the FDA.

Also around 1997, Coumadin began to be replaced with aspirin (acetylsalicylic acid, ASA) as the mainstay of post-MI therapy. Coumadin's effect is to reduce the plasma activity of coagulation factors II, VII, IX, and X, reducing the risk of venous thromboembolic disease but exerting little platelet suppression. Many adverse events that occur subsequent to MI are arterial, and arterial thrombosis results mostly from platelet activation, thus pushing post-MI therapy towards ASA.

A number of clinical trials conducted between 1995 and 2010 have shown that dual antiplatelet therapy (DAPT) employing ASA and clopidogrel, provides slightly improved efficacy and safety with fewer side effects than Coumadin and clopidogrel, ASA alone, or clopidogrel alone.⁶ DAPT is now the mainstay for prevention of secondary adverse events such as repeat MI or stent thrombosis in "uncomplicated" acute coronary syndrome (ACS). The typical dosages are 81 mg ASA (one baby aspirin) and 75 mg clopidogrel per day. Coumadin is still used in ACS when complicated by reduced ventricular ejection fraction (coronary insufficiency).⁷

Clopidogrel Mechanism

Clopidogrel is distributed as a prodrug that is absorbed within one hour of ingestion and becomes metabolized through the liver cytochrome P450 C19 pathway to produce 2-oxo-clopidogrel.⁸ The 2-oxo-clopidogrel has a half-life of 20 minutes, during which time it reversibly binds the platelet membrane ADP receptor P2Y₁₂, competing with ADP access to its receptor and thus suppressing platelet activation.

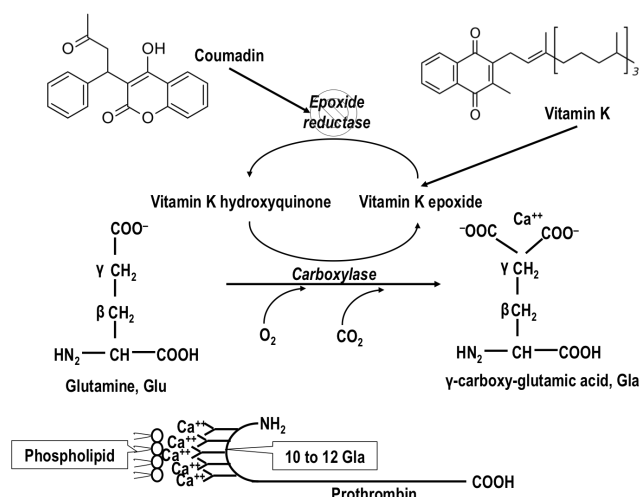


Figure 2. Vitamin K post-translational γ -carboxylation of coagulation factors II (prothrombin), VII, IX, and X, and control proteins C, S, and Z. Vitamin K hydroxyquinone transfers a carboxyl (COO⁻) group to the γ -carbon of glutamic acid, creating γ -carboxyglutamic acid. The negatively charged pocket formed by the pair of carboxyl groups attracts ionic calcium, enabling the molecule to bind phosphatidyl serine. Vitamin K hydroxyquinone is oxidized to vitamin K epoxide by carboxylase in the process of transferring the carboxyl group but is subsequently reduced to the hydroxyquinone form by epoxide reductase. Warfarin suppresses epoxide reductase, slowing the reaction, preventing γ -carboxylation. "Des-carboxy" proteins are unable to participate in coagulation. There are typically 12 to 18 γ -carboxyglutamic acid molecules near the amino terminus of the vitamin K-dependent factors.

Aspirin Mechanism

In 1948, Lawrence Craven provided the first evidence of ASA's ability to reduce AMI risk.⁹ Doubtful at the time, his findings were confirmed by HD Lewis in 1983 and subsequently confirmed in numerous large studies.^{10,11} For approximately 30 minutes subsequent to absorption, ASA releases its acetyl group to cyclooxygenase (COX), an essential enzyme in the prostaglandin (eicosanoid) synthesis pathway (Figure 4).¹² The acetyl group occupies COX molecule serine

529 and blocks arachidonic acid access to the enzymatically active site at tyrosine 385 (Figure 5). Normally, arachidonic acid becomes converted to prostaglandin G_2 (PGG_2), then prostaglandin H_2 (PGH_2), and subsequently to thromboxane A_2 , the short-lived product that binds adenylate cyclase and activates the platelet. COX acetylation is irreversible and prevents arachidonic acid to PGG_2 conversion, thereby inactivating the platelet. All platelets exposed to clopidogrel or ASA circulate inactivated through their lifespan.

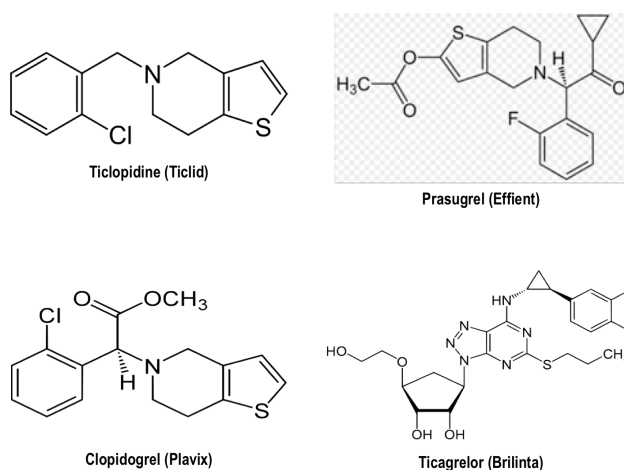


Figure 3. Structures of the thienopyridines ticlopidine, clopidogrel, prasugrel, and the purine analogue ticagrelor.

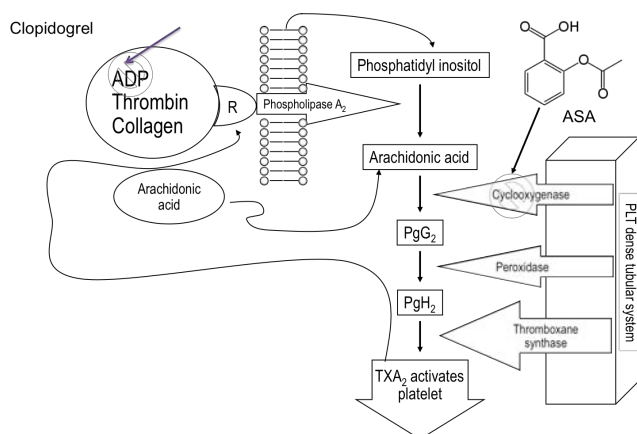


Figure 4. The prostaglandin (eicosanoid) synthesis pathway. Agonists ADP, thrombin, and collagen bind their respective membrane receptors and activate phospholipase A₂, which releases arachidonic acid from membrane phosphatidyl inositol. Arachidonic acid is converted by cyclooxygenase to a series of prostaglandins that generate the short-lived product, thromboxane A₂, which activates the platelet (Figure 5). ASA blocks cyclooxygenase (COX1). Clopidogrel and the thienopyridines reversibly bind the platelet membrane ADP receptor.

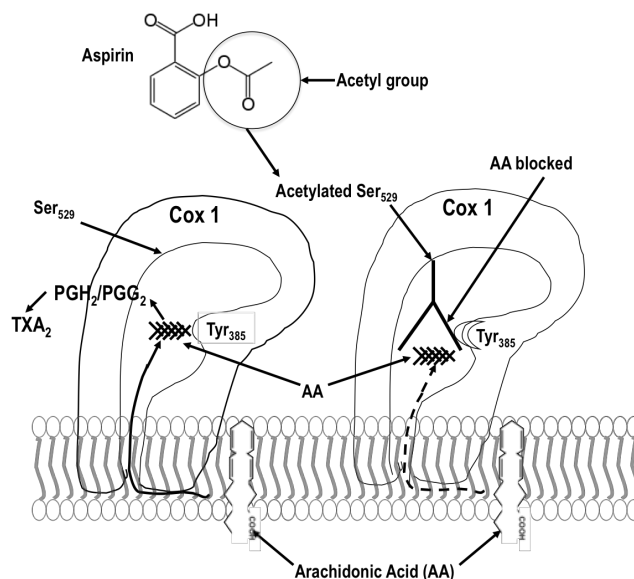


Figure 5. COX1 inhibited by aspirin. Arachidonic acid is released from membrane phospholipids and is converted by COX1 at the Tyr₃₈₅ active site to prostaglandins G_2 , then H_2 , and finally to the platelet activating molecule, thromboxane A₂. The acetyl group, released from aspirin, binds serine 529 and blocks tyrosine 385, preventing arachidonic acid access to the active site.

Aspirin Resistance

Between 10 and 20% of people who take 81 mg ASA daily do not generate the anticipated platelet function laboratory response.¹³ This phenomenon is called ASA resistance and the subjects are termed “ASA low responders (ALRs).” There exist multiple models to account for ALR, one of which contrasts the cyclooxygenase variants, COX1 and COX2. COX1 is constitutive, whereas COX2, a target of non-steroidal anti-inflammatory drugs (NSAIDs, celecoxib, Celebrex®, for instance) becomes induced during inflammatory conditions like diabetes, arthritis, and AMI. COX2 becomes acetylated in the same fashion as COX1, however arachidonic acid may nonetheless gain access to the tyrosine 385 active site, as the COX2 physical configuration is broader than COX1. Consequently, as COX2 becomes induced, ASA resistance may correlate with inflammation. A second model postulates that NSAIDs other than ASA may temporarily block ASA access to COX1, causing ALR during the time interval in which they are active.¹⁴

Clopidogrel Resistance

Likewise, patients respond differentially to clopidogrel. Here the reason is clear, the cytochrome P450-2 C19

polymorphism 2 (CYP2C19*2) codes for cytochrome pathway enzymes that metabolize the prodrug more slowly than the wild-type gene, a loss-of-function allele.¹⁵ Patients possessing the CYP2C19*2 polymorphism are termed clopidogrel low responders (CLRs) and comprise 20–30% of all clopidogrel patients.

Platelet Function Monitoring

In response to the established protocol that requires Coumadin to be monitored, a requirement regarded as a nuisance by patients, pharmaceutical manufacturers attempt to develop antithrombotics that function on a fixed-dose basis and thus require no laboratory monitoring. DAPT appears to meet this goal. Subsequent to percutaneous intervention (PCI, cardiac catheterization with stenting) or coronary artery bypass graft surgery (CABG), cardiologists start patients on a loading dose of 500 mg ASA followed by one 81 mg tablet per day in parallel with a loading dose of 600 mg clopidogrel followed by one tablet of 75 mg/day. Clopidogrel treatment is continued for 6 months to two years and ASA is continued indefinitely. Trusting the theoretical efficacy of DAPT, few clinicians order laboratory follow-up.

Patients are pleased to avoid regular laboratory visits; however, laboratory monitoring and dose adjustment appears to provide a clinical advantage. Neubauer et al in 2011 used whole blood impedance lumiaggregometry (WBA, Chrono-Log Model 590*, Chrono-Log Corporation, Havertown, PA, USA impedance aggregometer) to assay fresh whole blood specimens from 504 patients with stable coronary artery disease or ACS.¹⁶ All patients were tested 48–72 hours following PCI and the initiation of DAPT. The maintenance dose for clopidogrel was 75 mg/day; ASA, 100 mg/day. The investigators used as agonists 5 μ M ADP and 0.5 mmol/L arachidonic acid (Chrono-Par*, Chrono-Log Corporation, Havertown, PA, USA) to identify CLR and ALR, respectively. Reagent ADP normally triggers in vitro platelet aggregation and secretion, both measured in impedance lumiaggregometry, by occupying the P2Y₁₂ receptor site. This is the site that is blocked by clopidogrel. Reagent arachidonic acid enters the eicosanoid synthesis pathway where it is converted to active TXA₂, the pathway that is blocked by COX1 acetylation. Using upper limits of 5 Ω impedance for ADP-tested CLR and 0 Ω for arachidonic acid-tested

ALR, the investigators identified a CLR rate of 30.8% and an ALR rate of 19.4%, with dual low response (resistance in both reactions) of 8.5%.

To treat for ALR, Neubauer's group raised the ASA dose from 100 to 300 mg/day, reducing the ALR rate by 94.6%. The residual 5.4% were effectively treated at 500 mg/day ASA. By raising the clopidogrel dose from 75 to 150 mg/day, they reduced CLR rates by 69%. The remaining CLR patients were treated using prasugrel (Effient®) at 10 mg/day, effectively reducing the high-dose CLR rate by 92%, and the remaining high-dose CLR patients responded to 20 mg/day prasugrel.

Prasugrel is an alternative thienopyridine prodrug (Figure 3) that was FDA-released in 2009. Prasugrel is hydrolyzed in the intestine to a thiolactone, which is then converted to its active metabolite by the cytochrome pathways CYP3A4, CYP2B6, CYP2C9, and CYP2C19. Absorption takes place in less than 1 hour from ingestion and the active drug reaches its peak plasma level within 1 hour following absorption. Polymorphisms within these pathways have not been shown to affect the prasugrel response; hence laboratory monitoring of prasugrel may be unnecessary.¹⁷

Though not available at the time of the Neubauer study, ticagrelor (Brilinta®, AstraZeneca, Wilmington, Del) is a third antiplatelet drug, FDA-cleared in 2011 (Figure 3). Though loosely categorized with the thienopyridines, ticagrelor is a nucleoside analogue incorporating a nucleic acid base that resembles purine. The overall molecule resembles adenosine. Ticagrelor is administered in its pharmacologically active form. It is absorbed by the intestine and reaches its plasma peak within 2.5 hours. In contrast to clopidogrel and prasugrel, ticagrelor suppresses platelet activity by generating a conformational change of the P2Y₁₂ ADP receptor, though it does not directly fill the receptor site.¹⁸ The modified receptor does not respond to ADP. As ticagrelor does not require conversion at the time of absorption, like prasugrel, there appears to be no need for laboratory monitoring.

Platelet Function Testing

In addition to WBA, platelet functional response to antiplatelet therapy may be monitored using time-honored light transmittance aggregometry (LTA).

FOCUS: ANTIPLATELET DRUGS AND PLATELET FUNCTION TESTING

Historically, many have regarded LTA as the reference platelet function assay, however it requires a 9–12 mL whole blood specimen, low-force centrifugation to produce platelet-rich plasma, plasma with a platelet count of approximately 250,000/ μ L, and an interval of platelet function recovery following centrifugation. Most LTA aggregometers do not provide a luminescence channel to measure platelet secretion (Table 1).

The Chrono-Log corporation aggregometer series includes a low-cost, single channel whole blood

lumiaggregometer with disposable electrodes designed for near-patient testing, the Chrono-Log 591A*.

Accumetrics (Accriva Diagnostics, San Diego, CA) offers the VerifyNow whole blood assay system with arachidonic acid-impregnated cartridges for ASA response (VerifyNow Aspirin Test) and ADP cartridges for clopidogrel response (VerifyNow PRUTest).

The PRUTest adds prostaglandin E1 to the ADP-impregnated cartridge. PGE1 raises platelet cyclic AMP

Table 1. CLR and ALR Assays

Method	Assays	Advantages	Disadvantages
Whole blood impedance lumiaggregometry	ADP-induced aggregation for CLR	Reference method, whole blood, reports aggregation and secretion	High complexity, requires interpretation
Light transmittance aggregometry	Arachidonic acid (AA)-induced aggregation for ALR	Widely available since 1960	Large sample volume, requires centrifugation, no secretion, high complexity, requires interpretation
AspirinWorks	Immunoassay for urinary 11-dehydrothromboxane B ₂ tests for ALR	Random urine specimen, available since 2001	High complexity batch-wise assay provided by reference lab, no CLR
VerifyNow Aspirin	AA-induced aggregation for ALR on fibrinogen-coated microparticles	POC, CLIA-waived, direct sample transfer	
VerifyNow PRUTest	ADP/PGE ₁ -induced aggregation for CLR on fibrinogen-coated microparticles	POC, CLIA-waived, direct sample transfer	
Multiplate impedance aggregometry	ADPTest for CLR and ASPITest for ALR using lyophilized ADP and AA cartridges, respectively	Whole blood, semi-automated POC with 5 simultaneous channels	No luminescence channel
PFA-100	High-shear whole blood flow through aperture, COL-EPI, COL-ADP agonists used for ASA	Widely available POC device since 1997	Not FDA-cleared for ALR, large sample volume, no specific ALR agonist, no CLR assay
PlateletWorks	Sample tubes coated with ADP, AA, and collagen, tests for ALR and CLR	Inexpensive, widely available since 1999	Requires syringe collection and sample transfer, requires cell counter
TEM, TEG	Global viscoelastic hemostasis assay, amplitude reflects platelet function, assays for ALR and CLR	POC, widely available since 1948, reflects also coag factor deficiencies, heparin, fibrinogen concentration, and fibrinolysis	Not FDA-cleared for ALR or CLR
VASP	VASP-phosphorylation enzyme immunoassay detection assays for CLR		High-complexity batch-wise assay provided by reference lab, not FDA-cleared

and renders the test more specific for the effects of ADP on the P2Y₁₂ receptor. The PRUtest measures platelet function based upon the ability of ADP-activated platelets to bind to fibrinogen. Fibrinogen-coated microparticles aggregate in whole blood in response to platelet aggregation. If the P2Y₁₂ inhibitor has produced the expected antiplatelet effect, aggregation is slowed. The VerifyNow PRUtest reports the degree of platelet aggregation in P2Y₁₂ reaction units (PRUs). The PRU value reflects the degree of ADP-mediated aggregation specific to the platelet P2Y₁₂ receptor, and is calculated as a function of the rate and extent of platelet aggregation in the ADP channel, with a reference interval of 194–418 PRUs. In CLR, aggregation is inadequately suppressed, as indicated by a PRU value less than 418 or a similar, locally derived limit.

The VerifyNow Aspirin Test impregnates the activating cartridge with arachidonic acid and measures platelet function based upon the fibrinogen particle aggregation principle as in the PRUtest. If ASA has produced the expected antiplatelet effect, aggregation is slowed. The VerifyNow Aspirin Test reports the degree of platelet aggregation as ASA reaction units (ARUs). Given a reference interval of 350–700 ARUs, ARU values greater than 700 are consistent with ASA-induced inhibition of platelet function, whereas values less than 700 ARUs are evidence for ALR. The limit given here is from the manufacturer package insert and is confirmed locally.

The Multiplate® multiple electrode platelet analyzer, Roche Diagnostics, Basel, Switzerland, is a semiautomated five-channel whole blood impedance aggregometer. Each channel consists of a disposable cuvette with two electrodes; impedance readings from the electrodes are averaged. There is no luminescence channel, but the Multiplate can assay five separate samples or can use up to five agonists to test a single sample. The Multiplate offers the ADPtest® and the ASPITest® using lyophilized ADP and arachidonic acid respectively, both FDA-cleared in 2012.¹⁹ Specimen, diluent, and agonist pipetting is computer-aided and automatic.

AspirinWorks®, Corgenix Medical Corporation, Denver, CO, USA is an enzyme immunoassay that provides a quantitative measurement of urinary 11-dehydrothromboxane B₂, a stable, soluble endpoint of

eicosanoid synthesis (prostaglandin pathway) that reflects platelet activity with minimal interference. ALR is reflected in normal to elevated 11-dehydrothromboxane B₂ levels instead of the expected reduced values during ASA therapy. The 11-dehydrothromboxane B₂ value is normalized on urine creatinine, thus the assay may be performed on randomly collected, non-timed urine.

The Siemens Corporation PFA-100® is a time-tested point of care (POC) instrument designed to screen for platelet function disorders by creating high-shear blood flow through a narrow aperture. The PFA-100 uses collagen-epinephrine (COL-EPI) and collagen-ADP (COL-ADP) cartridges in tandem, each requiring 800 µL of whole blood. Testing begins with the COL-EPI cartridge. Normal whole blood clots under shear forces within 175 seconds of exposure to the COL-EPI, the “closure time (CT).”²⁰ Patients who have taken ASA should produce a CT longer than 175 seconds; a normal CT in ASA usage is presumptive for ALR. The operator confirms COL-EPI cartridge results with the COL-ADP cartridge, using a limit of 125 seconds. CT limits are established locally using normal specimens and may differ from manufacturer-recommended limits. The PFA-100 offers no assay for clopidogrel response and the aspirin response assay awaits FDA clearance. PFA-100 results may be influenced by von Willebrand factor activity.²¹

The Helena PlateletWorks® POC concept, FDA-cleared in 1999, requires that whole blood be collected in a syringe and transferred to tubes coated with either ADP, collagen, or arachidonic acid, plus a standard EDTA tube. The operator then performs a platelet count on each tube. If platelets are functioning normally, results in the three agonist-coated tubes are reduced by 50% compared to the EDTA tube value. Results that differ by less than 50% in the ADP tube indicates CLR, and in the arachidonic acid tube, ALR. PlateletWorks exploits available complete blood count instrumentation and the opportunity to simultaneously produce a complete blood count.

Rotational thromboelastometry (TEM, ROTEM Delta®, TEM Systems, Inc., Durham, NC USA) and thromboelastography (TEG, Thromboelastograph®, Haemonetics Corp, Braintree, MA USA), introduced in 1948, are whole blood POC global viscoelastic

hemostasis assays that are popular in operating suites around the world. These instruments provide clot onset, rate, and amplitude (strength) measures derived from a tracing (Figure 6). Clot amplitude reflects normal platelet function (as well as fibrinogen concentration), and is reduced in antithrombotic therapy. The TEG offers an assay called PlateletMapping®, FDA-cleared, that uses maximum amplitude to detect ALR and CLR.²² ROTEM assays for ALR and CLR await FDA clearance.

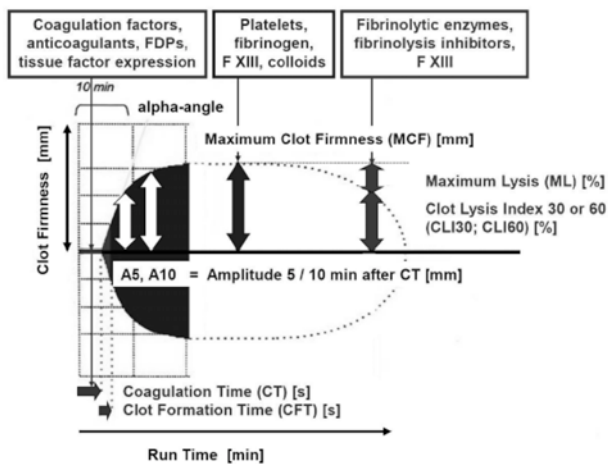


Figure 6. Thromboelastogram. Coagulation time and clot formation time reflect normal coagulation factor activity, anticoagulants, and coagulation factor deficiencies. Amplitude and maximum clot firmness reflect fibrinogen concentration, platelet count, platelet function, and factor XIII activity. Amplitude may be used as a research use only assay to detect ALR or CLR. Clot lysis index and maximum lysis reflect fibrinolysis activity.

Diagnostica Stago, Inc, offers the research use only intracellular vasodilator-stimulated phosphoprotein (VASP) phosphorylation enzyme immunoassay, Cy-Quant VASP/P2Y12. The assay uses a 96-well plate and plate reader that detects phosphorylation of platelet VASP, an indicator of platelet activation. Cy-Quant is P2Y₁₂-specific and is unaffected by ASA or platelet count. It is designed to detect CLR.²³

Disease Prediction

WBA, LTA, VerifyNow, Multiplate, and PlateletWorks detect ALR and CLR; and AspirinWorks, PFA-100, TEM, and VASP detect ALR. Laboratory practitioners know that all are surrogate measures for secondary adverse events, however several prospective randomized trials have linked ALR and CLR singly and together with ACS, diabetes mellitus, and elevated troponin

levels (Neubauer study). ALR and CLR predict each other and elevated HgBA1C predicts both. ALR predicts elevated C-reactive protein levels, a marker of inflammation. The CHARISMA trial used the Corgenix AspirinWorks assay to establish ALR as an independent predictor of secondary stroke, MI, and cardiovascular death.²⁴ Buonamici et al showed a positive correlation between CLR and stent thrombosis using VerifyNow PRUtest.²⁵ The ADEPT-DES trial also used VerifyNow PRUtest to find that CLR was related to stent thrombosis and AMI, was inversely related to bleeding, but was not related to mortality. Also, ALR using VerifyNow was not significantly associated with stent thrombosis, myocardial infarction, or death, but was inversely related to bleeding.²⁶ Perhaps the discrepancy in ALR findings relates to cross-platform variations.²⁷

Conclusion

When Boehringer Ingelheim released the oral direct thrombin inhibitor dabigatran in 2009, they experienced the largest first-year market growth in the history of new drug launches. Patients prefer anticoagulants that require no laboratory monitoring, and distributors choose to capitalize on that convenience. Though laboratory monitoring may enhance efficacy, unless monitoring also reduces risk, it is difficult to support studies that establish or rule out laboratory monitoring. DAPT appears to provide improved protection from secondary thrombotic events using with no monitoring. We now possess ASA, clopidogrel, prasugrel, and ticagrelor, an expansion of therapeutic options. The next stage is the application of individualized platelet function management using technically accessible methods and instrumentation. We can establish the therapeutic window for the best dose response and develop personalized antiplatelet therapy to achieve incremental improvements in adverse event-free outcomes. Perhaps a single, or two-step laboratory measurement approach, rather than monthly monitoring will achieve the outcome and will achieve antiplatelet regimens beyond P2Y₁₂ and COX 1 inhibition that reduce and eventually eliminate treatment failures.

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