Approximately 50% of ischemic strokes are caused by either atherosclerotic or small vessel ischemic disease, processes to which platelet activity is integral. In people with acute ischemic stroke or transient ischemic attack (TIA), acute management focuses on reducing risk of early neurologic deterioration as well as preventing stroke recurrence and consists of careful blood pressure adjustment, glucose management, and initiation or adjustment of statin and antiplatelet therapies. Interventions such as these may reduce recurrent stroke risk by as much as 80%, with the benefit mainly attributable to antiplatelet medication therapy. However, these drugs are not a panacea, and meta-analyses have demonstrated that despite optimal medical therapy, the risk of stroke recurrence at 1 year ranges between 5.7% and 17.7% depending on stroke subtype. Antiplatelet drug resistance may contribute to stroke recurrence. Various in vitro methods of platelet function testing (PFT) have been developed and validated. In this article, we discuss the clinical milieu of PFT and propose an algorithm for when to use PFT based on current evidence.

Mechanisms of Antiplatelet Resistance

Platelets express cyclooxygenase (COX), an enzyme that under normal circumstances converts arachidonic acid (AA) to thromboxane A2 (TXA2) and promotes vasoconstriction and platelet activation, resulting in platelet plug formation. This process, which is integral to the pathogenesis of ischemic stroke, is counteracted by aspirin, which irreversibly acetylates a serine residue of COX and inhibits this enzyme’s activity for the entire 10-day life of the platelet. COX exists as 2 isoforms (ie, COX-1 and COX-2), and aspirin is estimated to be as much as 170 times less effective against COX-2 than COX-1. This is especially relevant in inflammatory states, because the COX-2 isoform is more prevalent under these conditions. The redundancy in the COX pathway allows for alternative means of TXA2 production despite aspirin administration, especially in conditions that are known to promote inflammation, such as obesity and type 2 diabetes. Common medications also interfere with aspirin’s ability to inhibit COX activity. Aspirin’s activity is irreversible, but the drug has an estimated half-life of only 20 minutes. Therefore, if aspirin’s action is temporarily blocked by reversible, competitive inhibitors of COX, such as non-steroidal anti-inflammatory drugs (NSAIDs), the drug may miss its opportunity to suppress platelet function. Additionally, studies have demonstrated that administration of ibuprofen or naproxen 2 hours before aspirin inhibited aspirin’s ability to suppress the COX-1 pathway.

Unlike aspirin, clopidogrel is a prodrug and must be converted by CYP450 to its active metabolite before irreversibly binding and blocking the P2Y12 platelet surface receptor. CYP450 loss of function (LOF) sequence variations, which have been shown to decrease clopidogrel response by as much as one-third, are not uncommon, but vary by population. In one analysis, 15% to 17% of Chinese, 13% of Korean, 1% to 6% of White, and 7.5% of Black participants were poor metabolizers of clopidogrel. Commonly prescribed drugs also play a role in reducing clopidogrel function, and certain selective serotonin reuptake inhibitors (SSRIs) and proton pump inhibitors are known to interfere
with clopidogrel activation by virtue of their effect on CYP450.

Medication nonadherence is a common cause of poor therapeutic response to vascular disease.\(^8\) In a study by Schwartz et al.,\(^8\) among 190 participants with a history of myocardial infarction (MI), 17 failed to show acetylsalicylic acid (ASA) inhibition of arachidonic acid–dependent platelet aggregation while receiving their usual daily ASA dose. When retested 2 hours after witnessed aspirin ingestion, ASA inhibition of platelet aggregation was observed in all but 1 individual. This study highlights the fact that interventions are only as effective as individual adherence to therapies, and careful counseling that addresses medication nonadherence may be an important factor in improving individual outcomes.

**PFT Types**

PFT enables clinicians to evaluate the efficacy of antiplatelets in their patients, and a variety of testing methodologies are available. The first and most straightforward assay to evaluate aspirin response involves measurement of serum TXB2 levels. Under normal circumstances, COX-1 and thromboxane synthase convert AA to TXA2, which serves to enhance platelet activation and recruit additional platelets. However, because TXA2 has a very short half-life, its role as a surrogate for evaluating aspirin resistance is limited. TXA2’s metabolite, TXB2, is more stable and can be quantified in both blood and urine. Therefore, measurement of TXB2 serum levels is the most direct means of evaluating COX-1 activity. In comparison, light transmission aggregometry (LTA) is an in vitro method that is considered the standard of PFT and can be used to assess both aspirin and clopidogrel response. LTA involves combining an individual’s platelet-rich plasma with AA and quantifying the optic density. As the platelets aggregate, the fluid suspension becomes less turbid, allowing for more light to travel through the suspension which decreases the optical density. Like TXB2 quantification, LTA assesses the COX pathway, but it requires a great deal of expertise to perform. As such, it is not as practical as other platelet function assays on the market.

VerifyNow (Werfen; Bedford, MA) aspirin and Plavix resistance tests involve loading whole blood into cartridges with fibrinogen-coated beads and AA (in the aspirin resistance test) or adenosine diphosphate (in the Plavix resistance test). Platelets aggregate on the beads in proportion to the number of activated glycoprotein (GP) I/IIa receptors on their surface, and photometry is used to measure the change in optical signal intensity as aggregation proceeds. This assay uses whole blood; therefore, there is much less processing required compared with LTA. The results of VerifyNow tests have been shown to correlate with clinical outcomes in various investigations, as described in the following section.

**Implications**

The incidence of aspirin resistance ranges from 5% to 24% among different studies.\(^9\) For example, in a study of Colombian patients the rate of aspirin resistance was 7.4%, whereas in an Iranian study the rate was as high as 75.3%.\(^10\) One explanation for this heterogeneity may be genetic variability among differing populations. Some examples include a polymorphism within the COX-1 gene affecting serine 529, the site of aspirin activity, or polymorphisms of the genes encoding GP Ia/IIa and GP Ila.\(^11\) Other variables may include differences in PFT used, comorbidities, and participant cohorts (ie, those with stable vs unstable coronary artery disease, and those with recent ischemic stroke). Laboratory indicators of aspirin resistance appear to correlate with clinical outcomes. For example, Zheng et al.\(^12\) found that aspirin resistance was associated with increased stroke severity and infarct size during the acute stroke phase, whereas Ozben et al.\(^13\) identified aspirin resistance as an independent predictor of 2-year mortality. Similarly, in their 2023 study of 190 Taiwanese participants, Lee et al.\(^14\) demonstrated that high-on-clopidogrel platelet P2Y12 reactivity was associated with a 2.5 times greater risk of recurrent stroke in the follow-up period of 21 days.

Given the correlations between antiplatelet resistance and stroke outcomes, PFT seems an attractive option for guiding therapy and improving outcomes in ischemic stroke. However, data supporting the utility of PFT in clinical practice have been mixed and are largely derived from studies of people with coronary artery disease rather than ischemic stroke. For example, in the Gauging Responsiveness With a VerifyNow Assay—Impact on Thrombosis and Safety (GRAVITAS) trial (NCT00645918), people deemed to be resistant to clopidogrel by VerifyNow testing were randomized to either high-dose or standard-dose clopidogrel and evaluated for 6-month incidence of cardiovascular death, nonfatal MI, or stent thrombosis.\(^15\) The subgroup analysis of GRAVITAS showed that people with Plavix resistance units <208 were at a significantly lower risk of experiencing the primary endpoint of cardiovascular death, MI, or stent thrombosis after percutaneous coronary intervention. Conversely, in the Assessment by a Double Randomization of a Conventional Antiplatelet Strategy versus a Monitoring-Guided Strategy for Drug-Eluting Stent Implantation and of Treatment Interruption versus Continuation One Year After Stenting (ARCTIC) trial (NCT00827411), participants scheduled for coronary stenting were randomly assigned to either platelet function monitoring with subsequent drug adjustment or to a conventional strategy without monitoring or drug adjustment.\(^16\) No significant improvements in clinical outcomes, including death, MI, stent thrombosis, stroke, or urgent revascularization, were found when PFT was used to guide clopidogrel dosage in coronary stenting. Given
Regardless of these results, PFT is used frequently in endovascular therapy. In a cohort of individuals who were candidates for neurovascular stenting for management of aneurysms for whom aspirin and clopidogrel therapy were considered, about half of the participants were tested for platelet inhibition, and those found to be clopidogrel-resistant were switched to ticagrelor. Individuals whose regimen was adjusted based on PFT had fewer neurologic deficits and thromboembolic events than did those whose regimen was not adjusted. Findings such as these have generated interest in using PFT to guide ischemic stroke management. In a retrospective study of people with acute ischemic stroke who were found to be aspirin-resistant, those who had their antiplatelet regimen modified based on PFT had a lower rate of subsequent ischemic stroke compared with those who received no modification in antiplatelet therapy (hazard ratio [HR] = 0.70; P = .03). As with any other clinical test used in modern health care, a salient question is whether the test will be cost-effective. In 2023, Micieli et al evaluated individuals in Canada who had a TIA or minor stroke and received dual antiplatelet therapy (DAPT) for 3 weeks. Researchers analyzed the costs and health benefits of testing for the CYP450 LOF allele with switch to ticagrelor when appropriate vs standard of care (ie, no LOF testing). Results showed that compared with the standard of care, LOF testing led to an increase in life-years gained and quality-adjusted life years. The researchers also found that LOF allele testing was cost-effective in more than 99.99% of simulations using a willingness-to-pay threshold. This study provides compelling, albeit local and preliminary, evidence that LOF allele testing has the potential to be both cost-effective and clinically beneficial. Given the incidence of both aspirin and clopidogrel resistance and the clinical implications of reduced efficacy,
PFT may be beneficial in people who are starting antiplatelet treatment for the first time or who have had a TIA or stroke despite compliance with their treatment regimen without concurrent use of medications that may reduce their efficacy. We propose an algorithmic approach to PFT (Figure) that could be applied to these individuals and that is clear enough for all frontline providers in a stroke department to follow with little need for additional instruction. This algorithm should not be applied for people assigned to receive DAPT as per trials such as Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events (CHANCE), Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT), and Stenting and Aggressive Medical Management for Preventing Recurrent Stroke in Intracranial Stenosis (SAMMPRIS), as these studies have already validated their specific DAPT regimens; because data regarding PFT are scarce, it would be unwise to deviate from these protocols until more data can be collected.

Summary

Antiplatelet resistance has a variable prevalence because of a multitude of factors but is a real phenomenon with tangible clinical implications within the realm of ischemic stroke. PFT is a valuable addition to the stroke treatment landscape that empowers physicians to tailor antiplatelet regimens more effectively with the goal of improving outcomes. An understanding of antiplatelet pharmacokinetics and platelet function assays is essential to implementing these tools effectively. Confirming medication compliance and assessing for any interacting medications (both prescription and over-the-counter) in presumed failed antiplatelet treatment is a simple but high-yield practice and may obviate the need for testing. For those cases in which testing is warranted, we propose an algorithmic approach to PFT and medication adjustment.

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