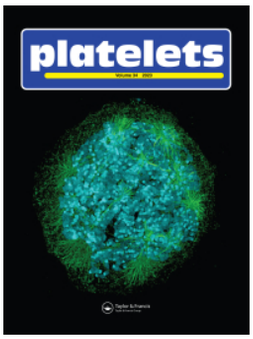

Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: informahealthcare.com/journals/iplt20

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To cite this article: Soon Tjin Lim, Catherine A. Coughlan, Stephen J. X. Murphy, Israel Fernandez-Cadenas, Joan Montaner, Vincent Thijs, Lars Marquardt & Dominick J. H. McCabe (2015) Platelet function testing in transient ischaemic attack and ischaemic stroke: A comprehensive systematic review of the literature, Platelets, 26:5, 402-412, DOI: 10.3109/09537104.2015.1049139

To link to this article: https://doi.org/10.3109/09537104.2015.1049139

platelets

http://informahealthcare.com/plt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

Platelets, 2015; 26(5): 402–412
 DOI: 10.3109/09537104.2015.1049139

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INVITED ARTICLE: Celebrating the achievements of Professor Stan Heptinstall, Founder and Editor-in-Chief of Platelets (1990–2015)

Platelet function testing in transient ischaemic attack and ischaemic stroke: A comprehensive systematic review of the literature

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Abstract

The majority of patients with ischaemic cerebrovascular disease (CVD) are not protected from further vascular events with antiplatelet therapy. Measurement of inhibition of platelet function *ex vivo* on antiplatelet therapy, using laboratory tests that correlate with the clinical effectiveness of these agents, would potentially enable physicians to tailor antiplatelet therapy to suit individuals. A systematic review of the literature was performed to collate all available data on *ex vivo* platelet function/reactivity in CVD patients, especially those treated with aspirin, dipyridamole or clopidogrel. Particular emphasis was paid to information from commonly available whole blood platelet function analysers (PFA-100®, VerifyNow® and Multiplate®). Data on pharmacogenetic mechanisms potentially influencing high on-treatment platelet reactivity (HTPR) on antiplatelet therapy in CVD were reviewed. Two-hundred forty-nine potentially relevant articles were identified; 93 manuscripts met criteria for inclusion. The prevalence of *ex vivo* HTPR in CVD varies between 3–62% with aspirin monotherapy, 8–61% with clopidogrel monotherapy and 56–59% when dipyridamole is added to aspirin in the early, subacute or late phases after TIA/stroke onset. The prevalence of HTPR on aspirin was higher on the PFA-100 than on the VerifyNow in one study ($p < 0.001$). Furthermore, the prevalence of HTPR on aspirin was lower when one used 'novel longitudinal' rather than 'cross-sectional, case–control' definitions of HTPR on the PFA early after TIA or stroke ($p = 0.003$; 1 study). Studies assessing the influence of genetic polymorphisms on HTPR in CVD patients are limited, and need validation in large multicentre studies. Available data illustrate that an important proportion of CVD patients have *ex vivo* HTPR on their prescribed antiplatelet regimen, and that the prevalence varies depending on the definition and assay used. Large, adequately-sized, prospective multicentre collaborative studies are urgently needed to determine whether comprehensive assessment of HTPR at high and low shear stress with a range of user-friendly whole blood platelet function testing platforms, in conjunction with pharmacogenetic data, improves our ability to predict the risk of recurrent vascular events in CVD patients, and thus enhance secondary prevention following TIA or ischaemic stroke.

Keywords

Ischaemic stroke, pharmacogenetic influences, platelet function, systematic review, transient ischaemic attack

History

Received 8 April 2015
Revised 30 April 2015
Accepted 1 May 2015
Published online 4 June 2015

Background

Stroke is the commonest cause of acquired disability in adults in higher-income countries [1] and the second commonest cause of death worldwide [2]. Although the incidence of stroke has fallen over the last decade in higher-income countries [3], the global burden of stroke-related disability is rising [4, 5].

Cerebral or ocular ischaemia/infarction are the underlying pathogenic mechanisms responsible for all transient ischaemic attacks (TIAs) and 80–90% of first strokes [6–8]. Several studies, including important early work by Mulley, Heptinstall and colleagues on ADP-induced platelet reactivity, have shown that platelets are excessively activated or hyper-reactive in the early [9–14], subacute [12] or late phases [10, 11, 13, 14] after TIA or ischaemic stroke.

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As the majority of TIAs/ischaemic strokes are 'non-cardioembolic' in origin, antiplatelet agents play a key role in secondary prevention in patients with ischaemic cerebrovascular disease (CVD) [15]. Aspirin has traditionally been the most commonly prescribed antiplatelet drug for secondary prevention following TIA/ischaemic stroke, but the majority (82–87%) of patients are not protected from further vascular events with aspirin alone [16, 17]. Clinical trials in CVD have shown that aspirin–dipyridamole combination therapy is superior to aspirin alone at preventing recurrent stroke [18] or recurrent vascular events overall [19]. There may also be a benefit of clopidogrel over aspirin in subgroups of ischaemic CVD patients, especially those with co-existing ischaemic heart disease (IHD) [20]. However, the PRoFESS trial did not subsequently show any difference in the incidence of recurrent stroke or vascular events between CVD patients on aspirin–dipyridamole combination therapy and those on clopidogrel [21]. Therefore, the optimal secondary preventive antiplatelet regimen for individuals following TIA or ischaemic stroke is unclear, and most CVD patients are not protected from further vascular events with currently available, 'non-monitored' antiplatelet treatment regimens.

Several groups have employed established laboratory tests to assess *ex vivo* 'non-responsiveness/resistance' [22] or 'high on-treatment platelet reactivity' (HTPR) to antiplatelet therapy in the physiological milieu of whole blood in IHD patients [23–25]. Most definitions of HTPR are 'cross-sectional, case–control' definitions whereby patients' results at a single time point are compared with those obtained from a group of healthy controls or the manufacturer's normal range. Very few robust, prospective, 'longitudinal studies' in CVD, in which the same patients are tested before and after starting or changing antiplatelet therapy have been performed to date (see below) [26–33]. Longitudinal definitions of HTPR in CVD patients commencing or changing antiplatelet therapy have the potential to provide more clinically meaningful information than traditional cross-sectional definitions of HTPR [26]. Nevertheless, cross-sectional data may be clinically informative when longitudinal data are unavailable [26, 27].

One recent meta-analysis reported a higher incidence of poor outcomes (stent thrombosis, myocardial infarction or death) in patients with higher on-treatment platelet reactivity compared with those with lower on-treatment platelet reactivity on the VerifyNow platelet function analyser after percutaneous coronary intervention [34]. However, altering antiplatelet therapy based on *ex vivo* platelet function testing with the VerifyNow has not been shown to improve 'vascular outcomes' in two large recent trials in IHD [35, 36]. Despite extensive interest in platelet function/reactivity testing in IHD, relatively little attention has been paid to the phenomenon of '*ex vivo* HTPR' in CVD. None of the aforementioned landmark clinical trials in CVD [16–18, 21] routinely incorporated platelet function testing into the study paradigm [37]. Due to the heterogeneous aetiology of TIA or stroke that may be caused by several different embolic, thrombotic or other mechanisms [38], one cannot assume that one may extrapolate data on *ex vivo* HTPR from IHD patients to those with TIA or stroke.

To investigate the potential importance of *ex vivo* platelet function/reactivity testing, and the potential role such testing could play in optimizing secondary preventive therapy in CVD, we performed a detailed systematic review of the literature to collate available data on *ex vivo* platelet function/reactivity in blood in CVD patients.

Methods

A systematic review of the literature was performed by searching PubMed, Medline, Ovid, Embase and Web of Science/Web of Knowledge for human studies published in English between 1993 and February 2015 on *ex vivo* platelet function/reactivity in CVD patients who were treated with aspirin, dipyridamole or clopidogrel. The following search terms were used in different combinations: transient ischaemic attack, TIA, stroke, platelet function, platelet reactivity, platelet aggregation, platelet aggregometry, flow cytometry, antiplatelet resistance, high on-treatment platelet reactivity, aspirin, clopidogrel, dipyridamole, Platelet Function Analyser-100 (PFA-100®), VerifyNow®, Multiplate®, aspirin TIA polymorphisms, aspirin stroke polymorphisms, acetylsalicylic acid TIA polymorphisms, acetylsalicylic acid stroke polymorphisms, clopidogrel TIA polymorphisms, clopidogrel stroke polymorphisms, antiplatelet TIA polymorphisms, antiplatelet stroke polymorphisms. We excluded review articles, studies assessing platelet function *in vitro* or *ex vivo* platelet activation, reports in which it was unclear whether haemorrhagic stroke patients were included and pharmacogenetic studies including <200 patients. Data on *ex vivo* platelet reactivity/function testing in the subgroup with moderate–severe symptomatic or asymptomatic carotid atherosclerotic stenosis were excluded because this is the subject of a separate systematic review in preparation.

Two authors (STL and IF-C) personally read all abstracts and/or relevant articles, hand-searched reference lists of published articles, and identified papers suitable for inclusion in this review. 1162 manuscripts were initially retrieved, including 246 articles on platelet function in CVD, and 84 on the impact of pharmacogenetics on HTPR in CVD; 93 were deemed suitable for final review and inclusion. Although multiple technology platforms are available to assess *ex vivo* HTPR (Table I), we focused on data obtained from some of the most commonly available platelet function assays, namely, platelet aggregometry, the PFA-100, VerifyNow and Multiplate analyser. For consistency, the terms 'non-responsiveness' or 'resistance' used in prior studies were replaced by HTPR in this review, unless specified. Data on HTPR on aspirin, dipyridamole or clopidogrel on each of these devices are reviewed, in turn, followed by a brief overview of available data on the influence of pharmacogenetic factors on HTPR in CVD.

Prevalence of *ex vivo* HTPR/'non-responsiveness' in TIA or ischaemic stroke patients on antiplatelet therapy

Platelet aggregometry

Thirteen studies focused on aggregometry in platelet rich plasma (PRP) or whole blood in CVD.

Helgason et al. investigated *ex vivo* 'aspirin responsiveness' in ischaemic stroke [39, 40]. The first study included 113 outpatients with prior ischaemic stroke, and 24 in-patients with acute ischaemic stroke on aspirin 325–1300 mg, titrated as per protocol [39]. 'Aspirin-HTPR', defined as incomplete inhibition of platelet aggregation in PRP in response to arachidonic acid (AA; 500 μM), ADP (5 μM), epinephrine (5 μM) and collagen (0.8 μg/ml) on up to 1300 mg of aspirin daily, was seen in 3–4% of CVD patients. Of interest, the majority of inpatients (79%) who had a stroke on aspirin had complete inhibition of platelet aggregation *ex vivo*, suggesting that thromboxane-independent mechanisms were involved in the pathogenesis of these strokes. Therefore, this study did not enforce the argument that platelet aggregometry in PRP might help identifying patients at high risk of stroke recurrence on aspirin. The authors subsequently measured *ex vivo* inhibition of platelet aggregation in PRP in 306 ischaemic stroke patients on ≥325 mg of aspirin daily [40]. 26% had HTPR on the initially prescribed aspirin dose. Of 171 patients who underwent repeat testing, 8% had aspirin-HTPR despite dose-escalation to 1300 mg daily. 31% of patients initially

Table I. Comparison of characteristics of commonly available platelet function technology platforms (Some data are adapted from Michelson AD [98]).

Platelet function test	Principle employed	Advantages	Disadvantages
Bleeding Time [99]	*In vivo* screening test	Cheap; physiological	Invasive; not sensitive or reproducible; not specific for particular antiplatelets or platelets alone
Optimal Aggregometry [40, 41]	Responsiveness to Agonists	Specific	PRP only and as outlined in text above
Impedence Aggregometry [44, 76]	Responsiveness to Agonists	Whole blood test; several agonists	Trained personnel required; labour intensive; high blood volume; semi-quantitative; expensive
Aggregometry and Luminescence [100, 101]	Aggregation and ADP release	Informative re platelet pathways affected	Trained personnel required; labour intensive; expensive
Laser Platelet Aggregometer (PA-200®) [102, 103]	Platelet micro-aggregates	Sensitive; studies hyper-reactivity	PRP only; no widespread experience
Thromboelastography (TEG®)/ Thromboelastometry [76, 104]	Global Haemostasis	Whole blood; may predict bleeding	Measures clot properties only; not specific for effects of particular antiplatelets or platelets alone
Glass Filterometer [105]	Shear induced platelet reactivity	Whole blood; user friendly; reproducible	Requires platelet counter; not widely used; results not predictive of MI or stroke risk in one study
Clot Signature Analyser (CSA) [106]	Global haemostasis, high shear platelet function	Whole blood; global haemostasis	Not platelet specific; sensitivity at detecting effects of all common antiplatelet agents unproven
Haemodyne [107]	Platelet contractile force	Whole blood or PRP; rapid; simple	Measures clot properties only; not specific for effects of particular antiplatelets or platelets alone
HemoSTATUS® [108]	Platelet procoagulant function	Whole blood; simple	Insensitive to aspirin and GpIb function
Thrombotic Status Analyser (TSA) [109]	Measure antiplatelet and thrombolytic effects at high shear stress	Whole blood; Simple	Little widespread experience
Cone & Plate Analyser (CPA) [110, 111]	High shear stress induced platelet adhesion/aggregation	Whole blood; physiological; small volume	Requires image analysis; not widely available; limited experience
ICHOR (Plateletworks®) [112]	Platelet counting pre- and post-platelet activation	Whole blood; rapid; simple, point-of-care; small blood volume	Need preparation of reagents; need access to analyser within minutes of venepuncture, so impractical in many routine clinical settings; indirectly measures aggregation inhibition
Platelet Function Analyser (PFA-100®) [37, 58]	Moderately high shear stress-induced adhesion/aggregation	Whole blood; rapid; user-friendly; reproducible; may detect antiplatelet effects of aspirin and clopidogrel, and additional effects of dipyridamole over aspirin	Fixed shear rate; fixed dose agonists; some cartridges expensive, VWF dependent, one pipetting step required
Verify Now® [73, 76]	Platelet aggregation at low shear; Aspirin effect; P_2Y12 antagonists; GpIIb/IIIa-dependent aggregation	Whole blood; user-friendly; No specific expertise required once trained; reproducible; may detect antiplatelet effects of aspirin, clopidogrel	2 ml blood volume per cartridge; some cartridges are expensive; no specific assays identified for dipyridamole
Multiplate® Impedance Aggregometry [83, 84]	Platelet aggregation at low shear; Responsiveness to panel of agonists	Whole blood; User-friendly; No specific expertise required once trained; may detect antiplatelet effects of aspirin and clopidogrel; reproducible; easily available	Specialized equipment; costs; pipetting steps required; no specific assays identified for dipyridamole
Flow Cytometry [65]	Platelet glycoprotein expression; platelet activation markers and platelet reactivity in response to agonist stimulation	Whole blood or PRP; Sensitive and specific; flexible; centralized assays available to detect aspirin and clopidogrel effects (Platelet Solutions UK)	Trained personnel required; labour intensive; expensive equipment and reagents required on site unless centralized assays performed, no specific assays identified for dipyridamole
Cellix I Diagnose platform	Platelet adhesion and aggregation	Whole blood; reproducible; variable shear rates and concentrations of agonists; multiple simultaneous agonists	Not yet widely available
Serum thromboxane B_2 or urinary 11-dehydro-thromboxane B2/ Creatinine ratio [75]	Thromboxane biosynthesis	'Standard assay' for inhibition of thromboxane biosynthesis by aspirin	ELISA technique; serum or urine; indirect measure of effects of aspirin; not platelet-specific, requires specialized equipment and trained personnel; very time consuming; expensive

on a dose of aspirin sufficient to completely inhibit platelet aggregation had HTPR on the same dose at some stage during follow-up. The mechanisms responsible for fluctuation in the *ex vivo* response to aspirin during follow-up were not determined.

Ex vivo 'aspirin responsiveness' was also measured in 14 ischaemic stroke patients with stroke recurrence on aspirin ('clinical aspirin failures') and 25 patients without stroke recurrence on aspirin ('clinical aspirin responders') after 7 days on 300 mg and 600 mg of aspirin daily, respectively [41]. A daily dose of 300 mg of aspirin inhibited 1.4 μM AA- and 10 μM epinephrine-induced platelet aggregation less effectively in 'clinical aspirin failures' than in 'clinical aspirin responders' ($p < 0.01$). More complete inhibition of platelet aggregation was seen in clinical aspirin failures when the dose was increased from 300 to 600 mg daily ($p < 0.01$). Due to the limited number of subjects and the lack of clinical follow-up after dose escalation, these results cannot be generalized to recommend higher aspirin doses in patients with recurrent stroke on 300 mg daily.

Sztriha et al. performed a cross-sectional optical platelet aggregation study in PRP on 241 patients on aspirin 100–250 mg daily who had at least one TIA or stroke in the preceding 5 years, 78 of whom had >1 preceding recurrent vascular event (stroke, MI or angina) [42]. The degree of aggregation did not differ between patients with and without a history of recurrent events on collagen- or epinephrine-induced aggregation. The retrospective clinical analysis in this study does not allow one to conclude whether HTPR status predicts risk of recurrent vascular events over time.

Gengo et al. performed a cross-sectional study on 653 patients with prior TIA/stroke on aspirin (<81 mg to >325 mg daily) [43]. Aspirin-HTPR was seen in 14% of patients with 0.5 mM AA, and 17% in response to 1 μg/mL collagen on whole blood impedance aggregometry; 98% retained the same aspirin-HTPR status at 7 months follow-up. Patients were not prospectively followed to assess the risk of recurrent vascular events, so this study did not inform us whether one should alter antiplatelet therapy based on baseline aspirin-HTPR status [43].

Fong et al. performed a retrospective analysis of optical platelet aggregometry data in PRP in 465 CVD patients [44]. Twenty-eight per cent of patients on 81–325 mg of aspirin daily had aspirin-HTPR in response to 0.5 mg/mL AA or 10 mM ADP. Twenty-eight per cent of patients on clopidogrel 75 mg daily had clopidogrel-HTPR in response to 10 mM ADP. Amongst those on dual antiplatelet therapy, 9.3% had both aspirin- and clopidogrel-HTPR.

Schwammenthal et al. performed an observational study on 105 patients within 36 hours of acute stroke; 40% were on aspirin for >1 week prior to presentation, and all were treated with 100–325 mg of aspirin daily for at least 6 hours before blood sampling [45]. 'Aspirin HTPR', assessed with 1.6 mmol/L AA-induced optical platelet aggregometry in PRP, was observed in 31% within 36 hours and in 45% of the 87 patients retested at days 4–5 after stroke onset. Fifty-three per cent had consistent HTPR results over time. Patients with aspirin-HTPR at baseline had more severe strokes at baseline, a more unfavourable clinical course and worse functional outcome during follow-up than those without HTPR after adjusting for age ($p \leq 0.02$). However, one could not reliably comment on whether aspirin-HTPR had any impact on the risk of recurrent vascular events ($n = 7$) during a median follow-up of 11.5 months.

HTPR was subsequently assessed with whole blood impedance aggregometry in 416 acute stroke patients on 100 mg or 200 mg of oral aspirin, 500 mg of IV aspirin or 75 mg of clopidogrel daily [46]. Based on response to stimulation with AA or ADP, aspirin-HTPR was identified in 36% of patients on 100 mg of aspirin, 33% on 200 mg of aspirin, 18% on 500 mg of intravenous aspirin and clopidogrel-HTPR was seen in 46% of patients on clopidogrel. However, as this study was not designed to assess clinical outcomes in patients with HTPR, one cannot conclude that one should use higher dose IV rather than oral aspirin in acute stroke. The same authors reported the results of another cross-sectional whole blood impedance aggregometry study in 737 patients with CVD, IHD or peripheral vascular disease (PVD) on 100–200 mg of oral aspirin or 500 mg of IV aspirin daily [47]. Aspirin doses ranged between 100 and 500 mg daily in the CVD subgroup [47]. The prevalence of aspirin-HTPR was 28% in patients with CVD, 18% in those with IHD and 22% in those with PAD. However, different groups of patients were prescribed different treatment regimens and doses of aspirin, so it was unclear whether aspirin-HTPR was more common in CVD than IHD patients.

Depta et al. analysed retrospective data on 324 patients with TIA ($n = 74$) or ischaemic stroke ($n = 250$) to assess clinical outcomes associated with 'optical platelet aggregometry-guided modifications in antiplatelet therapy' [48]. 'Antiplatelet therapy modification' was defined as any increase in the dose of existing antiplatelet therapy, addition of another agent or switching antiplatelet therapy (e.g. aspirin to clopidogrel) within 24 hours of platelet function testing. In this CVD population, 43% had aspirin-HTPR in response to stimulation with AA or ADP, and 35% had clopidogrel-HTPR in response to stimulation with ADP. After platelet function testing, antiplatelet therapy was altered/increased in 23%, but this decision was not necessarily based on the results of platelet function testing, and only 24 patients had repeat platelet function testing after modifying antiplatelet therapy. Patients who underwent modifications in therapy had higher rates of ischaemic events, bleeding or death than those who had no modifications of antiplatelet therapy (hazard ratio: 2.24; $p = 0.02$). However, one cannot conclude that treatment changes 'based on platelet function testing' influenced outcomes in CVD patients because the study was retrospective and observational, and most importantly, the results of platelet function testing were not the only criteria used to alter treatment. Furthermore, diverse modifications in antiplatelet therapy were left to the discretion of treating physicians, and only 7% had follow-up platelet function testing.

Ex vivo HTPR was evaluated with optical aggregometry in PRP in 72 patients within 7–62 days of non-cardioembolic TIA/ischaemic stroke who were on 75 mg of clopidogrel daily for ≥1 week [49]. Clopidogrel-HTPR was seen in 8.3% with 1 μmol/L ADP and 18.1% with 4 μmol/L ADP. Patients were studied on one occasion and the relationship between aggregometry data and recurrent events was not assessed.

Lago et al. performed a cross-sectional, observational study on 56 patients within 72 hours of ischemic stroke who were either on aspirin (100–300 mg daily in 87%, or 450 mg of intravenous acetylsalicylate of lysine daily in 13%; $n = 30$), 75 mg of clopidogrel daily ($n = 16$) or aspirin and clopidogrel ($n = 10$) [50]. Patients on clopidogrel exhibited a small (13%) reduction in 3 μm ADP-induced optical platelet aggregation in PRP, and as expected, had less pronounced inhibition of 1 mM arachidonic acid-induced aggregation, and shorter C-EPI closure times on the PFA-100 than patients on aspirin or aspirin–clopidogrel combination therapy ($p < 0.05$). However, the proportion of patients with HTPR on each regimen was not reported, and the C-EPI data are not informative because the C-EPI cartridge is not sensitive at detecting the antiplatelet effects of clopidogrel. Only one patient had a recurrent event on aspirin, and outcomes after discharge were not reported.

A recent cross-sectional study in CVD patients with prospective follow-up assessed *ex vivo* 'aspirin resistance' in 634 Chinese stroke patients on 200 mg of aspirin daily with optical aggregometry using PRP [51]. Aspirin resistance (AR) was

defined as a mean aggregation of ≥20% with 0·5 mg/ml AA and ≥70% with 10 μM ADP. 'Aspirin semi-resistance (ASR)' was defined as a mean aggregation of ≥20% with 0·5 mg/ml AA or ≥70% with 10 μM ADP. AR was detected in 20% and ASR in 4% of patients. During a median follow-up of 19.4 months ($n = 600$), in which all patients received 100 mg of aspirin daily, recurrent stroke, myocardial infarction, death or vascular events occurred more frequently in patients with AR or ASR than patients with 'aspirin responsiveness' (31% vs. 12%, $p < 0.001$). AR/ASR (pooled data) was an independent risk factor for ischaemic vascular events during follow-up (observed ratio 3:2, $p < 0.001$). Larger studies in non-Chinese populations, ideally with more 'user-friendly' tests of platelet function in whole blood, and retested on their actual maintenance dose of aspirin are needed to determine whether HTPR data predict outcome in CVD patients overall.

Data from commonly used whole blood platelet function analysers in CVD

PFA-100®

The PFA-100 activates platelets by exposure to moderately-high shear stress (5000–6000 second^{-1}) and biochemical stimulation, traditionally with collagen and epinephrine (C-EPI) or ADP (C-ADP). Previous studies have shown that aspirin prolongs C-EPI closure times in 83–100% of healthy controls [52–55]. The C-ADP cartridge is not sensitive at detecting platelet function inhibition with Clopidogrel [28, 37], but the INNOVANCE® PFA P2Y cartridge is reported to have overcome this issue [56, 57]. Fifteen studies assessing HTPR with the PFA-100 in CVD met criteria for inclusion in this review.

Alberts et al. reported that 37% of patients with 'acute stroke', TIA or asymptomatic extracranial or intracranial arterial stenosis had aspirin-HTPR using the C-EPI cartridge on aspirin doses between 81 mg alternate days/daily and 325 mg daily/twice daily [58]. Aspirin-HTPR was more common amongst patients receiving 81 mg than 325 mg daily (56% vs. 28%; $p = 0.001$), and 7% still had aspirin-HTPR after empirically increasing the dose to 650 mg BD. However, the precise interval between symptom onset and study inclusion in symptomatic patients was not reported, an unspecified number of patients were also taking non-steroidal anti-inflammatory drugs or cyclooxygenase-2 inhibitors in combination with aspirin, and the exact proportion of non-responders in the patient subgroup on aspirin monotherapy was not specified [58, 59].

Harrison et al. performed a cross-sectional study to simultaneously assess aspirin-HTPR with the PFA-100, the Ultegra® RPFA (RPFA) and optical aggregometry in 100 patients with recent TIA or minor ischaemic stroke on 75–150 mg of aspirin daily for ≥4 weeks [55]. Six patients were also taking 75 mg of clopidogrel daily, and two were on 600 mg of dipyridamole daily. The prevalence of Aspirin-HTPR was 22% on the PFA-100, 17% on the RPFA, 12% with AA-induced aggregation and 14% with ADP-induced aggregation. There was a higher prevalence of aspirin-HTPR with both point-of-care devices than with aggregometry, but only 2% had HTPR on all three tests. The co-prescription of aspirin or dipyridamole in a minority of patients may have influenced the overall results, and this initial study could not assess the value of these tests at predicting risk of recurrent events during follow-up. These assays were repeated by the same authors a year later in 72 patients from the original study cohort who were still on aspirin 75–150 mg daily [60]. The prevalence of aspirin-HTPR during follow-up, compared with baseline prevalence data from the initial study, was reported to be 25% vs. 19.4% on the PFA-100, 10% vs. 17% on the VerifyNow and 1% vs. 7% on optical aggregometry in response to AA stimulation. Only one patient was identified as having HTPR by all three tests. Levels of agreement in test results between the two time points were 'moderate' for the PFA-100 (kappa = 0.44, 95% CI: 0.19–0.68), 'fair' for the VerifyNow (kappa = 0.34, 95% CI: 0.04–0.64) and 'poor' for optical aggregometry (kappa = 0.14, 95% CI: −0.11 to 0.39 for ADP; kappa = 0.09, 95% CI: −0.21 to 0.39 for arachidonic acid) [60]. This follow-up study was not designed or powered to assess the predictive ability of these tests during clinical follow-up.

Prior to the introduction of the INNOVANCE® PFA P2Y cartridge, a pilot case-crossover study assessed 31 patients in the late phase after lacunar or 'atherothrombotic' ischaemic stroke on 100–300 mg of aspirin daily [28]. Patients were treated with 75 mg of clopidogrel daily, or a combination of clopidogrel and 300 mg of aspirin daily for 4 weeks at a time. About 16% had aspirin-HTPR on the C-EPI cartridge, clopidogrel-HTPR on the C-ADP cartridge was observed in 94% of patients on clopidogrel monotherapy and 72% on aspirin–clopidogrel combination therapy. Therefore, the C-ADP cartridge was not sensitive at detecting the antiplatelet effects of clopidogrel in CVD [28].

A subsequent randomized trial involving 70 patients within 3 months of ischaemic stroke showed that combination therapy with aspirin (81 mg daily) and clopidogrel (75 mg daily; $n = 35$) led to greater inhibition of platelet function on the C-ADP cartridge than aspirin alone ($n = 35$; $p = 0.01$) [32]. The addition of clopidogrel to aspirin also resulted in greater inhibition of platelet aggregation on optical aggregometry with 5 μmol/L ADP ($p = 0.00001$) or 5 μmol/L collagen ($p = 0.021$), but the percentage of patients with HTPR on each treatment was not specified.

Grundmann et al. studied patients with the C-EPI cartridge who were on 100 mg of aspirin daily for secondary prevention of vascular events [61]. Using a cross-sectional definition at one time-point, aspirin-HTPR was noted in 34% of patients within 3 days of TIA or ischaemic stroke ($N = 35$), but in none of the patients who were free of cerebrovascular events for >2 years ($N = 18$).

McCabe et al. subsequently performed a prospective, observational case–control study in patients in the early (≤4 weeks, $n = 57$) and late phases (≥3 months, $n = 46$) after TIA or ischaemic stroke. Sixty per cent of patients in the early phase and 43% in the late phase had aspirin-HTPR on the C-EPI cartridge on 75–300 mg of aspirin daily. None of the CVD patients studied in the late phase were defined as having aspirin-HTPR on sodium arachidonate- or ADP-induced platelet aggregometry in PRP ($n = 10$). Overall, a high proportion of CVD patients had aspirin-HTPR, and cyclooxygenase-independent mechanisms, including TIA/stroke subtype, appeared to play an important role in mediating aspirin-HTPR on the PFA-100. However, the study was not designed to assess the ability of the PFA-100 to predict risk of recurrent vascular events during follow-up.

Godeneche et al. identified aspirin-HTPR in 15% of acute ischaemic stroke patients on treatment with 160 mg of aspirin daily for ≥3 days in a cross-sectional study with the C-EPI cartridge in 3.8% citrate-anticoagulated blood ($n = 100$) [62]. As noted previously, C-ADP closure times were significantly shorter [63], and hypertension was more common in patients with than in those without aspirin-HTPR ($p \leq 0.018$). However, medium–long term clinical or laboratory follow-ups were not performed.

Boncoraglio et al. assessed 129 stable CVD patients with either vascular cognitive impairment, or TIA or stroke within the preceding 1–12 months who were on 75–300 mg of aspirin daily [64]. The composite outcome of recurrent TIA, stroke, myocardial infarction or cardiovascular death occurred in 15.4% ($N = 4$) of patients with aspirin-HTPR and 14.6% ($N = 15$) without aspirin-HTPR ($p = 1.0$) on the C-EPI cartridge during a mean follow-up of 56 months [64].

A further retrospective study assessed 142 CVD patients with the C-EPI cartridge who received 100 or 300 mg of aspirin daily, clopidogrel 75 mg daily or both (100 mg aspirin + 75 mg clopidogrel daily) [65]. Platelet aggregation in PRP was measured by optical aggregometry using 5 mg/mL collagen or 10 mmol/L ADP. 58–62% of patients had aspirin-HTPR on the C-EPI cartridge vs. 27–33% with collagen-induced aggregation. Clopidogrel-HTPR was seen in 44% with ADP-induced aggregation. Agreement between the PFA-100 and collagen-induced aggregometry was poor, and aggregometry could not reliably detect the individual antiplatelet effects of aspirin or clopidogrel in patients on combination therapy.

Lai et al. prospectively recruited 269 Taiwanese patients within 7 days of ischaemic stroke onset who were on 100 mg aspirin daily for >5 days before assessment [66]. Thirty-one per cent of patients had aspirin-HTPR on the C-EPI cartridge using a cross-sectional definition of HTPR in 3.8% citrate-anticoagulated blood. Patients with aspirin-HTPR were less likely to have a favourable outcome [modified Rankin Scale (MRS) score ≤ 2] at 30 days (47% vs. 60%, $p = 0.047$) or 90 days (58% vs. 71%, $p = 0.037$) than those without aspirin-HTPR. However, after controlling for differences in CRP levels between groups, aspirin-HTPR did not predict 90 day outcomes. Recurrent ischaemic stroke rates at 90 days were similar in patients with and without HTPR (3.6% vs. 3.8%). A lower prevalence of aspirin-HTPR was observed in this compared with previous studies [58, 63]; this may reflect ethnic differences between studies, and the higher concentration of sodium citrate used in this study that may prolong closure times and reduce the prevalence of 'cross-sectional HTPR' on the PFA-100 [67].

The TRinity AntiPlatelet responsiveness (TRAP) study was designed to assess HTPR at baseline within 4 weeks of TIA or ischaemic stroke, and then at $\geq$14 days and $\geq$90 days after starting/changing antiplatelet therapy. One arm of this study prospectively assessed patients changing from no medication to aspirin (75–300 mg daily; $n = 26$), or from aspirin to clopidogrel monotherapy (75 mg daily; $n = 22$) [26]. A novel 'longitudinal definition of HTPR' was defined as failure to prolong relevant closure times compared with the patient's 'baseline value' before undergoing an antiplatelet change by more than twice the coefficient of variation of the assay. Twenty-four per cent of patients at 14 days and 18% at 90 days demonstrated aspirin-HTPR with the C-EPI cartridge; 41% at 14 days and 35% at 90 days demonstrated clopidogrel-HTPR with the C-ADP cartridge [26]. Using this novel, scientifically valid longitudinal definition, the prevalence of aspirin-HTPR was much lower than that anticipated from a study employing a 'cross-sectional definition' in the early phase after TIA/stroke (24% vs. 60%, $p = 0.003$), and there was a trend towards a lower prevalence of aspirin-HTPR in the late phase after symptom onset also (18% vs. 43%, $p = 0.3$) [63]. The number of patients included in this pilot study was small, and the reportedly more sensitive INNOVANCE® PFA P2Y cartridge was not available to assess clopidogrel-HTPR in CVD patients in this study.

A pilot randomized trial showed that 30 days treatment with aspirin–dipyridamole combination therapy may lead to enhanced inhibition of platelet function compared with aspirin alone in Japanese patients with ischaemic CVD [33]. A further longitudinal, randomized study comprehensively assessed platelet activation and function in type II diabetic patients in the late stages after TIA, allocated to receive aspirin–dipyridamole combination therapy, clopidogrel monotherapy or aspirin–clopidogrel combination therapy [29]. There were no significant differences in platelet function on the PFA-100 between the three treatment groups.

Another pilot longitudinal, observational study from the TRAP investigators revealed that 59% of CVD patients at approximately 14 days, and 56% at $\geq$ 90 days after symptom onset did not have additional inhibition of platelet function on the C-ADP cartridge when 200 mg of dipyridamole MR BD was added to aspirin ($n = 52$) [27]. Using the novel longitudinal definition alluded to above, these patients were deemed to have 'dipyridamole HTPR' on the PFA-100 C-ADP cartridge, but did not undergo simultaneous platelet function testing at low shear stress. [27]. However, this study illustrated that carefully designed longitudinal studies enable identification of additional inhibition of platelet function with dipyridamole in 41–44% of patients with a whole blood platelet function analyser in response to stimulation with collagen and ADP that might not be identified at all in cross-sectional studies or in other whole blood assays [68]. The concept of doing longitudinal studies to assess the ability of dipyridamole to inhibit *ex vivo* platelet function in whole blood was also clearly illustrated by Heptinstall et al. 29 years ago in a novel study in healthy volunteers who were tested at baseline, and 30 and 60 minutes after receiving 200 mg of dipyridamole [68]. Using a platelet counting method in whole blood, dipyridamole monotherapy was shown to inhibit platelet function/reactivity especially after simulation with 0.08 μM platelet activating factor in the presence of 1 μM adenosine.

VerifyNow®

The VerifyNow® whole blood platelet function analyser employs a modified optical aggregometry paradigm to assess platelet function inhibition at low shear stress in a stirred solution in response to stimulation with different agonists. AA is used in the 'Aspirin cartridge' which is sensitive at detecting *ex vivo* aspirin-HTPR, and ADP, iso-thrombin receptor activating peptide, and PAR-4 activating peptide in the '$P2Y_{12}$ cartridge' that may detect HTPR to $P2Y_{12}$ ADP-receptor antagonists (Accumetrics Inc., San Diego, CA) [37]. Five studies in CVD employed the precursor to the VerifyNow, called the Ultegra® Rapid Platelet Function Assay (RPFA), including the study by Harrison et al. described above [55]. These data will be discussed first, followed by data from six studies that used VerifyNow.

Platelet function was assessed with the RPFA-Aspirin, PFA-100 C-ADP cartridge and optical platelet aggregometry (5 μM ADP and 5 μM epinephrine) in aspirin-free patients within 2–6 months after ischaemic stroke ($n = 40$), and in patients within 6 months of stroke on aspirin (27–650 mg daily; $n = 40$) [69]. Aspirin-treated stroke patients exhibited inhibition of platelet function on the RPFA ($p = 0.02$), prolongation of C-ADP closure times ($p = 0.03$) and inhibition of epinephrine-induced aggregation ($p = 0.0001$) compared with unmatched stroke patients not on aspirin. However, the proportion of patients with HTPR was not reported in this study. In another pilot cross-sectional study using the RPFA, aspirin-HTPR was noted in 30% of 50 CVD patients who were on 100 mg of aspirin daily for 2 years [70], but prospective assessment of the relationship between HTPR and recurrent vascular events was not performed.

Seok et al. identified aspirin-HTPR in 12% of 88 Korean ischaemic stroke patients on 100 mg of enteric-coated aspirin daily with the RPFA [71]. In patients in whom urinary thromboxane B_2 levels were also available, the prevalence of aspirin-HTPR was 25% on urine testing, suggesting that the RPFA was more sensitive at detecting the antiplatelet effects of aspirin than urinary thromboxane B_2. Clinical and laboratory follow-up were not performed.

Ozben et al. reported a 33% prevalence of aspirin-HTPR on the RPFA in acute stroke patients on 100 mg of aspirin daily for $\geq$1 week ($N = 106$) [72]. The National Institutes of Health Stroke Scale (NIHSS) score was higher ($p = 0.006$), and in-hospital (20% vs. 5.6%, $p = 0.038$) and 2-year mortality rates (60.0% vs. 31.0%,

$p = 0.004$) were higher in patients with *vs*. those without aspirin HTPR. Aspirin-HTPR was also an independent predictor of 2-year mortality (odds ratio 3.1; $p = 0.037$) [72]. However, aspirin-HTPR was only assessed once, so it is uncertain whether these results apply to late phase CVD patients or not. Furthermore, one other limitation of this study is that 'all-cause mortality' was recorded rather than vascular death, as is often assessed in TIA and stroke studies.

Kinsella et al. showed that the prevalence of antiplatelet-HTPR was significantly lower on the VerifyNow than on the PFA-100 in a cross-sectional study in the late phase after TIA/ischaemic stroke ($p < 0.001$) [37]. The authors identified aspirin-HTPR in 8% of patients on aspirin–dipyridamole combination therapy (75 mg of aspirin daily and 200 mg of dipyridamole MR BD), and clopidogrel-HTPR in 44% on 75 mg of clopidogrel monotherapy daily on the VerifyNow.

Further studies identified aspirin-HTPR in 21% of patients on 100–200 mg of aspirin monotherapy > 1 week after TIA/ischaemic stroke onset [73], and clopidogrel-HTPR in 29% on 75 mg of clopidogrel daily within 1 week of TIA/ischaemic stroke with the VerifyNow [74].

Aspirin-HTPR on the VerifyNow aspirin cartridge was identified in 6% of 101 Thai CVD patients on 81–325 mg of aspirin daily [75]. During prospective follow-up over 17 months, there was no difference in the risk of recurrent transient ischaemic attack, ischaemic stroke, unstable angina, myocardial infarction, cardiac interventions, cardiovascular death or all-cause mortality between patients with and without HTPR ($p = 0.06$). The outcome measure in this study was more widely encompassing than that employed in other studies, thus limiting comparisons between studies. Of interest, data from simultaneous urinary 11-Dehydrothromboxane B_2 assays identified 40% of patients with aspirin-HTPR, again indicating that the VerifyNow is more sensitive at detecting the antiplatelet effects of aspirin [71].

Aspirin-HTPR was assessed in 66 patients with acute ischemic stroke who were on long-term aspirin therapy (81–325 mg/day), and clopidogrel-HTPR was assessed in these patients 26 and 64 hours after administering clopidogrel (300 mg loading dose, followed by 75 mg daily) [76]. Whole blood samples were tested with the VerifyNow, Thrombelastograph Platelet Mapping System and whole blood impedance aggregometry. Aspirin-HTPR was identified in 23% of patients at baseline, and clopidogrel-HTPR in 40% of patients at 26 hours and 26% at 64 hours on the VerifyNow. The prevalence of aspirin-HTPR on the VerifyNow in this study was similar to other studies in the early phase after stroke [73]. The prevalence of clopidogrel-HTPR was higher than that reported in the early phase after stroke [74], but similar to another study in late phase CVD patients. [37]

Jover et al. assessed clopidogrel-HTPR in 18 TIA/stroke patients with the VerifyNow P2Y12 assay, INNOVANCE® PFA P2Y cartridge, 5 μmol/l ADP-induced optical aggregometry and vasodilatorstimulated phosphoprotein 7 and 90 days after commencing clopidogrel 75 mg daily as monotherapy or in combination with aspirin 100–300 mg daily [77]. Clopidogrel-HTPR was seen in 56% at day 7 and 61% at day 90 on the VerifyNow. On the INNOVANCE PFA P2Y, clopidogrel-HTPR was observed in 39% (7/18) at day 7 and appears to have increased to 56% (10/18) at day 90. Corresponding figures for clopidogrel-HTPR on optical aggregometry were 50% at day 7 and 90. This pilot cross-sectional study was the first to compare the VerifyNow P2Y12 and INNOVANCE PFA P2Y assays in early and late phase CVD patients. However, much larger studies are required to validate these findings, and to assess the impact of long-term compliance on the results, especially as the prevalence of HTPR unexpectedly increased over time.

Multiplate® assay

The Multiplate whole blood platelet aggregation assay is based on measurement of impedance at low shear stress as platelets adhere to two adjacent electrodes and aggregate to one another within a cuvette [78, 79]. The extent of platelet adhesion and aggregation is recorded as the area under the curve (AUC) up to 6 minutes after the addition of either arachidonic acid (AA) or ADP to measure the antiplatelet effects of aspirin or clopidogrel, respectively [78, 79]. There are few data on the assessment of HTPR with the Multiplate assay in CVD patients on aspirin or clopidogrel [80–82]; two studies met criteria for inclusion in this review.

One prospective, pilot, cross-sectional study included 89 patients within 72 hours of TIA/ischaemic stroke onset who had received at least one dose of 75 mg of aspirin, 33 of whom had been on long-term aspirin (dose unspecified) [83]. Blood sampling was performed within 12–24 hours of the first 75 mg aspirin dose in hospital and >48 hours later. Thirty-two per cent had aspirin-HTPR on the Multiplate with no significant differences in measurements between time-points. There was no clear relationship between HTPR status and the risk of recurrent cardiovascular events or all-cause mortality at 1 year, but there were too few events to make any definitive conclusions, and events rates were calculated by chart review rather than by in-person or telephone assessment. Although this study shows that the Multiplate provides reproducible results in CVD patients in a university hospital setting, the short duration of low-dose aspirin therapy in the majority and the absence of late-phase laboratory assessment of HTPR do not allow one to comment on the long-term prevalence of aspirin-HTPR in CVD [83].

A prior cross-sectional study, revealed HTPR in 42% of patients with recent ischaemic stroke ($n = 133$) on 150 mg of aspirin daily for ≥7 days [84]. The aspirin dose was increased to 300 mg daily in a proportion of those with HTPR who 'qualified for increased treatment', but 67% of this subgroup had persistent aspirin-HTPR 7 days later. Baseline NIHSS scores were slightly higher in patients with than in those without HTPR (3.7 vs. 2.5, $p < 0.01$), and the data suggested that increasing levels of CRP and VWF activity enhanced platelet reactivity in the subgroup with HTPR [84]. The authors changed patients to a 'thienopyridine antiplatelet agent' if they had persistent aspirin-HTPR on 300 mg daily, but there was no clear clinical evidence-base behind this decision, long-term laboratory re-assessment was not subsequently performed and clinical outcome events during short-term follow-up were not reported. Therefore, one cannot conclude that one should alter antiplatelet therapy in CVD patients based on these data.

Potential influence of pharmacogenetic factors on HTPR on antiplatelet therapy in CVD

The response to antiplatelet agents may be influenced by genetic factors [85–87]. However, pharmacogenetic studies in CVD have mainly focused on candidate gene analysis rather than on Genome Wide Association Studies (GWAS).

Data on aspirin

A recent study in 859 Chinese stroke patients, including one of the largest cohorts treated with aspirin, found that the rs1330344 single nucleotide polymorphism (SNP) of COX-1 (CC genotype) was associated with a higher incidence of non-fatal ischaemic stroke, myocardial infarction and death from cardiovascular causes during follow-up compared with non-carriers of this genotype. The authors reported that this genotype may upregulate

COX-1 RNA and protein expression, and in theory, enhance conversion of arachidonic acid to thromboxane A_2. However, simultaneous assessment of HTPR was not reported, and replication of the genetic analysis in another population was not performed [88].

Data on clopidogrel

Pharmacogenetic studies have linked alleles of cytochrome P450 genes, mainly CYP2C19 polymorphisms, with platelet reactivity in patients on clopidogrel [89]. These polymorphisms may affect metabolism of clopidogrel from its pro-drug to an active thiol derivative. In a meta-analysis of 42 016 patients on clopidogrel who had 3545 major vascular events during follow-up, certain CYP2C19 alleles were associated with recurrent vascular events, including ischaemic stroke. Simultaneous assessment of platelet function/reactivity was only performed in four included studies, and indicated that patients with the CYP2C19 2*/2* genotype (associated with reduced drug metabolism) exhibited platelet hyper-reactivity on 600 mg of clopidogrel compared with the CYP2C19 1*/1* genotype (normal drug metabolism). However, different assays were used to assess clopidogrel-HTPR, and we have no evidence regarding the safety or efficacy of this dose of clopidogrel in CVD patients. Furthermore, when the overall meta-analysis was restricted to studies containing >200 patients, the association with CYP2C19 polymorphisms and outcome events was not significant [90].

In another recent study, CYP2C19 *2/*3 loss of function (LOF) alleles were associated with recurrent non-fatal ischaemic stroke, non-fatal MI or vascular death in a cohort of 625 Chinese ischaemic stroke patients on clopidogrel [91]. This CYP2C19 *2/*3 LOF genotype was associated with clopidogrel-HTPR on ADP-induced aggregometry (59%), and worse outcomes on the MRS at 3 and 6 months in another study in 259 Chinese ischaemic stroke patients on clopidogrel [92]. A significant association between LOF alleles and worse outcome on MRS has been observed in another study in 211 stroke patients on clopidogrel, in which the CYP2C19 *2/*3 genotype was associated with higher platelet reactivity on ADP-induced platelet aggregation than patients without these LOF alleles [93]. However, the reason for the association with poor outcome is unexplained and needs validation and reassessment in larger studies.

Discussion

This systematic review has shown that the prevalence of *ex vivo* HTPR in patients after TIA or ischaemic stroke on commonly prescribed antiplatelet therapy varies according to the definitions and platelet function devices employed. The relatively limited, available literature indicates that the prevalence of HTPR in CVD varies between 3–62% with aspirin monotherapy [40, 65], 8–61% with clopidogrel monotherapy [46, 49, 77] and 56–59% when dipyridamole is added to aspirin [27] in the early, subacute or late phases after TIA/stroke onset. These summary figures for clopidogrel-HTPR exclude data from two studies that confirmed that the standard C-ADP cartridge on the PFA-100 is not sensitive at detecting the antiplatelet effects of clopidogrel if one uses a cross-sectional, case–control definition of HTPR [28, 37]. Most studies in CVD employed cross-sectional definitions of HTPR which may underestimate the effects of antiplatelet therapy on platelet function in individual patients compared with novel longitudinal definitions, in which patients act as their own baseline controls [26, 27]. However, one has no evidence as yet that novel longitudinal definitions of HTPR are more clinically informative at predicting recurrent vascular events during follow-up after TIA/stroke than more commonly applied cross-sectional definitions of HTPR.

There are some conflicting data about the consistency of HTPR measurements on optical platelet aggregometry over time [40, 43]. In general, the prevalence of aspirin-HTPR appears higher on the 'moderately high-shear stress' PFA-100 C-EPI system that assesses platelet adhesion and aggregation than, e.g. on 'low shear stress' platelet aggregometry assays [37, 55, 63, 65]. None of the studies published to date have been adequately powered to definitively comment on whether *ex vivo* HTPR status on platelet function testing in CVD patients predicts the risk of recurrent vascular events. Evidence pertaining to the relationship between antiplatelet-HTPR status and functional outcome, stroke severity and mortality on antiplatelet therapy following TIA or stroke is emerging, but available data from small-medium sized studies need to be validated in larger studies [51, 64, 66, 92, 93]. Furthermore, with the exception of three studies [45, 51, 64], duration of clinical follow-up has been relatively short.

No adequately powered studies have comprehensively assessed the impact of pharmacogenetic factors on platelet reactivity/HTPR in diverse geographical populations of TIA or stroke patients on antiplatelet therapy outside China. Most small-medium sized studies in CVD were performed in the era before GWAS, and findings have not been replicated. Different international consortia, such as the International Stroke Genetics Consortium (ISGC; http://www.strokegenetics.org/), or the International Clopidogrel Pharmacogenomics Consortium (ICPC; https://www.pharmgkb.org/page/icpc) are in a position to investigate the relationship between pharmacogenetic factors and HTPR in CVD in collaboration with translational platelet scientists and vascular neurologists/stroke physicians with expertise in this area.

In summary, assessment of *ex vivo* HTPR at high and low shear stress, preferably in the physiological milieu of whole blood, has the potential to play a significant role in facilitating optimal, 'individualized antiplatelet treatment' in CVD patients. However, at present, one cannot justify altering antiplatelet therapy in individual TIA or stroke patients in routine clinical practice based on *ex vivo* measurements of HTPR or based on specific genetic polymorphisms outside the setting of a research study or clinical trial. Large, adequately-sized, prospective multicentre collaborative studies are urgently needed to address this critical public health issue to determine whether comprehensive assessment of HTPR at high and low shear stress with a range of user-friendly whole blood platelet function testing platforms, in conjunction with pharmacogenetic data, improves our ability to predict the risk of recurrent vascular events in CVD patients. Such data from existing and emerging platelet function testing platforms [94–97] should improve our understanding of the mechanisms responsible for HTPR, and hopefully enhance secondary prevention in TIA or ischaemic stroke patients requiring long-term antiplatelet treatment.

Declaration of interest

Dr Lim's research is funded by The Meath Foundation, The Irish Institute of Clinical Neuroscience/Novartis Ireland Fellowship Grant, The Vascular Neurology Research Foundation Ireland, and an Irish Heart Foundation Stroke Prevention Bursary. Dr Coughlan is funded by The Vascular Neurology Research Foundation Ireland. Dr Murphy's research is funded by the Trinity College Dublin Innovation Bursary, The Meath Foundation, Joint Irish Institute of Clinical Neuroscience/Merck Serono Fellowship in Neuroscience Grant, The Vascular Neurology Research Foundation Ireland and by unrestricted educational grants from Bayer HealthCare Ireland, Elitech UK and Verum Diagnostica GmbH. Dr Fernandez-Cadenas is supported by the Miguel Servet programme (CP12/03298), Instituto de Salud Carlos III. None of the other authors have any relevant disclosures/conflicts of interest to declare.

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