

Prevalence of Ex Vivo High On-treatment Platelet Reactivity on Antiplatelet Therapy after Transient Ischemic Attack or Ischemic Stroke on the PFA-100[®] and VerifyNow[®]

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Background: The prevalence of ex vivo high on-treatment platelet reactivity (HTPR) to commonly prescribed antiplatelet regimens after transient ischemic attack (TIA) or ischemic stroke is uncertain. **Methods:** Platelet function inhibition was simultaneously assessed with modified light transmission aggregometry (VerifyNow; Accumetrics Inc, San Diego, CA) and with a moderately high shear stress platelet function analyzer (PFA-100; Siemens Medical Solutions USA, Inc, Malvern, PA) in a pilot, cross-sectional study of TIA or ischemic stroke patients. Patients were assessed on aspirin–dipyridamole combination therapy ($n = 51$) or clopidogrel monotherapy ($n = 25$). **Results:** On the VerifyNow, HTPR on aspirin was identified in 4 of 51 patients (8%) on aspirin–dipyridamole combination therapy (≥ 550 aspirin reaction units on the aspirin cartridge). Eleven of 25 (44%) patients had HTPR on clopidogrel (≥ 194 P2Y12 reaction units on the P2Y12 cartridge). On the PFA-100, 21 of 51 patients (41%) on aspirin–dipyridamole combination therapy had HTPR on the collagen–epinephrine (C-EPI) cartridge. Twenty-three of 25 patients (92%) on clopidogrel had HTPR on the collagen–adenosine diphosphate (C-ADP) cartridge. The proportion of patients with antiplatelet HTPR was lower on the VerifyNow than PFA-100 in patients on both regimens ($P < .001$). **Conclusions:** The prevalence of ex vivo antiplatelet HTPR after TIA or ischemic stroke is markedly influenced by the method used to assess platelet reactivity. The PFA-100 C-ADP cartridge is not sensitive at detecting the antiplatelet effects of clopidogrel ex vivo. Larger prospective studies with the VerifyNow and with the PFA-100 C-EPI and recently released Innovance PFA P2Y cartridges (Siemens Medical Solutions USA, Inc) in addition to newer tests of platelet function are warranted to assess whether platelet function monitoring predicts clinical outcome in ischemic cerebrovascular disease. **Key Words:** Antiplatelet therapy—high on-treatment platelet reactivity—ischemic stroke—PFA-100—platelet function—transient ischemic attack—VerifyNow.

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Antiplatelet agents play a key role in the secondary prevention of vascular events in patients with ischemic heart disease^{1,2} or noncardioembolic ischemic stroke.³ Several groups have investigated the controversial topic of ex vivo nonresponsiveness to antiplatelet therapy in patients with ischemic heart disease, including those undergoing percutaneous coronary intervention (PCI),⁴⁻⁷ in whom symptoms are usually believed to be caused by thrombotic subtotal or total occlusion of a coronary artery.⁸ More recent studies have assessed the newly termed concept of high on-treatment platelet reactivity (HTPR) in patients with ischemic heart disease.⁹⁻¹¹ This term accounts for the fact that patients might have some degree of inhibition of platelet function with a particular antiplatelet regimen, but are still considered to have hyperreactive platelets compared with an established normal range; this information may still be clinically informative in ischemic cerebrovascular disease (CVD) patients in whom longitudinal data are not available from the same patients before and after starting a particular antiplatelet regimen.¹² However, because of the heterogeneous etiology of ischemic CVD,¹³ one cannot assume that one may extrapolate data on ex vivo HTPR from ischemic heart disease patients to those with TIA or stroke.

Aspirin is the most commonly prescribed antiplatelet drug for secondary prevention after TIA or ischemic stroke, but the majority (82-87%) of patients are not protected from additional vascular events with aspirin alone.^{14,15} This has led to clinical trials of aspirin and dipyridamole combination therapy versus aspirin monotherapy,^{16,17} aspirin versus clopidogrel monotherapy,¹⁸ and more recently aspirin and dipyridamole combination therapy versus clopidogrel monotherapy¹⁹ in patients with ischemic CVD. None of these landmark clinical trials routinely incorporated platelet function testing into the study paradigm.

The limited, available literature indicates that the prevalence of ex vivo antiplatelet nonresponsiveness in ischemic CVD varies between 5% and 66% with aspirin monotherapy,²⁰⁻²⁴ 5% to 44% with clopidogrel monotherapy,^{21,25} 0% to 73% on aspirin and clopidogrel combination therapy,²¹ and 56% to 59% when dipyridamole is added to aspirin¹² in the early, subacute,^{12,20,24} or late phases^{12,20,25} after symptom onset. Studies in ischemic CVD patients have assessed inhibition of platelet function with platelet aggregometry in either platelet-rich plasma (PRP)²⁶ or whole blood²⁷ with the whole blood Ultegra rapid platelet function analyzer (RPFA)^{24,27,28} or VerifyNow^{26,29,30} (Accumetrics Inc, San Diego, CA) or the moderately high shear stress whole blood platelet function analyzer PFA-100^{12,20,24,26,31,32} (Siemens Medical Solutions USA, Inc, Malvern, PA). The reported prevalence of nonresponsiveness varied according to the definition used.

Because aspirin and clopidogrel combination therapy is not routinely recommended for long-term secondary pre-

vention in ischemic CVD,^{33,34} it needs to be established whether one can reliably detect the inhibition of platelet function with commonly prescribed antiplatelet regimens (aspirin and dipyridamole combination therapy or clopidogrel monotherapy) in individuals after TIA or ischemic stroke using established^{32,35} and relatively novel laboratory techniques.²⁶ In addition, the controversy over whether one can reliably detect the inhibition of platelet function on long-term clopidogrel with the PFA-100 and whether the collagen-adenosine diphosphate (ADP) cartridge could serve to monitor platelet reactivity in these patients needs to be resolved.^{32,36} We therefore assessed the ability of established and relatively novel point of care laboratory tests to simultaneously detect ex vivo inhibition of platelet function in whole blood in patients on aspirin and dipyridamole combination therapy or clopidogrel monotherapy in the late phase after TIA or ischemic stroke. We hypothesized that there would be a substantial proportion of patients with ex vivo HTPR to their prescribed antiplatelet regimen, and that the prevalence of HTPR would be higher with the PFA-100 assessment than the VerifyNow assessment.

Methods

This pilot cross-sectional, observational, translational platelet science study was performed at our secondary and tertiary referral university teaching hospital.

Clinical Assessment

Eligible patients who were >18 years of age, in the late stable phase (≥ 3 months) after TIA or ischemic stroke, and who had been prescribed aspirin and dipyridamole combination therapy or clopidogrel monotherapy by their treating physician were identified from our Vascular Neurology Research database. All patients had undergone thorough clinical and neurovascular work-up by either an experienced consultant vascular neurologist or consultant stroke physician, per European Stroke Organisation guidelines at the time of symptom onset, and were fully reassessed by a vascular neurology resident (Drs. Tobin or Kinsella) at a special outpatient study visit at study entry.³⁷ Local research ethics committee approval was secured, and all participants gave written informed consent. The treatment regimen was left to the discretion of the attending consultant vascular neurologist or stroke physician and was not altered as part of this study. TIA or stroke subtyping was performed according to Trial of Org 10172 in Acute Stroke Treatment criteria.¹³ Exclusion criteria for patients included the following: active infection, inflammation, or neoplasia; platelet count < 120 or $> 450 \times 10^9/L$; recurrent TIA or stroke within the preceding 3 months; myocardial infarction, pulmonary embolism, deep vein thrombosis, or major surgery within the preceding 3 months; ongoing unstable coronary or peripheral arterial disease; renal impairment (urea > 10

mmol/L); or other nonsteroidal anti-inflammatory drug (NSAID) intake (apart from aspirin in the patients on aspirin and dipyridamole) within the preceding 2 weeks.

The importance of antiplatelet compliance was reinforced by phoning patients the day before their study visit to ensure that they had remembered to take their medication over the preceding 10 days. Assessment was deferred for 10 days in patients in whom there was any initial concern about full adherence to their antiplatelet regimen at the time of recruitment.

We explored potential demographic and vascular risk factors that may have influenced nonresponsiveness to either antiplatelet therapy regimen.

Laboratory Methods

Blood was collected from a free-flowing vein via a 21 G butterfly needle and a Vacutainer (Becton Dickinson, Franklin Lakes, NJ) system with a luer adaptor after resting for at least 20 minutes, as described previously.³⁸

A 3-mL lithium-heparin tube was taken first. The next four 2-mL 3.2% sodium citrate–anticoagulated tubes were used for assessment of platelet function with modified light transmission aggregometry (VerifyNow aspirin, P2Y12, and glycoprotein IIb/IIIa [GpIIb/IIIa] cartridges; see below). The next two 3-mL 3.2% sodium citrate tubes were used for assessment of platelet function at moderately high shear stress with the PFA-100 (see below), and for estimation of the platelet count, mean platelet volume (MPV), and platelet distribution width (PDW) in citrate-anticoagulated blood. A 2-mL ethylenediaminetetraacetic acid EDTA tube was also taken for all full blood count measurements.

VerifyNow is a cartridge-based platelet function analyzer that uses a modified light transmission aggregometry paradigm to assess the inhibition of platelet function in response to stimulation with different platelet agonists, depending on the cartridge used: arachidonic acid in the aspirin cartridge; adenosine diphosphate (ADP), isothrombin receptor activating peptide (iso-TRAP), and protease activated receptor (PAR)-4 activating peptide in the P2Y12 cartridge; and iso-TRAP alone, which activates the platelet PAR-1 receptor in the GpIIb/IIIa assay. We also analyzed platelet aggregation units (PAUs) obtained from the VerifyNow GpIIb/IIIa cartridge to assess whether the antiplatelet effects of aspirin and dipyridamole or clopidogrel could influence the results obtained from this cartridge and, therefore, to indirectly assess its specificity for detecting platelet inhibition with a GpIIb/IIIa inhibitor.

PFA-100 activates platelets by exposure to moderately high shear stress ($5000\text{--}6000\text{ s}^{-1}$) and biochemical stimulation with collagen and epinephrine (C-EPI cartridge) or ADP (C-ADP cartridge).^{39,40} The time taken for activated platelets to occlude an aperture in the cartridge is called the closure time (maximum closure time 300 seconds), and we arbitrarily defined closure times above 300

seconds as 301 seconds.²⁰ Aspirin prolongs C-EPI closure times in 83% to 100%,^{41–44} with prolongation of C-ADP closure times in 0% to 24% of healthy controls.^{41,43,44}

Blood samples were analyzed with the VerifyNow GpIIb/IIIa cartridge within 15 minutes of venipuncture. PFA-100 and VerifyNow aspirin and P2Y12 assays were performed simultaneously between 2 and 3 hours after venipuncture. Intra-assay coefficients of variation (CV) on the VerifyNow and PFA-100 were established in our own laboratory based on control data (VerifyNow aspirin [0.1%], P2Y12 [5.5%], and GpIIb/IIIa [4.0%]; PFA-100 C-ADP [7.0%] and C-EPI 7.5%), indicating a high level of reproducibility for all tests. Therefore, we did not perform analyses in duplicate. Full blood counts were performed between 2 and 4 hours after venipuncture on a Sysmex XE-2100 hematology analyzer (Sysmex UK Ltd, Milton Keynes, UK).

Because all clopidogrel-treated patients reported being treated for at least 3 months, this study had the potential to resolve the uncertainty regarding the ability of the PFA-100 to reliably detect inhibition of platelet function with long-term clopidogrel in ischemic CVD.^{36,45}

Statistical Methods

All statistical analyses were performed with R software (version 2.9.2; Statistics Department of the University of Auckland, Auckland, New Zealand). On the VerifyNow, aspirin HTPR was defined by aspirin reaction units (ARUs) ≥ 550 on the aspirin cartridge, based on the manufacturer's definition of aspirin HTPR. Clopidogrel HTPR on the VerifyNow was defined as P2Y12 reaction units (PRU) ≥ 194 , based on the manufacturer's definition of clopidogrel HTPR. Antiplatelet HTPR on the GpIIb/IIIa cartridge was defined as PAU < 125 based on the manufacturer's definition of GpIIb/IIIa HTPR.

For the purpose of this study, we considered patients to have ex vivo antiplatelet HTPR on the PFA-100 if they had evidence of platelet "hyperreactivity" on the relevant cartridge despite stable antiplatelet therapy. Therefore, antiplatelet HTPR was defined as failure to prolong the closure time beyond the mean + 2 standard deviations of our control range for the C-EPI and C-ADP cartridges (i.e., failure to prolong [a] C-EPI closure times with aspirin and dipyridamole beyond 176 seconds and [b] C-ADP closure times with clopidogrel beyond 160 seconds, as per the usual cross-sectional, case control definitions in the literature).^{20,32}

Descriptive statistical calculations assessed the percentage of patients on aspirin and dipyridamole combination therapy or clopidogrel monotherapy who had HTPR on each device. Chi-square testing compared proportions between groups. Unweighted Cohen's kappa statistics (κ) were calculated to assess interdevice agreement in the definition of antiplatelet HTPR. A κ value of 0 to 0.20 indicates poor agreement; 0.21 to 0.40 fair agreement; 0.41 to 0.60 moderate agreement; 0.61 to 0.80 good agreement; and

0.81 to 1.00 very good/excellent agreement. Unpaired Student *t* tests compared means, and the Wilcoxon rank sum test compared medians between antiplatelet nonresponsive and responsive patients. Chi-square or Fisher exact tests were used to compare proportions between groups. $P < .05$ was considered statistically significant.

Results

Between August 2009 and February 2010, 76 eligible TIA or ischemic stroke patients were recruited. Fifty-one patients were on combination therapy, with a median dose of 75 mg of aspirin daily and 200 mg of modified release dipyridamole twice daily; 25 patients were taking 75 mg of clopidogrel daily (Table 1). None had recurrent vascular events on their prescribed antiplatelet regimen in the preceding 3 months at the time of recruitment. The median interval between index cerebrovascular event and study inclusion was 401 days (range 95-821 days) in patients taking aspirin and dipyridamole and 462 days (range 93-774 days) in patients taking clopidogrel.

On VerifyNow, 4 of 51 patients (8%) on aspirin and dipyridamole combination therapy had HTPR on the aspirin cartridge per the manufacturer's definition; 2 of these patients had experienced a stroke of undetermined etiology, and 2 a lacunar stroke (Tables 2 and 3). Eleven of 25 (44%) patients on clopidogrel monotherapy had HTPR on the P2Y12 cartridge (Table 3). No patients on aspirin and dipyridamole combination therapy, and only 1 of 25 (4%) patients on clopidogrel monotherapy, had an antiplatelet effect detected on the GpIIb/IIIa cartridge, defined by PAU <125.

Twenty-one of 51 patients (41%) on aspirin and dipyridamole combination therapy had HTPR on the PFA-100 C-EPI cartridge using our cross-sectional definition (Table 3). The median C-ADP closure time was shorter in aspirin and dipyridamole patients with HTPR than in those without HTPR on the C-EPI cartridge (80 *v* 106 seconds; $P < .001$). Twenty-three of 25 patients (92%) on clopidogrel had HTPR on the PFA-100 C-ADP cartridge (Table 3).

The proportion of those with HTPR was lower on the VerifyNow than PFA-100 on both aspirin and dipyridamole combination therapy ($P < .001$) and clopidogrel monotherapy ($P < .001$). Interdevice agreement in the definition of HTPR was fair for aspirin and dipyridamole ($\kappa = 0.33$) and moderate for clopidogrel-treated patients ($\kappa = 0.46$). Three of 4 patients with HTPR on aspirin and dipyridamole combination therapy on the VerifyNow also had HTPR on the PFA-100 C-EPI cartridge. Interestingly, of the 11 patients with HTPR on clopidogrel on VerifyNow, 8 also had HTPR on PFA-100 C-ADP cartridge.

There were no significant differences in demographic or vascular risk factors between patients with and those without HTPR on either device. Of note, post hoc analysis of our VerifyNow data did not reveal any significant differences in the use of proton pump inhibitors (2/11 [18%

Table 1. Patient demographic and vascular risk factor profiles

	ASA/DP (n = 51)	Clopidogrel (n = 25)
Mean age (y)	60	71
Male gender	40 (78%)	15 (60%)
Mean days since CVE	458	461
Stroke as qualifying event	22 (43%)	14 (56%)
History of TIA/stroke before qualifying event	10 (20%)	9 (36%)
Diabetes mellitus	4 (8%)	7 (28%)
Atrial fibrillation	1 (0.5%)	2 (8%)
Hyperlipidemia	29 (57%)	24 (96%)
Hypertension	23 (45%)	22 (88%)
Ischemic heart disease	6 (12%)	9 (36%)
Previous DVT/PE	2 (4%)	2 (8%)
Peripheral arterial disease	5 (10%)	3 (12%)
Migraine	13 (25%)	7 (28%)
Family history of stroke/IHD	23 (45%)	16 (64%)
Smoker (current or previous)	30 (59%)	13 (52%)
TOAST subtype		
Large artery atherosclerotic	9	1
Small vessel occlusion (lacunar)	13	4
Cardioembolic	8	5
Undetermined etiology	20	14
Other determined etiology	1	1

Abbreviations: ASA/DP, aspirin and dipyridamole; CVE, cerebrovascular event; DVT, deep venous thrombosis; IHD, ischemic heart disease; PE, pulmonary embolism; TIA, transient ischemic attack; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

v 6/14 [43%]; $P = .23$) or calcium channel blockers (5/11 [45%] *v* 1/14 [7%]; $P = .06$) between patients with HTPR versus those without HTPR on clopidogrel.

In the aspirin and dipyridamole group, the median platelet count was higher in patients with HTPR than in those without HTPR on the VerifyNow aspirin cartridge ($P = .037$). The median PDW and MPV were higher in patients with than in those without HTPR on the PFA-100 C-EPI cartridge ($P = .001$). There were no other differences in platelet parameters between patients with and those without HTPR on either antiplatelet regimen on PFA-100 or VerifyNow (Table 4).

Discussion

To our knowledge, this is the first cross-sectional study to use a parallel testing paradigm to simultaneously assess ex vivo HTPR on aspirin and dipyridamole combination therapy or clopidogrel monotherapy in ischemic CVD patients with the PFA-100 and this version of the VerifyNow.

The finding that 8% of ischemic CVD patients on aspirin and dipyridamole and 44% of patients on clopidogrel monotherapy exhibited ex vivo HTPR on the

Table 2. Comparison of demographic and vascular risk factors between patients with and without high on-treatment platelet reactivity taking aspirin and dipyridamole combination therapy or clopidogrel monotherapy

	Aspirin and dipyridamole				Clopidogrel			
	VerifyNow		PFA-100		VerifyNow		PFA-100	
	Aspirin		C-EPI		P2Y12		C-ADP	
	No HTPR (n = 47)	HTPR (n = 4)	No HTPR (n = 30)	HTPR (n = 21)	No HTPR (n = 14)	HTPR (n = 11)	No HTPR (n = 2)	HTPR (n = 23)
Vascular risk factors								
Previous stroke/TIA	10 (21%)	1 (25%)	6 (20%)	5 (24%)	5 (36%)	4 (36%)	2	7 (30%)
Diabetes mellitus	4 (9%)	0	1 (3%)	3 (14%)	2 (14%)	5 (45%)	1	6 (26%)
Atrial fibrillation	1	0	1	0	2 (14%)	0	0	2 (9%)
Hyperlipidemia	27 (57%)	2	14 (47%)	15 (71%)	13 (93%)	11	1	23
Hypertension	21 (45%)	2	12 (40%)	11 (52%)	12 (86%)	10 (91%)	1	21 (91%)
Ischemic heart disease	5 (11%)	1	4 (13%)	2 (10%)	6 (43%)	3 (27%)	0	9 (45%)
Current or ex-smoker	27 (57%)	3 (75%)	18 (60%)	12 (57%)	10 (71%)	3 (27%)	1	12 (52%)
Stroke as qualifying event	19 (40%)	3 (75%)	13 (43%)	9 (43%)	6 (43%)	8 (73%)	0	14 (61%)
TOAST subtype								
Large artery atherosclerotic	9 (19%)	0	7 (23%)	2 (10%)	1 (7%)	0	0	1 (4%)
Small vessel occlusion (lacunar)	11 (23%)	2	7 (23%)	6 (29%)	2 (14%)	2 (18%)	1	3 (13%)
Cardioembolic	8 (17%)	0	4 (13%)	4 (19%)	3 (21%)	2 (18%)	0	5 (22%)
Undetermined etiology	18 (38%)	2	12 (40%)	9 (43%)	7 (50%)	7 (64%)	0	14 (61%)
Other determined etiology	1	0	1	0	1	0	1	0

Abbreviations: C-ADP, collagen-adenosine diphosphate; C-EPI, collagen-epinephrine; HTPR, high on-treatment platelet reactivity; PFA-100, platelet function analyzer; TIA, transient ischemic attack; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

None of the differences between subgroups were statistically significant.

VerifyNow is slightly higher than the prevalence range for HTPR reported in chronic, stable ischemic heart disease patients on aspirin monotherapy (range 0-6.7%)^{7,46} or aspirin and clopidogrel combination therapy (range 2.4-40%).⁴⁷⁻⁵⁰ However, the prevalence of HTPR on the VerifyNow assessment in patients on aspirin and dipyridamole in our study is lower than that reported by others who studied patients on aspirin monotherapy in either the subacute²⁹ or late phases after TIA or stroke (range 10.1-12.8%).^{26,29} One cannot conclude that dipyridamole enhances the antiplatelet effects of aspirin on the VerifyNow aspirin cartridge because data from this and previous studies are not directly comparable; an earlier version of the VerifyNow aspirin cartridge was used in 1 study,²⁶ 31% of patients were studied within 3 months of symptom onset in another,²⁹ and there are no longitudinal data comparing ex vivo ARUs in CVD patients taking aspirin and subsequently taking aspirin and dipyridamole. In addition, the limited available experimental evidence indicates that the addition of dipyridamole to aspirin in vitro does not affect the prevalence of HTPR to aspirin in patients in the subacute or late phases after ischemic stroke, as measured with the aspirin cartridge on the Ultegra-RPFA.²⁸

The prevalence of HTPR on aspirin and dipyridamole on the PFA-100 C-EPI cartridge (41%) in this study is

slightly higher than in ischemic heart disease patients on aspirin (range 19-32.4%),^{51,52} higher than that reported in patients taking 75 to 150 mg of aspirin daily at least 11 months after TIA or ischemic stroke (25%),²⁶ and slightly lower than reported previously in patients in the late stable phase after TIA or stroke (43%) on aspirin monotherapy.²⁰ We did not measure simultaneous serum thromboxane B₂, salicylate, dipyridamole, or urinary dehydrothromboxane B₂ levels as additional measures of aspirin and/or dipyridamole adherence, absorption, or HTPR, because this was not the purpose of this study.

The 92% prevalence of apparent clopidogrel HTPR on the C-ADP cartridge in our study is higher than reported in ischemic heart disease patients on clopidogrel (24.4%)⁵⁰ but in keeping with reported figures for clopidogrel HTPR in pilot studies in patients with previous ischemic CVD taking 75 mg of clopidogrel monotherapy daily (range 75-93.5%).^{32,45} Our data are in contrast to a small, longitudinal pilot study in 9 acute ischemic stroke patients that suggested a lag time of at least 12 days before seeing an initial ex vivo antiplatelet effect with 75 mg of clopidogrel monotherapy daily, and that clopidogrel prolongs C-ADP closure times in the majority of patients.³⁶ Our data indicate that the C-ADP cartridge is not a sensitive tool for detecting inhibition of collagen and ADP-induced platelet adhesion

Table 3. *VerifyNow and PFA-100 data from study patients*

	ASA/DP (n = 51)	Clopidogrel (n = 25)
VerifyNow cartridge		
Aspirin		
Mean ARUs (range)	476 (350-660)	648 (546-674)
P2Y12		
Mean PRUs (range)	325 (233-428)	204 (35-372)
GpIIb/IIIa		
Mean PAUs (range)	210 (126-288)	198 (113-318)
PFA-100 cartridge		
Median C-EPI CT (range in secs)	202 (81->300)	120 (72-243)
Median C-ADP CT (range in secs)	95 (54->300)	102 (62-236)

Abbreviations: ARU, aspirin reaction unit; ASA/DP, aspirin and dipyridamole; C-ADP, collagen-adenosine diphosphate; C-EPI, collagen-epinephrine; CT, closure time; GpIIb/IIIa, glycoprotein IIb/IIIa; PAU, platelet aggregation unit; PFA-100, platelet function analyzer; PRU, P2Y12 reaction unit.

and aggregation with clopidogrel if one uses a “cross-sectional definition” of clopidogrel HTPR on the PFA-100; we conclude that the C-ADP cartridge should not be used for this purpose. The recently licensed Innovance PFA P2Y cartridge has been designed to address the limitations of the C-ADP cartridge in detecting the antiplatelet effects of clopidogrel.^{48,53,54} This cartridge was not available at the time of this study, but does deserve future study.

The prevalence of ex vivo aspirin HTPR in patients on aspirin and dipyridamole and clopidogrel HTPR in pa-

tients on clopidogrel monotherapy was significantly lower on the VerifyNow than on the moderately high-shear stress PFA-100 ($P < .001$). The discordance between the devices is in keeping with that reported previously by Harrison et al²⁶ in chronic stable ischemic CVD patients, predominantly on aspirin monotherapy, who were tested with an earlier version of the VerifyNow aspirin cartridge and the PFA-100 C-EPI cartridge. The lower prevalence of aspirin HTPR on the VerifyNow than PFA-100 is likely multifactorial. For example, platelets are exposed to biochemical stimulation with arachidonic acid on the VerifyNow, compared with moderately high shear stress and biochemical stimulation with both collagen and epinephrine on the PFA-100 that may potentially overcome inhibition of platelet cyclo-oxygenase-1 by aspirin.²⁰ In addition, the VerifyNow only assesses platelet aggregation compared with assessment of both adhesion and aggregation on the PFA-100. Because von Willebrand factor (vWF) plays an important role in high shear stress-induced platelet adhesion and aggregation, the results of the PFA-100 in ischemic CVD are influenced by vWF antigen levels.²⁰

The elevated median platelet count in patients on aspirin and dipyridamole who had HTPR versus those without HTPR on the VerifyNow aspirin cartridge might be interpreted as indicating that an increased platelet count could predispose to platelet hyper-reactivity on this device. However, this result could also have arisen due to a type I error, and needs to be reassessed in a much larger population because only 4 of these patients had HTPR on this device (Table 4). In addition, patients who had HTPR on aspirin and dipyridamole on the PFA-100 C-EPI cartridge had larger platelets as measured by MPV and

Table 4. *Comparison of platelet parameters on the full blood count in aspirin and dipyridamole patients with and without HTPR versus those with HTPR on the VerifyNow aspirin and PFA-100 C-EPI cartridges, and in clopidogrel patients without HTPR versus those with HTPR on the VerifyNow P2Y12 and PFA-100 C-ADP cartridges*

	VerifyNow aspirin		PFA-100 C-EPI	
	No HTPR (n = 47)	HTPR (n = 4)	No HTPR (n = 30)	HTPR (n = 21)
Aspirin and dipyridamole				
Median platelet count ($\times 10^9/L$)	220	281 ($P = .037$)	244	218 ($P = .54$)
Median PDW (fl)	12.3	12.6 ($P = .9$)	11.7	13.4 ($P = .01$)
Median MPV (fl)	10.6	10.6 ($P = .9$)	10.3	11.0 ($P = .01$)
	VerifyNow P2Y12		PFA-100 C-ADP	
	No HTPR (n = 14)	HTPR (n = 11)	No HTPR (n = 2)	HTPR (n = 23)
Clopidogrel				
Median platelet count ($\times 10^9/L$)	207	225 ($P = .36$)	238	213 ($P = .73$)
Median PDW (fl)	13.1	13.2 ($P = .87$)	11.5	13.3 ($P = .46$)
Median MPV (fl)	10.9	10.8 ($P = .88$)	10.1	10.9 ($P = .5$)

Abbreviations: C-ADP, collagen-adenosine diphosphate; C-EPI, collagen-epinephrine; HTPR, high on-treatment platelet reactivity; PFA-100, platelet function analyzer; MPV, mean platelet volume; PDW, platelet distribution width.

PDW, than those without HTPR. Larger platelets may be more reactive to stimuli, and additional studies are required to determine whether the MPV and PDW influence ex vivo platelet reactivity in patients with cerebrovascular disease.

We postulated that the VerifyNow P2Y12 cartridge would be more reliable at detecting inhibition of ADP-induced platelet reactivity with clopidogrel than the PFA-100 C-ADP cartridge, and this was the case. Although platelets are exposed to ADP in both cartridges, the P2Y1 ADP platelet receptor is blocked by prostaglandin E₁ in the VerifyNow cartridge to allow one to specifically calculate the degree of P2Y12 receptor inhibition with clopidogrel,^{55,56} whereas ADP in the PFA-100 C-ADP cartridge may activate both P2Y1 and P2Y12 ADP platelet receptors.⁵⁷ The lower prevalence of clopidogrel HTPR on the VerifyNow may also relate to other factors outlined above, including the influence of vWF antigen levels on C-ADP closure times, but systematic analysis of the effects of vWF antigen levels was not performed in this pilot, cross-sectional, observational study.

At present, the main indicator of presumed HTPR to an antiplatelet regimen in patients with ischemic CVD, in the absence of platelet function monitoring, is the occurrence of a recurrent vascular event, such as TIA, stroke, or myocardial infarction. These patients have sometimes been referred to as “antiplatelet failures” despite the fact that apparent HTPR to the antiplatelet regimen in question may be related to several important factors. Although compliance with antiplatelet therapy was verbally confirmed in all of our patients, if one uses VerifyNow data to assess whether patients had ingested the antiplatelet agents in question, we know that the vast majority of our patients were definitely taking their prescribed antiplatelet medication. In addition to the factors outlined above, medication dosage, body weight, the extent of intestinal drug absorption, differences in platelet surface receptor expression, and genetic polymorphisms (e.g., mutations in polymorphisms involving the hepatic cytochrome P450 enzymatic system that convert clopidogrel to its active metabolite) may all play a role.^{58,59}

These findings emphasize that clinicians treating patients with ischemic CVD cannot compare data on ex vivo HTPR in one study with that in another without paying close attention to platelet function test used and the shear stress that platelets are exposed to during the laboratory test in question. Larger studies are required to explore whether the definitions of antiplatelet HTPR on cartridge-based devices from cross-sectional studies are more or less clinically informative than those derived from well-designed, longitudinal studies that investigate platelet function before and after altering antiplatelet therapy in ischemic CVD.¹² At present, clinicians should not routinely alter treatment in patients with ischemic CVD who are taking aspirin and dipyridamole combination therapy or clopidogrel monotherapy based on the

results of these cartridge-based devices. Large, adequately powered, prospective, multicenter studies using established and novel definitions of HTPR are needed to assess the usefulness of platelet function tests at predicting the risk of recurrent vascular events in ischemic CVD patients on commonly prescribed antiplatelet regimens. If it is found that patients with HTPR have a higher risk of recurrent vascular events than those with inhibition of platelet function on the device in question, this will provide a clear rationale to use platelet function monitoring to tailor antiplatelet therapy in order to optimize secondary prevention in this patient population.

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