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A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease

Marie Lordkipanidzé[1,2,3], Chantal Pharand[1,2,3], Erick Schampaert[2,4,5], Jacques Turgeon[1], Donald A. Palisaitis[2,4,5], and Jean G. Diodati[2,4,5]*

[1]*Faculty of Pharmacy, Université de Montréal, Montréal, Québec, Canada;* [2]*Research Center, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada;* [3]*Department of Pharmacy, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada;* [4]*Faculty of Medicine, Université de Montréal, Montréal, Québec, Canada; and* [5]*Division of Cardiology, Hôpital du Sacré-Cœur de Montréal, 5400, boul. Gouin ouest, Montréal, Québec, Canada H4J 1C5*

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KEYWORDS
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Aims We sought to compare the results obtained from six major platelet function tests in the assessment of the prevalence of aspirin resistance in patients with stable coronary artery disease.
Methods and results 201 patients with stable coronary artery disease receiving daily aspirin therapy ($\geq$80 mg) were recruited. Platelet aggregation was measured by: (i) light transmission aggregometry (LTA) after stimulation with 1.6 mM of arachidonic acid (AA), (ii) LTA after adenosine diphosphate (ADP) (5, 10, and 20 μM) stimulation, (iii) whole blood aggregometry, (iv) PFA-100®, (v) VerifyNow Aspirin®; urinary 11-dehydro-thromboxane B_2 concentrations were also measured. Eight patients (4%, 95% CI 0.01–0.07) were deemed resistant to aspirin by LTA and AA. The prevalence of aspirin resistance varied according to the assay used: 10.3–51.7% for LTA using ADP as the agonist, 18.0% for whole blood aggregometry, 59.5% for PFA-100®, 6.7% for VerifyNow Aspirin®, and finally, 22.9% by measuring urinary 11-dehydro-thromboxane B_2 concentrations. Results from these tests showed poor correlation and agreement between themselves.
Conclusion Platelet function tests are not equally effective in measuring aspirin's antiplatelet effect and correlate poorly amongst themselves. The clinical usefulness of the different assays to classify correctly patients as aspirin resistant remains undetermined.

Introduction

Aspirin is one of the most widely used drugs worldwide.[1] First employed for its anti-inflammatory and antipyretic properties, it is now predominantly used in cardiology for its antiplatelet effects. Aspirin inhibits platelet aggregation through irreversible acetylation of platelet cyclooxygenase (COX) enzyme, blocking the transformation of arachidonic acid (AA) into thromboxane (Tx) A_2, a potent vasoconstricting and aggregating agent.[2] As a result, the use of aspirin reduces the risk of stroke, myocardial infarction, or death by approximately 25% in patients with cardiovascular disease.[3]

Despite its high efficacy, safety, and low cost, aspirin may not benefit all patients equally. Although there is no consensual definition of aspirin resistance, it is generally accepted that incomplete suppression of platelet aggregation as assessed by platelet function assays constitutes biochemical unresponsiveness of platelets to the inhibitory action of aspirin, a definition used herein.[4–7] An overview of the literature reveals that 0.4–83.3% of patients do not respond to this drug.[5] However, the exact prevalence of aspirin resistance in patients suffering from stable coronary artery disease (CAD) remains unclear; this may be attributable to differences in studied populations, lack of formal definition of aspirin response, and use of non-standardized diagnostic methods.[5]

A myriad of tests are currently available to assess inhibition of platelet function induced by aspirin and their methodologies are diverse.[8] Light transmission aggregometry (LTA), the current gold standard,[8] evaluates luminosity as aggregation occurs in platelet-rich plasma (PRP) following stimulation with a platelet agonist.[9,10] Although this test has been used for over 40 years and was shown to predict clinical outcomes in aspirin resistant patients, poor standardization and the requirement for manipulation by a skilled technician limit its use to specialized laboratories.[8–11] In order to palliate these shortcomings, various point-of-care

* Corresponding author. Tel: +1 514 338 2222 ext. 3420; fax: +1 514 338 2694.
E-mail address: jean.gino.diodati@umontreal.ca

assays have been developed, but scarcely validated in large patient cohorts. Nonetheless, little is known about the comparability or interchangeability of these tests to assess aspirin response.

Hence, we present the first study designed to compare results simultaneously obtained from six major platelet function tests in the assessment of the prevalence of aspirin resistance in patients with stable CAD.

Methods

Patients

Two hundred and one consecutive patients with stable CAD (diagnosis based on a positive stress test or angiographically documented coronary artery stenosis) were enrolled in this study from the outpatient cardiology clinic of Hôpital du Sacré-Coeur de Montréal, Canada, from June 2005 to March 2006. All patients had received daily aspirin therapy ($\geq$80 mg daily) for at least 1 month. Exclusion criteria were acute coronary syndrome or revascularization within the last 6 months; concurrent ingestion of non-steroidal anti-inflammatory drugs (NSAID, including COX-2 selective anti-inflammatory drugs), clopidogrel, ticlopidine, dipyridamole, warfarin, or acenocoumarol; self-reported use of non-prescription NSAID or drugs containing aspirin in the 10 days preceding enrolment; major surgical procedure within 1 month of enrolment; platelet count outside the 100 to 450 $\times$ 10^9/L range; hematocrit $<$25% or haemoglobin $<$100 g/L; and chronic renal failure requiring dialysis. This study, which complies with the Declaration of Helsinki, was approved by the local Scientific and Ethics Review Board and all patients gave written informed consent.

Urine and blood sampling

After enrolment, a morning urine sample was brought in by the patient, within 2 h of collection. Upon arrival, compliance with therapy was assessed by a personal interview. Blood samples were then obtained from patients, between 7 AM and noon, following a 12 h fast, 2 to 12 h after the ingestion of the last aspirin dose, in order to eliminate any effect of circadian variation on platelet function. The first 2 mL of blood, drawn by venipuncture through a 21-gauge needle, were discarded. Then, blood was drawn into five 3.5-mL evacuated tubes containing 3.2% sodium citrate. All blood samples were processed within 2 h of collection.

Platelet aggregation assessment

Light transmission aggregometry

Platelet aggregation was assessed in PRP at 37°C by LTA. PRP was obtained by centrifugation of citrated whole blood for 10 min at 1000 rpm and adjusted to 250–450 $\times$ 10^9/L with platelet poor plasma (obtained by centrifugation of the remaining blood for 10 min at room temperature at 3000 rpm) if needed. Aggregation was measured with a ChronoLog Aggregometer (540 model, PA, USA) and was expressed as the maximal percent change in light transmittance from baseline after the addition of AA, using platelet poor plasma as reference. Although no consensus exists on the optimal AA concentration to be used to induce reliable and reproducible platelet aggregation while minimizing interindividual variability, the use of 1.6 mM (0.5 mg/mL) has been suggested as appropriate for the study of COX inhibitors through aggregometry.[12,13] Consequently, the primary agonist used was AA (LTA_{AA}; ChronoLog, PA, USA) at such a concentration. Subjects having residual platelet aggregation $\geq$20% despite daily aspirin therapy were considered aspirin resistant, as this cut-off has been frequently used in the past and associated with increased risk of suffering from adverse cardiac events.[11,14–22] Because LTA_{AA} is considered the gold standard for the detection of patients resistant to aspirin, it was used as the phenotypic identifier for comparison with other concurrent tests.

Adenosine diphosphate (ADP; Sigma Aldrich, Ontario, Canada) was also used as an agonist (LTA_{ADP}; 5, 10, and 20 μM). Previous investigators have reported that subjects having residual ADP-induced platelet aggregation $\geq$70% despite daily aspirin therapy were aspirin resistant.[11,14–16,18,20–22] Some authors have used the combination of LTA_{AA} (residual platelet aggregation $\geq$20%) and LTA_{ADP} (10 μM; residual platelet aggregation $\geq$70%) criteria to define aspirin resistance.[14,15,18]

Whole blood aggregometry

Whole blood aggregometry (WBA) measures electrical impedance (maximal amplitude) between two electrodes immersed in whole blood 5 min after addition of a platelet agonist (AA, 1.6 mM), using a ChronoLog Aggregometer (560 model, PA, USA).[9,10,12,23] Although an impedance $>$0 Ω has been considered by some investigators as representative of inadequate response to aspirin,[24,25] a cut-off value of 3 Ω was chosen, based on previous results obtained in our laboratory from healthy volunteers, and recently used by other investigators.[26]

Platelet function analyzer (PFA-100®)

PFA-100® (Dade Behring, IL, USA) is a point-of-care assay that assesses platelet aggregation under high shear, mimicking platelet-rich thrombus formation after injury to a small vessel wall under flow conditions.[10,23,27] Whole blood was transferred into standard cartridges and time necessary to occlude a microscopic aperture in a membrane coated with collagen and epinephrine (CEPI) was measured. Subjects were considered aspirin resistant if their closure time was in the normal range ($<$193 s) despite aspirin treatment, as stipulated by the manufacturer.

VerifyNow Aspirin®

The VerifyNow Aspirin® point-of-care system (Accumetrics, CA, USA) is based on turbidimetric optical detection of platelet aggregation in whole blood.[9,10,23] Whole blood was transferred into standard cartridges containing a lyophilized preparation of human fibrinogen-coated beads and AA. As aggregation occurs, the system converts luminosity transmittance results into Aspirin Reaction Units. Subjects for which the assay yielded a result $\geq$550 Aspirin Reaction Units, cut-off value previously associated with increased risk of adverse ischaemic events, despite aspirin treatment were considered aspirin resistant.[28–30]

Urinary 11-dehydro-thromboxane B_2 measurement

Urinary 11-dehydro-thromboxane B_2 ($dTxB_2$) concentrations were measured using an enzyme immunoassay kit (11-dehydro-thromboxane B_2 EIA Kit, Cayman Chemical, MI, USA). Concentrations in the range of 10–1000 pg/mL can be measured with confidence, with a specificity approaching 100%.[31] Urinary $dTxB_2$ concentrations were normalized for urinary creatinine concentrations. Subjects presenting $dTxB_2$ levels $\geq$67.9 ng/mmol of creatinine were considered aspirin resistant, as previously suggested.[32]

Sample size and statistical analysis

A sample size of 193 subjects was predetermined to estimate the prevalence of aspirin resistance and to detect a correlation coefficient of at least 0.2 between any of the paired platelet aggregation data sets obtained from the different platelet function assays, with a power of 80% and level of significance of 0.05 (PASS 2002, NCSS 2004 Statistical software, UT, USA).

Continuous variables are presented as mean $\pm$ standard deviation and categorical variables are presented as frequencies and percentages. Correlations between results obtained with the various assays, irrespective of aspirin resistance classification, were

established using Spearman's correlation coefficient, where the null hypothesis was $\rho = 0$. The agreement between the aspirin resistance status assessed by the various platelet function tests in rapport with LTA_{AA} was evaluated with the use of the κ statistic. A two-sided *P*-value of <0.05 was considered significant. Analyses were performed with SPSS 14.0 for Windows (SPSS Institute, IL, USA).

Results

Of the 201 subjects studied, 155 (71.1%) were male. Mean age was 66.5 $\pm$ 10.4 years (range from 34 to 91 years). All subjects received daily aspirin therapy for at least 1 month (110 were on 80 mg daily, 10 on 81 mg daily, 1 on 162.5 mg daily, 79 on 325 mg daily, and 1 on 1300 mg daily). Due to technical fallbacks, 200 subjects underwent analysis of platelet aggregation by LTA and whole blood impedance, with AA as the agonist. ADP was also used at various concentrations as the agonist with LTA: 184 subjects were tested with 5 μM, 173 with 10 μM, and 178 with 20 μM. Platelet aggregation results were obtained by PFA-100® for 200 subjects and by VerifyNow Aspirin® for 195 subjects. Urinary analysis of $dTxB_2$ was carried out in all 201 subjects.

Eight subjects were found to be aspirin resistant, as defined by LTA_{AA} (prevalence of 4%, 95% CI 0.01–0.07). The measure of platelet aggregation by LTA_{AA} segregated patients into two exclusive and distinct groups according to their aspirin resistance status (*Figure 1A*).

The application of previously reported assay-specific cut-off values resulted in important variation in the prevalence of aspirin resistance, from 2.8 to 59.5% (*Figure 2*). When the results obtained with these assays were divided into two groups, patients resistant or sensitive to aspirin based on LTA_{AA} results, much overlap was noted above and below the specific cut-off value for each test (*Figure 1B–F*). This is supported by the Spearman's correlation coefficients and the κ statistics (*Tables 1* and *2*) that were calculated to assess correlation and agreement between the various platelet function tests. Overall, correlation between various platelet assays and LTA_{AA}, and among themselves was poor (from -0.12 to 0.29). The assay that provided the best correlation with LTA_{AA} was WBA ($r = 0.24$, $P = 0.001$), but correlation was weak. VerifyNow Aspirin®, the point-of-care assay marketed for the specific indication of detecting platelet inhibition by aspirin, demonstrated a poor correlation ($r = 0.13$, $P = 0.06$) with the gold standard, as well as poor agreement in the detection of aspirin resistant patients ($\kappa = 0.25$, $P < 0.0001$). The degree of agreement between the various assays and LTA_{AA} in relation to resistance status was weak at best (from -0.03 to 0.25). This was further reflected by the sensitivity and specificity of the various assays in reference to LTA_{AA} (*Table 3*). Most tests lacked sensitivity, or the capacity to detect aspirin resistant subjects, while reported higher specificity, or the capacity to correctly identify subjects responding to aspirin. Concordantly, the negative predictive values of the different platelet function assays were generally high, while their positive predictive values, or their capacity to predict truly aspirin resistant patients, were particularly low (between 2 and 23%).

Discussion

Our study is the first to compare simultaneously in the same population six different assays used to evaluate aspirin non-responsiveness. Using LTA_{AA}, patients suffering from stable CAD were found to present a low prevalence of aspirin resistance, prevalence that was highly variable when other

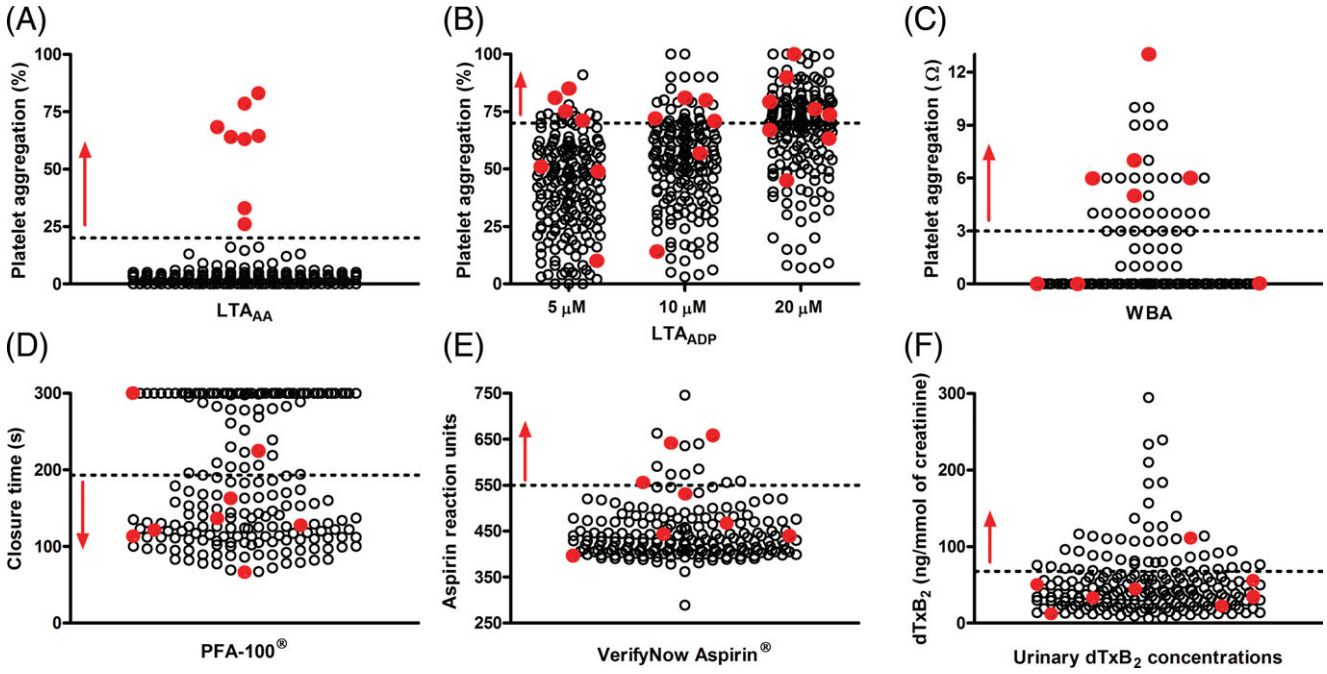

Figure 1 Distribution of platelet aggregation results as measured by various platelet function assays. (*A*) Platelet aggregation measured by light transmission aggregometry using AA as the agonist. (*B*) Platelet aggregation measured by light transmission aggregometry using ADP as the agonist, at concentrations of 5, 10, and 20 μM. (*C*) Platelet aggregation in whole blood measured by electrical impedance. (*D*) Closure time by PFA-100®. (*E*) Aspirin response by VerifyNow Aspirin®. (*F*) Urinary $dTxB_2$ concentration. Open circles indicate aspirin sensitive patients as per LTA_{AA}; closed red circles represent aspirin resistant patients as per LTA_{AA}. Horizontal dotted lines indicate test-specific cut-off values for aspirin resistance, as reported in the literature. The arrow indicates the zone within which patients are considered aspirin resistant.

assays were used. This was associated with overall poor correlations between the different platelet function tests and notably low agreement between tests in terms of classifying subjects as aspirin resistant or sensitive. In view of the fact that the various assays measured different aspects of platelet function, the apparent lack of correlation between assays should come as no surprise.

The major flaw of most studies assessing platelet response to aspirin is the use of aspirin resistance definitions based on arbitrary, clinically non-validated cut-off values. Although previous investigations have observed an association between lack of platelet function inhibition, as assessed by some of the platelet function assays, and worse clinical outcomes, the clinical relevance of the various tests and their respective thresholds remains to be established.[11,33–38]

Of the assays available to quantify the antiplatelet effect of aspirin, LTA_{AA} is considered the historical gold standard because of its relatively high specificity for platelet COX; AA is used as the agonist to exploit the specific pathway affected by aspirin (COX-dependent TxA_2 synthesis).[8,10,39]

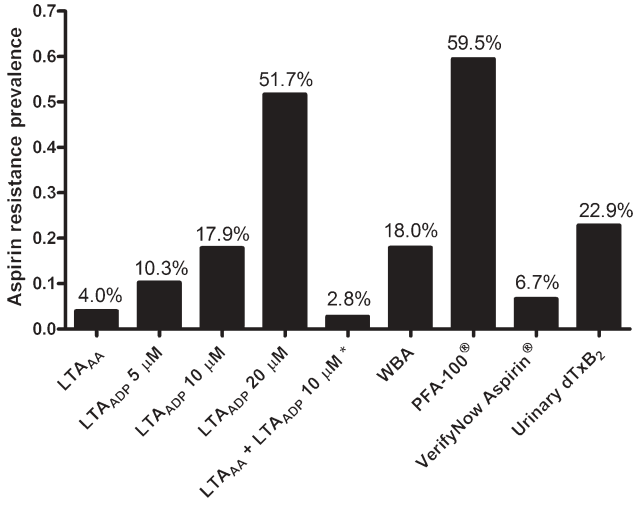

Figure 2 Prevalence of aspirin resistance determined by various platelet function assays *Definition used by Gum *et al.*,[11] Dussaillant *et al.*,[14] and Sadiq *et al.*[18]

Notwithstanding, LTA_{AA} presents inherent limitations that should not be ignored, mostly the fact that it requires operator expertise.[8,9] Moreover, AA can generate other platelet activating molecules than TxA_2 after transformation through the lipooxygenase pathway (lipid hydroperoxides) or through non-enzymatic oxidation (isoprostanes).[40,41] Although these alternative activating pathways are generally considered minor, the resulting molecules could hypothetically induce platelet aggregation in response to AA despite adequate COX inhibition and falsely lead to the conclusion that certain individuals are not responding to aspirin. Nevertheless, the 4% prevalence of aspirin resistance found in the current study via LTA_{AA} is in agreement with a recent systematic review, which has found a pooled unadjusted prevalence of 6% (95% CI 0–12%) with this methodology.[42] Furthermore, when all platelet function assays were considered, the mean prevalence of aspirin resistance was much higher, namely 24% (95% CI 20–28%), in conformity with current results.[42]

ADP has also been used as an agonist to assess response to aspirin by LTA, usually in conjunction with other platelet function assays.[11,14,15,18] Although it is not specific to the COX pathway, its administration in low concentrations (1–3 μM) requires an active COX to induce measurable irreversible platelet aggregation.[13,23,39] In contrast, platelet stimulation with higher ADP concentrations (10–20 μM) results in aggregation largely TxA_2-independent.[39] In our study, the use of high ADP concentrations translated into higher levels of platelet aggregation, no correlation with LTA_{AA} results, and a higher rate of falsely aspirin resistant patients. The use of a moderate concentration of ADP (5 μM), which depends only partially on TxA_2 synthesis, provided no added benefit in terms of correlation with LTA_{AA} results, but led to a slightly better agreement with the latter assay in identifying aspirin non-response in patients. Alternatively, some investigators have used results from a combination of assays, LTA_{AA} and LTA_{ADP}, to define resistance to aspirin in patients with stable CAD; all reported a low prevalence consistent with our results.[11,14,15,18]

In whole blood, platelet aggregation may be triggered by numerous mechanisms, and accordingly, WBA by electrical impedance was reported to convey certain advantages

Table 1 Correlation coefficients between platelet function tests[a]

	LTA			WBA	Point-of-care assays		Urinary $dTxB_2$
	ADP 5 μM	ADP 10 μM	ADP 20 μM	AA 1.6 mM	PFA-100®	VerifyNow Aspirin®	
LTA							
AA 1.6 mM	0.063	0.091	0.058	0.243*	−0.120	0.133	0.175*
ADP 5 μM	–	*0.827**	*0.718**	0.290*	0.101	−0.030	−0.064
ADP 10 μM	–	–	*0.714**	0.281*	0.086	−0.012	−0.071
ADP 20 μM	–	–	–	0.282*	0.094	0.023	0.014
WBA							
AA 1.6 mM	–	–	–	–	0.061	0.119	−0.025
Point-of-care assays							
PFA-100®	–	–	–	–	–	0.189*	−0.124
VerifyNow Aspirin®	–	–	–	–	–	–	0.151*

[a]It is generally accepted that a correlation coefficient between 0 and 0.2 shows no correlation, between 0.2 and 0.4 shows low correlation, between 0.4 and 0.6 shows moderate correlation, and superior to 0.8 shows good correlation.

*$P < 0.05$.

over LTA in detecting the effect of antiplatelet drugs in a more physiologically relevant way.[43–47] However, in our study as in others, results of platelet aggregation in whole blood and PRP were not closely correlated.[10,13] WBA may be less consistent and more difficult to interpret than LTA, partly due to unpredictable interactions between platelets and whole blood elements, including transcellular prostanoid formation by monocytes or direct stimulation of platelet degranulation by erythrocytes.[43–45] Because these mechanisms of platelet activation may bypass the inhibition provided by aspirin, platelet aggregation measured in whole blood may not be as sensitive to the effect of aspirin as that assessed in PRP.[48]

Although readily available, the capacity of PFA-100® and VerifyNow Aspirin® to adequately quantify platelet response to aspirin remains debatable. The PFA-100® device, US Food, and Drug Administration (FDA)-approved to detect platelet dysfunction, is one of the most widely used point-of-care assays to detect aspirin resistance, partly because of its rapidity and ease of use.[27] However, our results show that its methodology is insensitive to inhibition by aspirin. Most studies that have compared PFA-100® to LTA have also reported poor correlations between the two assays, independently of the agonist used, with higher proportions of aspirin resistance with the former.[15,16,49] It could be argued that PFA-100® measures a general state of platelet hyperactivity engendered by shear stress, collagen, and epinephrine stimulation, which cannot be expected to be completely inhibited by aspirin. Furthermore, von Willebrand factor levels, which are known to be elevated in patients with CAD, have been shown to modulate assay results; this could result in a falsely elevated prevalence of aspirin resistance in this population.[50] Since PFA-100® methodology is not specific to the aspirin-sensitive COX pathway, it seems less suitable for the detection of aspirin resistance.

The VerifyNow Aspirin® assay is a novel point-of-care assay, specifically designed and FDA-approved to detect platelet inhibition by aspirin, that has been shown to predict future clinical outcomes.[33] Because it is performed in whole blood, some of the limitations discussed with WBA apply. Notwithstanding, a good correlation ($r = 0.902$) was reported between results obtained with this assay and by LTA, however using epinephrine as the agonist.[28] Yet in our study, the correlation between VerifyNow Aspirin® and LTA_{AA} results was poor, and the test's sensitivity (0.38) was notably lower than that previously reported (0.87–0.95).[28,29] It should be noted however that previous comparisons were made with the earlier cartridges, which used cationic propyl gallate as agonist instead of AA. Moreover, our data showed that agreement between VerifyNow Aspirin® and LTA_{AA} in determining the aspirin resistance status was equally low. Although these results were surprising given the use of the same platelet agonist (AA), similar ones were also observed by Harrison *et al.*[16] in a population of patients suffering from transient ischaemic attacks and stroke.

It is well established that TxB_2 is the major metabolite of platelet TxA_2 in plasma.[51] The presence of its metabolite, $dTxB_2$, in urine is believed to be predominantly attributable to platelet activation and should decrease after aspirin treatment.[52] In our study however, urinary $dTxB_2$ measurements showed only mild correlation with LTA_{AA}. This poor reflection of platelet activity has been suspected in the literature following a report of a discrepancy between levels of platelet TxB_2 produced by collagen-stimulated platelet aggregation in plasma and urinary measurements of $dTxB_2$.[53] Urinary $dTxB_2$ is a global index of TxA_2 synthesis, which may originate from other blood elements such as erythrocytes and monocytes and from renal biosynthesis.[45,51,54] Accordingly, high levels of urinary $dTxB_2$ despite daily aspirin therapy may be a reflection of a larger non-platelet production, unaffected by cardioprotective aspirin doses, as opposed to increased platelet activity as it has been previously suggested.[34]

Table 2 Degree of agreement on aspirin resistance status between various platelet function assays and LTA_{AA} expressed in terms of the κ statistic[a]

Platelet function assays	κ statistic[a]	*P*-value
LTA		
ADP 5 μM	0.250	<0.0001
ADP 10 μM	0.168	0.002
ADP 20 μM	0.019	0.531
WBA, AA 1.6 mM	0.173	0.001
Point-of-care assays		
PFA-100®	0.019	0.392
VerifyNow Aspirin®	0.247	<0.0001
Urinary $dTxB_2$	−0.033	0.475

[a]It is generally accepted that a κ statistic between 0 and 0.2 translates into slight agreement, 0.2 to 0.4 into fair agreement, 0.4 to 0.6 into moderate agreement, 0.6 to 0.8 into substantial agreement, and 0.8 to 1 into almost perfect agreement.

Table 3 Sensitivity, specificity, and predictive values of various platelet function assays to detect aspirin resistance using LTA after AA stimulation as the standard

Platelet function test	Sensitivity ($CI_{0.95}$)	Specificity ($CI_{0.95}$)	Positive predictive value ($CI_{0.95}$)	Negative predictive value ($CI_{0.95}$)
LTA, ADP 5 μM ($n = 184$)	0.50 (0.43–0.57)	0.91 (0.87–0.95)	0.21 (0.15–0.27)	0.98 (0.96–1.0)
LTA, ADP 10 μM ($n = 173$)	0.67 (0.60–0.74)	0.84 (0.78–0.90)	0.13 (0.08–0.18)	0.99 (0.98–1.0)
LTA, ADP 20 μM ($n = 178$)	0.63 (0.56–0.70)	0.49 (0.42–0.56)	0.05 (0.02–0.08)	0.97 (0.94–1.0)
WBA, AA 1.6 mM ($n = 200$)	0.63 (0.56–0.70)	0.84 (0.79–0.89)	0.14 (0.09–0.19)	0.98 (0.96–1.0)
PFA-100® ($n = 200$)	0.75 (0.69–0.81)	0.40 (0.33–0.47)	0.05 (0.02–0.08)	0.97 (0.95–0.99)
VerifyNow Aspirin® ($n = 195$)	0.38 (0.31–0.45)	0.95 (0.92–0.98)	0.23 (0.17–0.29)	0.97 (0.95–0.99)
Urinary $dTxB_2$ ($n = 201$)	0.13 (0.08–0.18)	0.77 (0.71–0.83)	0.02 (0–0.04)	0.95 (0.92–0.98)

As we demonstrated, platelet function assays show great variability in differentiating between aspirin resistant and sensitive patients. We believe the non-standardized use of these assays and the absence of a formal definition explains much of the disparity reported in the literature in regards to the prevalence of aspirin resistance. As expected, platelet function assays that exploit the COX pathway (e.g. LTA with AA or low-dose ADP as the agonists, AA-induced WBA, and VerifyNow Aspirin®) are more sensitive in detecting aspirin inhibition and reveal lower proportions of aspirin resistance than non-specific platelet function tests (e.g. PFA-100®). However, none of these assays correlate strongly with the current gold standard, nor display relevant agreement in the determination of aspirin resistance status. Notwithstanding, their results have been shown to predict worse clinical outcomes in patients under chronic aspirin treatment, which can alternatively be attributed to increased platelet activity as opposed to aspirin resistance *per se*.[11,33–35] In fact, one could argue that comparing assay results to those of a gold standard is irrelevant; instead all assays should be tested to determine cut-off values that best predict clinical outcomes to then establish their validity as a test to detect aspirin resistance.

In principle, platelet function testing may be of great value to determine the efficacy of antiplatelet drugs. However, results from our study suggest that conclusions drawn could be highly dependent on the test used and results from various assays are clearly not interchangeable. Hence, the clinical usefulness of the different platelet function tests to detect appropriately aspirin resistant patients remains uncertain. Further research is warranted to better understand the platelet activation pathways involved in platelet response to aspirin, in order to allow specific targeting with various platelet function assays and to determine the threshold to be used to best predict clinical outcomes.

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