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 heterogenous 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 monotherapy[19] 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 aspirin[12] 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 blood[27] 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 prevention 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