Published in final edited form as:
Expert Rev Neurother. 2011 February ; 11(2): 251–263. doi:10.1586/ern.10.203.

Antiplatelet resistance in stroke

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Abstract

Although the exact prevalence of antiplatelet resistance in ischemic stroke is not known, estimates about the two most widely used antiplatelet agents – aspirin and clopidogrel – suggest that the resistance rate is high, irrespective of the definition used and parameters measured. Inadequate antiplatelet responsiveness correlates with an increased risk of recurrent ischemic vascular events in patients with stroke and acute coronary syndrome. It is not currently known whether tailoring antiplatelet therapy based on platelet function test results translates into a more effective strategy to prevent secondary vascular events after stroke. Large-scale clinical trials using a universally accepted definition and standardized measurement techniques for antiplatelet resistance are needed to demonstrate whether a 'platelet-function test-guided antiplatelet treatment' strategy translates into improved stroke care. This article gives an overview of the clinical importance of laboratory antiplatelet resistance, describes the challenges for platelet-function test-guided antiplatelet treatment and discusses practical issues about the management of patients with aspirin and/or clopidogrel resistance.

Keywords

antiplatelet; aspirin; clopidogrel; ischemic stroke; non-response; platelet function assay; point of care; resistance; stroke

The concept of antiplatelet resistance in stroke

Each year, almost 185,000 recurrent strokes occur in the USA and approximately a third to half of them develop while on antiplatelet therapy [1]. The occurrence of thrombotic events despite the use of antiplatelet drugs has led to the concept of 'antiplatelet resistance' [2]. Other commonly used terms for antiplatelet resistance include 'antiplatelet treatment failure', 'antiplatelet nonresponsiveness' and 'inadequate efficacy'.

The term 'antiplatelet resistance', in its most strict sense, refers to insufficient inhibition of platelet aggregation by antiplatelet agents *in vitro*. Laboratory resistance to aspirin is defined as a failure to achieve reduction in platelet thromboxane A2 (TxA2) formation following

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Financial & competing interests disclosure
Hakan Ay has been supported by NIH grant R01-NS059710. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

inhibition of platelet cyclooxygenase-1 (COX-1) enzyme. Laboratory resistance to thienopyridines refers to the inability to attain reduction in ADP-mediated platelet aggregation after blockade of $P2Y_{12}$ receptor signaling. Clinical antiplatelet resistance or treatment failure, on the other hand, designates the occurrence of ischemic stroke or other vascular events during antiplatelet treatment. It is important to notice that treatment failure does not necessarily indicate laboratory antiplatelet resistance. Antiplatelet agents commonly used in the prevention of vascular events do not inhibit all pathways of platelet activation and aggregation [3]. Platelets may continue to aggregate and cause a stroke despite full inhibition of one particular pathway as a result of the recruitment of compensatory pathways not blocked by the antiplatelet agent. Therefore, it is critical to test all aspects of platelet function before attributing a clinical recurrence to laboratory resistance. Another point that deserves consideration is that stroke is an etiologically heterogeneous disorder. The extent to which platelets contribute to stroke pathophysiology varies depending on the underlying cause. Mechanisms such as proximal embolism from a cardiac or venous source, inflammatory and noninflammatory vasculopathies, nonthrombotic occluding atheroma, iatrogenic causes or hemodynamic compromise, and arterial vasoconstriction or vasospasm require limited contribution of platelets to vascular occlusion, and therefore can result in clinical–laboratory dissociation (clinical recurrence in the absence of laboratory resistance).

Although insufficient platelet response to anti-platelet treatment has been recognized for a long time, antiplatelet resistance in ischemic stroke has only been the focus of increased interest in recent years. There are several reasons for this: first, acceptable alternatives to aspirin that inhibit alternative pathways of platelet activation, such as clopidogrel, ticlopidine, dipyridamole–aspirin combination and cilostazol, have become available. Second, conventional labor-intensive platelet function methods (light transmission or optical light transmission aggregometry) have been replaced by simple point-of care (POC) tests that rapidly measure platelet function in the whole blood at the bedside [4]. Third, evidence indicating that platelet activity measures provide a predictive value for clinical outcome after stroke has started to emerge [5]. Fourth, combination antiplatelet therapies in individuals with clinical recurrence while on single antiplatelet therapy have been shown to confer an increased risk of clinically significant intra- or extracranial hemorrhage [6,7]. Finally, indications for antiplatelet therapy have evolved to include conditions that are associated with substantial risk of vascular injury and thrombus formation, such as percutaneous neurovascular interventions.

Evaluation of platelet functions to guide antiplatelet therapy may translate into a reduced rate of ischemic and hemorrhagic complications, and thus improved patient outcome. To develop a platelet function test-guided antiplatelet treatment strategy, it is necessary to have a readily available, easily applicable, affordable, reliable and valid method of monitoring the biological effect of antiplatelet drugs. It is also necessary to gain a better understanding of potential sources of variance in platelet aggregation and stroke pathophysiology. The purpose of this article is to introduce the factors that play a role in the variability of platelet response in patients with ischemic stroke and to provide practical considerations for clinical and laboratory aspects of resistance to aspirin and clopidogrel.

Prevalence of laboratory antiplatelet resistance

The rate of antiplatelet resistance in patients with stroke or transient ischemic attack (TIA) is highly variable, ranging from 3 to 85% for aspirin and 28 to 44% for clopidogrel (Table 1) [8]. This high variance can be partly attributed to the lack of correlation among different measurement techniques. The prevalence of antiplatelet resistance is highly assay- and agonist-dependent [9–11]. In a study of 201 patients with stable coronary artery disease

using aspirin, laboratory-based aspirin resistance was 60% with Platelet Function Analyzer-100® (PFA-100®; Dade-Behring), 10–52% with light transmittance aggregometry (LTA) using different concentrations of arachidonic acid as agonist, 23% with urinary 11-dehydrothromboxane-B2 (TxB2) assay, 18% with whole-blood aggregometry (Chronolog®), 7% with VerifyNowAspirin® assay and 4% LTA with standard arachidonic acid as agonist [10]. The rate of clopidogrel resistance is also assay-dependent. In a study of 70 patients receiving 150 mg clopidogrel after percutaneous coronary intervention, the clopidogrel resistance was 13% with LTA and ADP as agonist, 39% with vasodilator-stimulated phosphoprotein assay and 33% with the VerifyNow $P2Y_{12}$®[12]. In general, tests that are directly related to the inhibition of the COX-1 or $P2Y_{12}$ receptor, such as LTA with arachidonic acid as agonist, VerifyNow Aspirin® and VerifyNow $P2Y_{12}$, show lower resistance rates compared with nonspecific assays (PFA-100) [13]. In addition to the type of test, multiple technical and clinical factors can also confound the results of platelet function tests. These include posture [14], quality of the blood draw [15], platelet response to endothelial injury that occurs during blood collection[16], time of day [9,17], sample transport conditions [15], platelet count [15], hemolysis [15], exercise [18], smoking [19,20], age [21], gender [22], presence of infection [23], obesity [24,25], glucose control in diabetics [25,26], hemoglobin [27], serum cholesterol [26] and triglyceride [28], as well as use of concomitant agents, such as anticoagulants [29].

Another source of variability in published rates of antiplatelet resistance is the lack of a standard definition for thresholds used to determine sufficient response [30]. Antiplatelet resistance is not an ‘all or none’ phenomenon; platelet response to antiplatelet treatment is a continuous parameter [31,32]. The definition of ‘resistance’ versus’ ‘nonresistance’ or ‘responder’ versus ‘nonresponder’ is largely dependent upon arbitrary cutoffs used in platelet function tests and is, therefore, highly variable; at least seven different thresholds have been used to define aspirin response in studies using the PFA-100 system [30,33,34]. Further complicating the problem is that antiplatelet resistance is not a stable phenomenon over time [35,36]. A patient who is ‘resistant’ at a specific time point can be found ‘responsive’ at another time point, despite the same treatment [37]. Platelet aggregability can recover despite sustained inhibition of one pathway due to strengthening of alternative pathways. Chronic aspirin use causes increased platelet response to TxA2-independent stimuli, such as ADP, thrombin, epinephrine, collagen and stress increases over time [31]. Similarly, clopidogrel treatment results in the upregulation of $P2Y_{12}$-independent pathways, such as thrombin, TxA2, collagen and $P2Y_1$ receptor-mediated platelet aggregation [31]. Variation in the timing of platelet function measurement relative to the index event (stroke, acute coronary syndrome, coronary or supra-aortic interventions) is also a potential contributor to the observed variability in the prevalence of antiplatelet resistance among the studies.

Proposed mechanisms of antiplatelet resistance

Several pharmacokinetic and pharmacodynamic factors, including reduced bioavailability, genetic polymorphisms, activation of alternate platelet-stimulation pathways, accelerated platelet turnover and factors associated with antiplatelet-resistant state, contribute to the variability in platelet inhibition by antiplatelet agents (Box 1). Accurate identification of the underlying mechanism of resistance, particularly a distinction between whether diminished platelet response to antiplatelet treatment is primarily due to the lack of antiplatelet drug’s effectiveness in inhibiting its target receptor or recruitment of alternative pathways for platelet activation, is key to the management. The following section describes potential causes of resistance to aspirin or clopidogrel based on their relative contribution to the inhibition of COX-1 and $P2Y_{12}$ systems.

Box 1

Possible causes of antiplatelet resistance

Antiplatelet resistance due to inadequate inhibition of COX-1 or $P2Y_{12}$

- Reduced bioavailability:
  - Poor compliance
  - Inappropriate dosing or underdosing
  - Reduced absorption
  - Increased metabolism
  - Drug–drug interactions
  - Aspirin: NSAIDs (ibuprofen, indometacin and naproxen), proton pump inhibitors
  - Clopidogrel: CYP3A4 substrates (atorvastatin, simvastatin and lovastatin) and inhibitors (amlodipine)
  - CYP2C19 substrates and inhibitors (omeprazole and esomeprazole)
- Genetic polymorphisms:
  - Aspirin:
    - Receptors: GPIa/IIa, GPIb α, GPIIIa (PlA1/A2), GPIIbIIIa, GPIb/V/IX, thromboxane and von Willebrand factor receptor
    - Enzymes: COX-1, COX-2, thromboxane A2 synthase and UDP-glucuronosyltransferases
    - Factor XIII Val34Leu polymorphism
  - Clopidogrel:
    - Receptors: $P2Y_{12}$
    - Enzymes: CYP3A4, CYP1A2, CYP2C19, ABCB1 (P-glycoprotein), etc.

Antiplatelet resistance despite adequate inhibition of COX-1 or $P2Y_{12}$

- Activation of alternate platelet stimulation pathways:
  - Increase epinephrine-mediated platelet activation
  - Stress-induced COX-2 expression in platelets (aspirin)
  - Increased platelet sensitivity to ADP and collagen
  - Increased release of ADP
  - Red-cell-induced platelet activation
  - Provide PGH2 to platelets (COX-1 bypass) or direct synthesis of TbXA2, by endothelium and monocytes (aspirin)
  - Increased $P2Y_1$-dependent platelet aggregation (clopidogrel)
- Accelerated platelet turnover:

- Stress, bleeding and surgery
- Acute ischemic syndromes
- Acute or chronic infection or inflammation

- Other:
  - Severity, duration and control of atherosclerosis
  - Diabetes mellitus and other vascular risk factors
  - Enhanced basal platelet reactivity

Data from [2,30,70].

Antiplatelet resistance due to inadequate inhibition of COX-1 or $P2Y_{12}$

Reduced availability of antiplatelet drugs—The most common cause of inadequate antiplatelet response in laboratory assays is noncompliance or nonadherence to anti-platelet treatment [38]. Approximately 50% of patients using aspirin or clopidogrel either stop taking medication or fail to adhere to their prescribed dose at 1 year [39–41]. Detection of plasma aspirin, clopidogrel or their metabolite levels (salicylate for aspirin, active thiol or inactive carboxyl metabolite for clopidogrel) can be considered to confirm drug intake when noncompliance is a concern [39]. Alternatively, a shift towards greater platelet inhibition after a period of supervised drug intake strongly indicates that noncompliance is the mechanism responsible for poor platelet response to treatment [42].

Reduced bioavailability due to poor absorption also blunts platelet response to aspirin. Aspirin is served in different pharmaceutical formulations, including plain, enteric-coated, buffered, soluble, suppository, mouth-dispersible and micro-encapsulated forms. Enteric-coated or slow-release aspirin formulations are absorbed in the small intestine. Optimal absorption of aspirin occurs in a pH range of 2–4. The neutral pH environment of the small intestine results in delayed and reduced absorption, and thus inadequate platelet inhibition [43]. Plain preparations are stable in the acidic environment of the stomach and are rapidly absorbed [44]. Soluble buffered, dispersible and chewable preparations are comparable to plain aspirin in terms of absorption rate, onset of effect and magnitude of platelet inhibition [45].

Potential drug interactions may also be important in explaining the reduced response of platelets to antiplatelet therapy. Concomitant use of NSAIDs, particularly ibuprofen, offsets the clinical benefit of aspirin by blocking its docking site on COX-1 [46]. Proton pump inhibitors (PPIs) can reduce absorption of the active form of aspirin through suppression of gastric acid secretion and activation of gastrointestinal mucosal esterases that hydrolyze aspirin to an inactive form [30]. PPIs are substrates and inhibitors of the hepatic enzyme (CYP2C19) that converts clopidogrel to its active metabolite. Therefore, concomitant use of PPIs with clopidogrel can negate clopidogrel's antiplatelet effect [47–50]. However, published data on the clinical relevance of this interaction are inconsistent; although smaller studies suggest that the clopidogrel–PPI interaction is associated with an increased risk of vascular events after acute coronary syndromes [51,52], more recent data from larger studies fail to provide conclusive evidence for a clinically significant interaction [53,54]. It has been demonstrated that lipophilic statins, such as atorvastatin [55,56], simvastatin [56] and lovastatin [31], impede the antiplatelet effects of clopidogrel. Lipophilic statins inhibit the metabolism of clopidogrel to its active form through competition for the CYP3A4 enzyme. Although registry studies suggest a worse outcome in patients using clopidogrel together with lipophilic statins [31], randomized trials refute these findings by revealing a fairly comparable risk reduction in patients with and without statin therapy [57–59].

Dihydropyridine calcium-channel blockers are also substrates and inhibitors of the CYP3A4 enzyme. In patients treated with percutaneous coronary intervention (PCI), concomitant dihydropyridine calcium-channel blocker therapy correlates with higher platelet reactivity [60–62]. Clinical outcome in patients using clopidogrel and dihydropyridine calcium-channel blockers appears to be worse compared with those who do not use dihydropyridine calcium-channel blockers [62]. Larger randomized trials that take into account other differences among treatment groups are needed to confirm or refute the clinical relevance of this interaction.

Genetic polymorphisms—It is estimated that up to 30% of variability in platelet activity can be explained by genetic factors [63]. Although several polymorphisms have been found to cause resistance to antiplatelet medications, the lack of validation in large studies limits their use in clinically identifying resistant individuals [63,64]. Common polymorphisms for aspirin resistance include polymorphisms in the platelet *COX-1* (C50T/A842G polymorphism) and *COX-2* genes, the platelet glycoprotein receptor genes (*P1A1/A2* polymorphism) [64] and the UDP-glucuronosyltransferase gene (*UGT1A6*2*). Clopidogrel resistance has been linked to polymorphisms in genes involved in hepatic metabolism (*CYP 1A2*, *CYP3A4* and *CYP2C19*2*), intestinal absorption (*ABCB1* gene, P-glycoprotein gene, C3435T genotype) [65] and platelet surface receptors ($P2Y_1$ and $P2Y_{12}$) [66]. Individuals with these polymorphisms exhibit higher residual platelet aggregation and increased risk of instent thrombosis and death from myocardial infarction (MI) and stroke after acute coronary syndromes [67,68]. The US FDA has issued a warning regarding clopidogrel, alerting that it can be less effective in individuals carrying the CYP2C19*2 loss-of-function allele (poor metabolizers) [69]. More studies are needed to determine the clinical relevance of genetic polymorphisms in patients with ischemic stroke.

Antiplatelet resistance despite adequate inhibition of COX-1 or $P2Y_{12}$

Mechanisms that activate platelets through COX-1- or $P2Y_{12}$-independent pathways can cause increased baseline platelet reactivity and antiplatelet resistance despite full inhibition of the COX-1 or $P2Y_{12}$ systems. Aspirin reduces TxA2 synthesis through the inhibition of the COX-1 enzyme. However, COX-2 enzyme is inducible under certain conditions, such as inflammation and atherosclerosis, and can continue to provide TxA2 to platelets. Infection and advanced atherosclerosis associated with the formation of macrophage-rich plaques blunt platelet response to aspirin and clopidogrel, despite adequate COX-1 and $P2Y_{12}$ inhibition [2,70,71].

Another mechanism of poor platelet response to antiplatelet treatment is increased availability of circulating platelets that are not exposed to antiplatelet medication in the bloodstream. The plasma half-life of aspirin is only 20 min and the plasma half-life of the active metabolite of clopidogrel is approximately 8 h. Increased production by the bone marrow, enhanced turnover induced by stress and bleeding, as well as platelet transfusions, thereby, result in circulating platelets that are capable of aggregating in spite of treatment [31].

Diabetes mellitus is also associated with enhanced baseline platelet reactivity [25,29,72]. Several mechanisms have been proposed for the development of the antiplatelet-resistant state in diabetes mellitus. These include a higher rate of circulating immature platelets [73], diabetes-associated hyperfibrinogenemia [74], hyperlipidemia [26], systemic inflammatory stress [23,74,75], reduced conversion of clopidogrel to its active metabolite [76], generalized platelet receptor overexpression [77], reduced formation of platelet-derived nitrous oxide [77], increased platelet sensitivity to ADP [77] and diminished acetylation of the target site of COX-1 [78]. Diabetes-associated antiplatelet resistance is associated with an increased

risk of cardiovascular events [72]. Although published data suggest that glycemic control results in improvement of platelet inhibition, translation of this finding to improvement in clinical outcome awaits further studies [25,26]. Other factors that are associated with increased platelet reactivity in spite of COX-1 and $P2Y_{12}$ inhibition include cigarette smoking, hyperlipidemia, obesity, hypertension, female gender and catecholamine surge in response to mental stress, exercise, sepsis and congestive heart failure [31].

Laboratory tests to assess platelet function

Although several platelet function tests are available to determine 'laboratory antiplatelet resistance', none are currently recommended for routine clinical use because of the lack of randomized trial data demonstrating that tailoring antiplatelet therapy based on platelet function tests improves clinical outcome. Nevertheless, the field is rapidly advancing and evidence from trials using newer, less labor-intensive and easy-to-apply tests is becoming available. Table 2 provides an overview of currently available tests to evaluate platelet function. There are two major categories of tests: specific and nonspecific tests. Each test serves a different purpose. Nonspecific tests reflect the global biologic response of platelets to various activators and inhibitors so that they provide an assessment of *in vivo* platelet activity (e.g., bleeding time and PFA-100®). Tests specific to COX-1 or $P2Y_{12}$ (e.g., LTA-arachidonic acid, serum TxB2 level and VerifyNow®), on the other hand, measure individual pathway- and drug-specific platelet inhibition.

The bleeding time is a simple bedside test used to assess platelet function, but it is not specific and poorly reproducible [4,31]. LTA measures changes in light transmittance that occur after platelet aggregation in response to the addition of agonists. LTA has been the gold standard test of platelet function and is still used to validate newer tests. There are several aspects of this technique that limit its use as a reliable and valid means to assess platelet function. These include operator dependency, poor reproducibility and lack of standardization of results [79]. Measurement of TxA2 pathway metabolites in the serum (TxB2) and urine (11-dehydroTxB2) can be used to assess the antiplatelet effect of aspirin [4]. However, TxA2 metabolites are also dependent on TxA2 production from COX-1-independent sources (e.g., monocytes/macrophages), and are therefore not necessarily platelet-specific. Nonplatelet sources are estimated to be responsible for up to 30% of urinary 11-dehydroTxB2 levels [80]. Flow cytometry is a conjugated monoclonal antibody-based technique that measures *in vitro* platelet reactivity to agonists. It is a highly specific assay for the assessment of activity of individual surface antigens (receptors) of platelets. However, it is poorly sensitive to changes in overall platelet aggregation [81].

Newer POC assays allow rapid assessment of platelet functions in whole blood at bedside. They are ideal for use in emergency departments or catheter laboratories because they do not require sample transport, pipetting, special handling or a specialized laboratory or technician. PFA-100® simulates the events occurring during a typical vessel wall damage under high shear conditions in the presence of erythrocytes. Shear stress is created by drawing anticoagulant blood through a capillary tube towards a small aperture coated with collagen/epinephrine or collagen/ADP cartridges. As blood flows through the system, a platelet plug forms and gradually occludes the aperture. The time needed to occlude the aperture is called closure time or *in vitro* bleeding time. The drawbacks of the PFA-100 system include limited sensitivity, dependence of platelet-independent factors, poor reliability and high variability in measurements [82]. Multiple Platelet Function Analyzer (Multiplate®, Munich, Germany) is an automated version of impedance aggregometry. Different reagents, including arachidonic acid (ASPItest®), ADP (ADPtest®), ADP plus prostaglandin E1 (ADP-HS® test), collagen (COLtest®), ristocetin and thrombin receptor activating peptide (TRAP-6) (TRAPtest®), are used to monitor the effect of aspirin,

thienopyridines and GPIIb/IIIa inhibitors. The VerifyNow® instrument (Accumetrics, San Diego, CA, USA), which is also known as the Ultegra Rapid Platelet Function Analyzer (RPFA), is a fully automated test using turbidometric aggregometry methodology. Three VerifyNow assays are available: the VerifyNow IIb/IIIa Assay® is sensitive to GPIIb/IIIa antagonists, the VerifyNow Aspirin Assay® is sensitive to aspirin and the VerifyNow $P2Y_{12}$ Assay® is sensitive to thienopyridines. The Impact® Cone and Plate(let) Analyzer (DiaMed) contains a microscope and performs staining and image analysis of the platelets that have adhered and aggregated on a specially designed plastic plate [83]. POC assays are still new systems. More data regarding correlation between results obtained with these systems and the gold standard LTA are needed [84].

Clinical significance of antiplatelet resistance

Although there is no unified definition for antiplatelet resistance, the published data to date suggest that platelet aggregability is associated with clinical outcome after stroke. Grotemeyer *et al.* studied platelet reactivity in 180 patients with anterior circulation infarcts 12 h after an oral dose of 500 mg aspirin [85]. A third of patients were diagnosed to have aspirin resistance. All patients were discharged with 500 mg aspirin three-times daily. After a 2-year follow-up, the incidence of stroke, MI or vascular death was 4.4% in aspirin responders and 40% in aspirin nonresponders. Grundmann *et al.* studied 53 patients using 100 mg/day aspirin for more than 5 years for secondary prevention of cardiovascular events [34]. The rate of aspirin resistance was 34% in patients with, and 0% in patients without, ischemic stroke or TIA during the previous 3 days. In a case–control study of 653 consecutive patients treated with aspirin for secondary stroke prophylaxis, aspirin resistance was diagnosed in 36% of patients with and 17% of patients without prior history of stroke or TIA while on aspirin treatment [86].

There are relatively more data on the link between antiplatelet resistance and clinical outcome in cardiac patients. A meta-analysis of 13 prospective cohort and three case–control studies, which comprised of 2781 patients with cardiovascular disease, demonstrated that the pooled odds ratio (OR) of cardiovascular outcome for laboratory antiplatelet resistance was 3.8 (95% CI: 2.3–6.1) [87]. In another meta-analysis of 20 studies (17 cohort, two case–control and one descriptive study) including a total of 2930 patients with cardiovascular disease, the cardiovascular event rate was 39% in aspirin-resistant patients and 16% in aspirin-sensitive patients (OR: 3.9; 95% CI: 3.1–4.8) [88]. A meta-analysis that included 3688 patients undergoing coronary angioplasty and stenting from 25 studies demonstrated that clopidogrel resistance detected by POC tests was associated with increased risk of cardiovascular events (OR: 8.0; 95% CI: 3.4–19.0) [89]. In a multicenter, prospective, controlled study of 162 patients undergoing coronary angioplasty and stenting, patients randomized to receive additional bolus doses of clopidogrel according to the results of platelet function tests before the procedure had a lower risk of cardiac events (10 vs 0%) during the 1-month follow-up compared with controls who received a standard bolus of clopidogrel not guided by platelet function test results [90]. Although these studies indicate that laboratory antiplatelet resistance may be a clinically important phenomenon, marked heterogeneity among the studies in aspirin dose (75–1500 mg/day), duration of follow-up, type of assay used to determine laboratory resistance, cutoff value to define resistance, distribution of withdrawals, definition of end points and the method of confirmation of compliance limits the validity of their conclusions. The lack of data from well-controlled large-scale randomized trials deters guidelines from recommending the use of platelet function tests for outcome prediction in routine clinical practice. The American College of Cardiology/American Heart Association Guidelines currently only recommend platelet function testing in high-risk patients for coronary stent thrombosis (class IIB, level of evidence C).

Management of laboratory antiplatelet resistance

It is not known whether treatment modification based on laboratory testing is safe, efficient or cost-effective in stroke. More data from large-scale studies are needed to support whether switching from one antiplatelet agent to another, increasing the dose of an antiplatelet drug or administration of a combination of antiplatelet drugs overcomes antiplatelet resistance. However, the lack of high-quality data should not cause a neglect on the side of treating physicians in addressing modifiable causes of antiplatelet resistance. Some simple measures to enhance platelet response to antiplatelet medication include improving patient compliance, selecting the right formulation, avoiding drug interactions and controlling vascular risk factors.

Subjects with antiplatelet resistance may be candidates for a more potent platelet inhibition. One option for this may be to increase the dose of the antiplatelet agent. Platelet response to aspirin seems to be dose-dependent; higher doses (e.g., 300 mg instead of 100 mg) are associated with a lower rate of aspirin resistance [91]. However, there is currently no evidence that platelet function test-guided dose escalation is associated with better outcome. On the contrary, different doses of aspirin (75–1500 mg) are known to provide similar benefit in reducing morbidity and mortality from stroke and other cardiovascular diseases [92]. Moreover, there is a clear dose–hemorrhage relationship for aspirin therapy. Trials are needed to assess the risk and benefit of aggressive aspirin dosing strategies based on platelet function test results. The same conclusion also applies to clopidogrel; although higher loading (1200 mg instead of the usual 600 mg) and maintenance dosing strategies (150 mg/day instead of the routine 75 mg/day) provide greater platelet inhibition in individuals with the CYP2C19 loss-of-function polymorphism [93], it remains to be determined whether increased dosing strategy is safe and effective in clopidogrel-resistant patients with stroke.

More potent platelet inhibition may also be achieved by transition to a different formulation or to a different class of antiplatelet agent. Buffered and enteric-coated aspirin preparations are often prescribed to minimize gastrointestinal side effects, yet, as mentioned before, these preparations suffer from lower bioavailability compared with regular or plain formulations [43]. It is not currently known whether switching from aspirin to clopidogrel on the basis of clinical aspirin failure is an effective strategy because most of the aspirin nonresponders are also less responsive to clopidogrel [94]. Patients with laboratory resistance to clopidogrel may be responsive to other thienopyridines, such as ticlopidine [95,96], prasugrel, cangrelor, ticagrelor and elinogrel [66,97].

Drug–drug interactions should also be carefully taken into consideration in order to ensure optimal platelet response. Elimination of ibuprofen or other NSAIDs, such as naproxen, tiaprofenic acid and indomethacin, may be emphasized in patients using aspirin. If discontinuation is not an option, recommendation for an NSAID that does not interfere with aspirin, such as sulindac [98] and diclofenac [99], can be considered. Alternatively, aspirin can be administered when the plasma concentration of ibuprofen is low. Avoidance of ibuprofen from 8 h before until 30 min after administration of an aspirin dose has been shown to enhance platelet response to aspirin. All PPIs (esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole) reduce the antiplatelet effect of thienopyridines to varying degrees owing to CYP2C19 interaction. The strongest interaction occurs with omeprazole. Pantoprazole exhibits relatively limited interaction with the CYP2C19 system, yet the clinical significance of this interaction is uncertain [48,100]; a recent retrospective cohort study of patients receiving clopidogrel after MI has demonstrated that all PPIs, including pantoprazole, are associated with an increased risk of rehospitalization for MI and coronary stent placement [101]. Until further studies become available, the risks and benefits of concomitant treatment with PPIs and thienopyridines should be carefully

considered for each individual patient [102]. Lipophilic statins (atorvastatin, lovastatin and simvastatin) reduce the anti-platelet effect of clopidogrel by inhibiting its activation via interaction with CYP3A4, yet clinical trials do not indicate an increased risk for adverse vascular events by concomitant use of statin and clopidogrel [103]. Switching from lipophilic statins to hydrophilic statins (rosuvastatin and pravastatin) is not justified at this time. Likewise, there is currently no convincing evidence from clinical trials that discontinuation of dihydropyridine calcium channel blockers reduces the rate of vascular events in stroke patients with clopidogrel resistance.

Expert commentary

Increasing evidence suggests that antiplatelet resistance is common and closely correlates with the risk of future vascular events in patients with stroke and coronary artery disease. While individualization of antiplatelet treatment by measuring their effect at the target site is certainly desirable to achieve optimal platelet inhibition, there are several unresolved issues that impede the transition of platelet function measures to routine clinical practice. Laboratory tests measuring platelet responsiveness are currently not optimal. Generally, their results poorly correlate with each other and are subject to high intra-assay variability. In addition, thresholds used to define antiplatelet resistance are inconsistent across the studies and highly assay-dependent. The dynamic nature of platelet aggregability and multiplicity of its activation mechanisms further complicates the problem in terms of when and in whom to measure platelet function. As a result, it is not currently known whether platelet function test-guided anti-platelet therapy translates into a more effective strategy to prevent secondary vascular events after stroke. Well-powered randomized controlled trials are needed to take antiplatelet therapy to the individual level. Such trials will require the availability of platelet function tests that are applicable to a clinical setting, not labor-intensive, have low intra-assay variability, define resistance based on a well-established cutoff, and provide results that correlate with the clinical outcome. Since platelet aggregation is a multidimensional process, there is probably no single test or definition for antiplatelet resistance. Therefore, such trials should consider measuring both global *in vivo* platelet activity using nonspecific tests and drug-specific platelet inhibition using specific tests. The knowledge of global and specific responses to antiplatelet therapy may help in the development of strategies to achieve maximum platelet inhibition, while keeping the bleeding risk to a minimum.

The vast majority of data published on antiplatelet resistance come from studies of patients with coronary artery disease. While stroke and coronary artery disease share common risk factors and, to some extent, have similar pathophysiology, caution should be exercised before indiscriminate application of data from cardiac literature to stroke. Stroke is an etiologically diverse disease. The relative contribution of platelet activation to the pathophysiology of stroke and coronary artery disease may be different. For instance, atherosclerotic plaque rupture with subsequent platelet aggregation and thrombus formation is the causative mechanism in less than half of all ischemic strokes, whereas up to 90% of acute coronary syndromes are caused by plaque rupture. The safety of treatment modification based on platelet function testing may also be different between stroke and coronary artery disease. Stroke is generally a higher risk condition for developing intracranial hemorrhage by antiplatelet treatment compared with coronary artery disease [104]. It is necessary to test the efficacy and safety of ‘platelet function test-guided antiplatelet therapy’ separately in patients with ischemic stroke before any specific recommendations for this population can be made.

Until evidence from large-scale good quality trials on the utility of platelet function test-guided antiplatelet treatment becomes available, platelet inhibition by antiplatelet treatment

can be optimized by careful consideration of assuring adherence to antiplatelet medication, selecting the right dose and formulation, avoiding drug interactions and controlling baseline vascular risk factors. An individualized approach based on platelet function testing may be considered in patients with high and imminent risk of intravascular thrombus formation to prevent a catastrophic stroke. For instance, instent thrombosis in a vertebral or carotid artery could be lethal if the contralateral artery is occluded. Platelet function test results may also influence selection of stent type (drug-eluting or not) and revascularization strategy, such as endarterectomy versus stenting, in patients scheduled for neurovascular intervention.

Five-year view

In recent years, there has been an increasing interest in antiplatelet resistance in ischemic stroke. The number of publications on the prevalence of antiplatelet resistance in ischemic stroke has substantially increased during the last 5 years. It is anticipated that small observational studies exploring the prevalence of anti-platelet resistance will be replaced by larger cohort studies that focus *a priori* on the clinical relevance of antiplatelet resistance in ischemic stroke. It is also anticipated that large clinical trials will be launched to examine the impact of treatment modification based on platelet function test results on the risk of subsequent vascular events after stroke. Several such trials are currently underway in cardiac patients. The Aspirin Nonresponsiveness and Clopidogrel Endpoint Trial (ASCET) investigates whether clopidogrel treatment in patients with coronary artery disease who are nonresponsive to aspirin according to the PFA-100 method will reduce the risk of composite of unstable angina, MI, stroke or death [201]. The safety and efficacy of platelet function test-guided antiplatelet treatment are also being evaluated in several trials in patients undergoing percutaneous cardiac interventions (Gauging Responsiveness With A VerifyNow Assay – Impact On Thrombosis And Safety [GRAVITAS] [202]; Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation and Interruption Versus Continuation of Double Antiplatelet Therapy [ARCTIC] [203]; and Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel [TRIGGER-PCI] [204]). The results of these cardiac trials are expected to provide potentially important implications for the management of antiplatelet therapy in ischemic stroke.

There are several emerging antiplatelet drugs that have more predictable pharmacokinetic and pharmacodynamic properties and, therefore, exhibit a more potent and predictable antiplatelet effect compared with currently available agents. Prasugrel, ticagrelor and cangrelor are new-generation $P2Y_{12}$ receptor antagonists. Their efficacy is relatively free of hepatic activation, resistant to esterases and less dependent on CYP2C19 oxidation. Accordingly, they exert a more potent and predictable antiplatelet effect. To date, none have been tested exclusively in patients with stroke in a well-powered trial. Therefore, their efficacy and safety in stroke remains to be studied in the future. Future research in stroke will also focus on identifying genes involved in platelet response to antiplatelet treatment. Currently ongoing genome-wide association studies of stroke may reveal additional prevalent and clinically significant genetic abnormalities in the near future.

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Table 1

Prevalence of antiplatelet resistance in patients with stroke.

Study (year)	Agent (mg)	n	Prevalence of antiplatelet resistance (%)				Ref.
			LTA	PFA100®	VerifyNow®	Other	
Grotemeyer (1991)	Aspirin (500)	82	26	–	–	–	[105]
Grotemeyer *et al.* (1993)	Aspirin (500)	180	33	–	–	–	[85]
Helgason *et al.* (1993)	Aspirin (<325)†	113	20.5	–	–	–	[106]
Helgason *et al.* (1994)	Aspirin (<325)†	228	25.5	–	–	–	[37]
Grundman *et al.* (2003)	Aspirin (100)	53	–	34	–	–	[34]
Grau *et al*. (2003)	Aspirin (100–300)	31	–	16.1	–	–	[107]
Grau *et al*. (2003)	Aspirin plus clopidogrel	31	–	3.4	–	–	[107]
Alberts *et al*. (2004)	Aspirin (75–1300)	129	–	37	–	–	[108]
Macchi *et al*. (2004)	Aspirin (160)	37	–	24.3	–	–	[109]
Harrison *et al*. (2005)	Aspirin (75–150)	100	12	22	17	14 (LTA with ADP)	[110]
McCabe *et al.* (2005)	Aspirin (75–300)	103	–	50.5	–	–	[111]
Berrouschot *et al.* (2006)	Aspirin (300)	291	7.2	–	–	–	[112]
Hohlfeld *et al*. (2007)	Aspirin (50–1500)	90	15	–	–	–	[113]
Bennet *et al.* (2008)	Aspirin (100)	50	–	–	30	–	[114]
Englyst *et al.* (2008)	Aspirin (75)	40	–	–	–	67 (TEG)	[115]
Seok *et al*. (2008)	Aspirin (100)	88	–	–	12	25 (Urinary TxB_2)	[116]
Cha *et al*. (2008)	Aspirin (100)	107	6.5	–	–	25.2 (LTA with ADP)	[117]
Gengo *et al*. (2008)	Aspirin (81–325)†	653	14	–	–	17 (LTA with ADP)	[86]
Boncoraglio *et al*. (2009)	Aspirin (75–325)	129	–	20.1	–	–	[118]
Bernstein *et al.* (2009)‡	Aspirin (NS)	60	8.3	–	–	–	[119]
Von Lewinski *et al*. (2009)	Aspirin (100)	69	33	62	–	84 (LTA with ADP)	[120]
Von Lewinski *et al*. (2009)	Aspirin (300)	26	27	58	–	85 (LTA with ADP)	[120]
Von Lewinski *et al*. (2009)	Clopidogrel (75)	36	44	–	–	64 (LTA with collagen)	[120]
Von Lewinski *et al*. (2009)	Aspirin plus clopidogrel	11	0	73	–	9 (LTA with ADP)	[120]
Lee *et al*. (2010)	Aspirin (100)	244	–	–	11.5	–	[121]

Study (year)	Agent (mg)	n	Prevalence of antiplatelet resistance (%)				Ref.
			LTA	PFA100®	VerifyNow®	Other	
Jeon *et al.* (2010)	Aspirin (100)	117	–	–	13.7	–	[5]
Fong *et al.* (2010)	Aspirin (81–325)	436	28	–	–	–	[122]
Fong *et al.* (2010)	Clopidogrel (75)	299	28	–	–	–	[122]
Fong *et al.* (2010)	Aspirin plus clopidogrel	270	9.3	–	–	–	[122]

† Included primarily aspirin users.

‡ Included patients with stroke, transient ischemic attack or vascular cognitive impairment.

LTA: Light transmittance aggregometry; NS: Not specified; PFA: Platelet function analyzer; TEG: Thromboelastography; TxB_2: 11-dehydrothromboxane-B2.

Table 2

Methods used for the evaluation of antiplatelet resistance.

Test	Drugs tested	Limitations	Advantages
Bleeding time	Aspirin, thienopyridines	Poor reproducibility Operator dependent Significant patient discomfort	Standardized clinical test
Light transmission optical aggregometry	Aspirin, thienopyridines, GPIIb/IIIa inhibitors	Labor intensive Time consuming Operator dependent Large sample volume Expensive Difficult to standardize Low sensitivity	Gold standard
Platelet Works®, Helena Laboratories, (TX, USA)	Aspirin, thienopyridines, GPIIb/IIIa inhibitors	Expensive Limited specificity and sensitivity	Point of care
Multiplate® Platelet function Analyser† (Munich, Germany)	Aspirin, thienopyridines, GPIIb/IIIa inhibitors (indirect)	Overlap between non-, low- and high- responders	Point of care
VerifyNow® Accumetrics (CA, USA)	Aspirin, thienopyridines, GPIIb/IIIa	Lack of clear cutoff between low and high responders	Point of care
Platelet Function analyzer (PFA-100®), Dade Behring	Aspirin	Dependence on hematocrit and von Willebrand factor Insensitive to clopidogrel	Point of care
Impact Cone and Plate(let) Analyzer, DiaMed (Cressier, Switzerland)	Aspirin	Not widely available	Point of care
Thromboelastography Thromboelastogram, Haemoscope Corporation, (IL, USA; TEG® or ROTEM®)	Aspirin, thienopyridines, GPIIb/IIIa inhibitors	Not exclusively platelet dependent, mostly evaluates clot properties	Point of care
Flow cytometry	Aspirin, thienopyridines, GPIIb/IIIa inhibitors	Expensive, operator dependent	Requires small volume of whole blood
Vasodilator-stimulated phosphoprotein phosphorylation (Flow cytometric)	Thienopyridines	Operator dependent Labor intensive	Requires small volume of whole blood
Serum thromboxane B2 level	Aspirin	Not platelet specific, prone to artifact	Widely available
Urinary 11-dehydrothromboxane B2 Immunoassay (AspirinWorks®)	Aspirin	Not platelet specific Renal function dependent	Widely available

† Automated design of impedance light transmission aggregometry for whole blood samples.

Data taken from [2,8,83,97,123,124].