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Assessment of on-treatment platelet reactivity at high and low shear stress and platelet activation status after the addition of dipyridamole to aspirin in the early and late phases after TIA and ischaemic stroke

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ABSTRACT

Background: Data are limited on the ability of dipyridamole to additionally inhibit platelet function/reactivity in ischaemic cerebrovascular disease (CVD) patients on aspirin.
Aims: To assess inhibition of platelet function/reactivity and platelet activation with dipyridamole in CVD.
Methods: This prospective, observational study assessed TIA/ischaemic stroke patients before (**baseline**; $N = 60$), at 14 ±7 days (**14d**, $N = 39$) and ≥ 90 days (**90d**, $N = 31$) after adding dipyridamole to aspirin. Platelet function/reactivity at high shear stress (PFA-100® C-ADP) and low shear stress (VerifyNow® P2Y12 and Multiplate® ADP assays), and platelet activation status (% expression of CD62P, CD63 and leucocyte-platelet complexes on whole blood flow cytometry) were quantified. 'Dipyridamole-high on-treatment platelet reactivity (HTPR)' was defined as failure to inhibit ADP-induced platelet aggregation +/- adhesion compared with the patient's baseline on aspirin monotherapy by more than twice the coefficient-of-variation of the assay after adding dipyridamole to aspirin.
Results: Dipyridamole-HTPR was identified in 71.4–75% of patients on PFA-100 C-ADP, 83.9–86.8% of patients on VerifyNow P2Y12, and 81.5–83.3% of patients on Multiplate ADP assays. There were no changes in CD62P/CD63 expression ($P \geq 0.18$), or consistent changes in leucocyte-platelet complexes in CVD patients overall at 14d or 90d vs. baseline after commencing dipyridamole. Monocyte-platelet complexes increased in the patient subgroup with dipyridamole-HTPR at 14d and 90d on PFA-100, and at 14d on VerifyNow ($P \leq 0.04$), but not in those without dipyridamole-HTPR.
Discussion: Additional antiplatelet effects of dipyridamole are detectable under high and low shear stress conditions with user-friendly platelet function/reactivity tests ex vivo. Increasing circulating monocyte-platelet complexes over time are associated with dipyridamole-HTPR.

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1. Introduction

The optimal 'non-monitored' antiplatelet regimen for secondary prevention in individual patients following non-cardioembolic transient ischaemic attack (TIA) and ischaemic stroke remains contentious. Aspirin-dipyridamole combination therapy has been shown to be more effective than aspirin monotherapy at preventing recurrent stroke [1,2] or recurrent ischaemic vascular events overall in patients with TIA or ischaemic stroke [3,4], and can be safely started within 24 h of symptom onset [5]. However, aspirin-dipyridamole combination therapy has not been shown to be superior to clopidogrel monotherapy in an overall ischaemic cerebrovascular disease (CVD) population [6]. Dipyridamole has been shown to exert its antithrombotic effects via a variety of pathways, including inhibition of platelet activation [7], platelet function [7–9], the reuptake of adenosine by erythrocytes (which can in turn lead to vasodilation) [10], expression of endothelial activation markers such as VWF antigen [11,12], and may have indirect 'anticoagulant effects' by reducing peak and total thrombin generation ex-vivo following TIA or ischaemic stroke [13].

More recent studies have shown that short-term treatment with aspirin-clopidogrel combination therapy can improve outcomes compared with aspirin monotherapy in selected patients with 'high-risk' TIA or minor ischaemic stroke [14–18], whereas triple antiplatelet therapy (aspirin-dipyridamole-clopidogrel) has been shown to be harmful due to an increased risk of bleeding on same [19]. The bleeding risks on long-term aspirin-clopidogrel combination therapy outweigh the benefits of antiplatelet monotherapy in CVD patients [20–22], unless required for cardiac or other vascular indications. Some patients may not benefit from antiplatelet combination vs. monotherapy due to a number of factors which may affect the efficacy of their prescribed antiplatelet regimen, with increasing interest in the phenomenon of 'high on-treatment platelet reactivity' (HTPR) ex vivo in CVD patients on antiplatelet therapy [8,9,23]. A randomised pilot study of 60 patients with type II diabetes and previous TIA suggested that the inhibition of ADP-induced aggregation in platelet rich plasma initially occurred more rapidly with clopidogrel than with aspirin-dipyridamole, but there was more marked inhibition of the expression of several markers of platelet activation over time with aspirin-dipyridamole vs. clopidogrel [24]. The pilot, longitudinal TRinity AntiPlatelet responsiveness (TRAP) study showed that 59% of CVD patients at approximately 14 days and 56% at ≥90 days following symptom onset did not have additional inhibition of platelet function on a 'moderately-high shear stress' test of platelet function (PFA-100®) in response to collagen and ADP when 200 mg of dipyridamole MR BD was added to aspirin; these patients were considered to have 'dipyridamole-HTPR' [8]. As interest in the concept of personalised/precision medicine has emerged [25], these preliminary findings on dipyridamole-HTPR warranted re-evaluation in an independent cohort of patients on user-friendly tests of platelet function/reactivity under both high and low shear stress conditions because none of the aforementioned, large multicentre randomised controlled trials employed platelet function/reactivity testing during follow-up [1,4,6].

1.1. Aims

The aims of this prospective, longitudinal, observational-analytical pilot study were:

1. To simultaneously compare the ability of established and relatively novel laboratory tests of platelet function/reactivity to identify patients with recent TIA or ischaemic stroke who do not have additional inhibition of platelet function/reactivity ex vivo after the addition of dipyridamole to aspirin using innovative longitudinal definitions of 'dipyridamole-HTPR'.

2. To improve our understanding of the underlying platelet activation pathways and clinical, demographic and pharmacodynamic mechanisms influencing ex vivo on-treatment platelet reactivity in CVD patients when dipyridamole is added to aspirin.

3. To collect pilot data to assess the potential ability of this 'novel longitudinal definition of dipyridamole-HTPR' to predict the risk of recurrent vascular events during long-term follow-up.

We hypothesised that:

1a. Inhibition of platelet function/reactivity would be enhanced in an important minority of patients on aspirin-dipyridamole combination therapy compared with aspirin alone, and the PFA-100 C-ADP cartridge would be confirmed to be able to detect such inhibition;

1b. Low shear stress ADP assays might be able to detect additional inhibition of platelet function/reactivity after the addition of dipyridamole to aspirin.

2. This study would provide further insights into the relationship between platelet activation and dipyridamole-HTPR status, and improve our understanding of the mechanisms influencing ex vivo on-treatment platelet reactivity on dipyridamole in CVD patients overall.

3. Pilot data from this study would inform the design a definitive multi-centre study assessing whether platelet reactivity testing improves our ability to predict the risk of recurrent vascular events in CVD patients treated with aspirin-dipyridamole combination therapy.

2. Methods

2.1. Inclusion criteria

Consecutive eligible patients ≥18 years of age, who were on aspirin monotherapy and whose treating physician decided to change their antiplatelet treatment regimen to aspirin-dipyridamole combination therapy within 4 weeks of onset of a recent clinical diagnosis of TIA or ischaemic stroke were recruited to this component of our prospectively-planned, single centre, observational analytical study (the **O**ptimal **A**ntiplatelet **T**herapy in TIA and Ischaemic **S**troke (**OATS**) study. Patients were intermittently recruited between October 2011 and January 2016 from the Rapid Access Stroke Prevention service, and from the inpatient population of the Neurology, Stroke, Age-Related Health Care and Vascular Surgery services at our secondary and tertiary referral university teaching hospital.

2.2. Exclusion criteria

Patients were excluded if they had a myocardial infarction, venous thrombo-embolism (DVT or PE) or recent surgery within the preceding three months; ongoing unstable angina or unstable symptomatic peripheral vascular disease; platelet count $<100 \times 10^9$/L; known bleeding or clotting diathesis, including known platelet-related bleeding disorders; active proven vasculitis; active neoplasia; recent or prior history of intracranial haemorrhage, active infection and non-steroidal anti-inflammatory drug (NSAID) intake other than aspirin in the preceding 11 days; were unlikely to be able to attend for clinical follow-up and repeat testing at 14 +/− 7 days.

Dipyridamole was added to aspirin entirely at the discretion of the collaborating consultant staff; the supervising author (DJHM) and his coordinating research team (STL, SJXM) did not influence decisions regarding the choice of optimal antiplatelet therapy in any patients who were not directly under their care. If patients in this component of the OATS study had their index TIA or ischaemic stroke on aspirin monotherapy, or if the attending consultant felt that secondary prevention could be optimised in an evidence-based manner [1,4] in patients who were antiplatelet-naïve on presentation and had initially been prescribed aspirin monotherapy whilst they were undergoing their urgent neurovascular work up, then dipyridamole MR was subsequently added to establish them on this combination antiplatelet regimen. Typically, if these patients presented with their first TIA or ischaemic stroke, aspirin was initially prescribed at a dose of 300 mg daily for 2 weeks and then reduced to 75 mg daily. One week after commencing aspirin, 200 mg of dipyridamole MR once daily was typically added for one week, thereafter increasing to 200 mg BD of dipyridamole MR, and then

combination treatment with 75 mg of aspirin daily and 200 mg BD of dipyridamole MR was continued thereafter. If a patient presented with a recurrent ischaemic cerebrovascular event on aspirin monotherapy, with no other obvious cause found that would warrant anticoagulation, and no history of ischaemic heart disease, aspirin therapy was usually continued and dipyridamole MR treatment was empirically added at a dose of 200 mg once daily for one week and then increased to 200 mg BD in combination with aspirin (usually 75 mg daily). If patients were deemed to be at 'higher risk' of recurrent vascular events whilst on aspirin monotherapy, e.g. in association with ≥50% extracranial internal carotid stenosis, some collaborators continued aspirin and added full dose dipyridamole MR 200 mg BD at the time of presentation (without a dose escalation phase) [1,5], once tolerated.

2.3. Ethical approval

Written informed consent was obtained from all participants. This OATS study was approved by the St. James's Hospital/AMNCH Research Ethics Committee (REC Ref: 2011/35/03).

2.4. Clinical assessment

All patients underwent detailed neurovascular assessment and were given a clinical diagnosis of a TIA or ischaemic stroke by their attending Consultant Neurologist or Stroke Physician after detailed investigations according to ESO recommendations [26,27]. The diagnosis was also confirmed in all cases by a clinically-experienced Vascular Neurology Research SpR (STL/SJXM) or by the supervising Vascular Neurologist (DJHM). Information regarding vascular risk factors, including hypertension, prior TIA or stroke, ischaemic heart disease, atrial fibrillation, valvular heart disease, diabetes mellitus, hyperlipidaemia, peripheral vascular disease, migraine, family history of stroke, medication intake (including anti-thrombotic therapy), smoking status, alcohol intake, illicit substance intake, and the method of detection of carotid stenosis and timing of any carotid intervention in patients with large artery atherosclerosis was collected prospectively [27]. Details regarding antiplatelet regimens, dose and duration of therapy were recorded. Results of routine haematological (FBC), coagulation (PT/APTT), biochemical and blood glucose testing were collected prospectively. CT and/or MRI brain and colour Doppler ultrasound (CDUS) of neck vessels were performed in all patients, as well as magnetic resonance angiography (MRA) or CT angiography (CTA) to establish concordance between CDUS and another non-invasive imaging modality in recently symptomatic carotid stenosis patients. A chest radiograph, electrocardiograph (ECG), 24-h ECG recording and transthoracic or transoesophageal echocardiograph were obtained in all patients [27]. The underlying mechanism responsible for TIA or ischaemic stroke was categorised according to both the TOAST classification [28] and ASCOD classification systems [29].

Patients underwent detailed clinical and laboratory assessment with venepuncture at baseline before changing treatment (**baseline**), with planned clinical and laboratory assessment at 14 +/− 7 days after (**14d**), and at least 90 days (**90d**) after their treating physician added dipyridamole to aspirin monotherapy. In patients with recently symptomatic ≥50% carotid artery stenosis, the 90d follow-up was performed at least 3 months following carotid surgery or endovascular treatment, unless intervention had been delayed for at least 3 months after symptom onset [27].

Adherence to prescribed antiplatelet therapy in inpatients was confirmed by checking their inpatient prescription chart to ensure that medications had been dispensed and taken. Adherence in all outpatients was checked by history taking alone, but all patients were phoned to emphasise the importance of medication-adherence in the week prior to reassessment. Reassessment was deferred for 14 days in any patients deemed possibly non-adherent to their antiplatelet treatment, and any issues potentially affecting adherence were addressed [27].

2.5. Clinical outcome events

Detailed information regarding the occurrence of the pre-specified, primary composite clinical endpoint of non-fatal ischaemic stroke, non-fatal MI or vascular death during follow-up was collected in person at each clinical and/or laboratory follow-up visit. Data on other pre-specified secondary outcomes, including haemorrhagic complications [6], were recorded. Clinical follow-up to assess the longer-term risk of recurrent vascular outcome measures was performed using a validated in-person or telephone questionnaire at ≥1 year after symptom onset. We prospectively planned to confirm any outcome events determined by telephone interview alone by contacting the GP, reviewing relevant hospital consultant's letters or notes, and if necessary, by reviewing death certificates if the patient had unfortunately died before the 1-year follow-up visit.

2.6. Laboratory assessments

Careful, atraumatic venepuncture was performed from a free-flowing vein using a 21G butterly needle and a Vacutainer® system with a luer adapter after resting participants for ≥20 mins [27,30] [31]. The first 3 ml citrate-anticoagulated blood sample was discarded. Two 2 ml sterile 3.2% buffered sodium citrate-anticoagulated samples were taken for analysis of platelet function/reactivity with the VerifyNow® system (Accriva Diagnostics, USA). Six further samples were taken into sterile 3 ml Vacutainers® containing 3.2% buffered sodium citrate. The first and second of these citrate-anticoagulated samples were used for whole blood flow cytometric analysis and for measurement of platelet function with the platelet function analyser (PFA-100®, Dade-Behring, Germany/Sysmex, UK), respectively, and the last sample was used to measure the platelet count, mean platelet volume (MPV) and platelet distribution width (PDW) in citrate between 2 and 4 h after venepuncture. Subsequently, one 3 ml double-walled Vacutainer® tube containing 'recombinant hirudin anticoagulant' was taken for platelet reactivity analysis on the Multiplate® system (Verum Diagnostica GmbH/Roche Inc., Germany). Three 3 ml sterile Vacutainer® tubes containing K_2EDTA were obtained, the first of which was used to measure the full blood count (FBC), including measurement of the MPV and PDW in EDTA between 2 and 4 h after venepuncture.

2.6.1. PFA-100® platelet function analyser

The degree of inhibition of platelet function in whole blood was assessed before and after adding dipyridamole to aspirin therapy at 'moderately high shear stress' following biochemical stimulation with collagen (2 μg) and 50 μg of ADP (Collagen-ADP cartridge), 10 μg epinephrine bitartrate (Collagen-EPI cartridge – sensitive to inhibition with aspirin), or 20 μg ADP/5 ng Prostaglandin E_1/459μg CaCl (INNOVANCE PFA P2Y™ cartridge - sensitive to inhibition with clopidogrel/ $P2Y_{12}$ antagonists), as previously described [32]. This device mimics the in vivo haemostatic process that one may see in a moderately stenosed artery. The time taken for activated platelets to occlude an aperture in the cartridge is called the 'closure time'. The maximum closure time recorded by the device is 300 s, so we arbitrarily defined closure times >300 s as '301 s' for statistical analyses and assumed that the data were not normally distributed (see statistical methodology section).

Innovative longitudinal definition of dipyridamole-HTPR on the PFA-100

In our laboratory, the intra-assay coefficient of variation (CV) was 7% for the C-ADP assay, 7.5% for the C-EPI assay and 7.8% for the INNOVANCE PFA P2Y assay. Although all 3 PFA-100 assays were performed, we only anticipated that the C-ADP cartridge would detect the antiplatelet effects of dipyridamole [8]. For the purpose of this study, 'dipyridamole-HTPR on the PFA-100' was also defined as failure to prolong C-ADP closure times compared with the patient's baseline on aspirin monotherapy by more than twice the CV of the assay when

dipyridamole was added to aspirin therapy i.e. failure to prolong C-ADP closure times by >14% of the patient's baseline C-ADP closure time [8].

2.6.2. VerifyNow® platelet function analyser

The VerifyNow® is a cartridge-based analyser which assesses ex vivo platelet reactivity at 'low shear stress' in response to stimulation with fixed doses of different platelet agonists in single-use cartridges containing fibrinogen-coated beads [33]. The reagents bound to the fibrinogen beads are arachidonic acid in the 'Aspirin cartridge', and adenosine diphosphate (ADP), iso- thrombin receptor activating peptide (iso-TRAP), and PAR-4 activating peptide in the '$P2Y_{12}$ cartridge'. During the test, a 2 ml 3.2% sodium citrate-anticoagulated whole blood sample tube is inserted into the cartridge. The whole blood is mixed with the platelet agonists and the fibrinogen-coated beads by the movement of an electromagnetically-driven steel ball. The platelets become activated by the specific agonist in the cartridge to a degree that is dependent on the level of inhibition by the antiplatelet agent which the patient is taking. Activated platelets will then bind to the fibrinogen-coated beads, cause agglutination and will fall out of the solution. Within the instrument, the light absorbance through the solution is measured 16 times per second. Both the rate and extent of platelet-induced agglutination over a fixed period of time are measured and combined with a proprietary algorithm to report the values in 'reaction units' [33].

Exploratory novel longitudinal definition of dipyridamole-HTPR on the VerifyNow $P2Y_{12}$ assay

Intra-assay CVs were measured in our laboratory and found to be 0.1% for the Aspirin cartridge and 5.5% for the $P2Y_{12}$ cartridge. Based on our experience with the PFA-100 C-ADP assay, we provisionally defined 'dipyridamole-HTPR on the VerifyNow' as failure to decrease the P2Y12 Reaction Units (PRU) on the $P2Y_{12}$ assay compared with the patient's baseline PRU on aspirin monotherapy by more than twice the CV of the assay when dipyridamole was added to aspirin i.e. failure to decrease the PRU by >11% of the patient's PRU at baseline.

2.6.3. Multiplate® assay

This whole blood platelet aggregation assay is based on measurement of impedance at 'low shear stress' as platelets adhere to 2 adjacent electrodes and aggregate to one another within a cuvette. The extent of platelet adhesion and aggregation is recorded as the Area Under the Curve (AUC) in 'units (U)' up to 6 min after the addition of either arachidonic acid (Aspirin test) or ADP (ADP test) to measure the antiplatelet effects of aspirin or clopidogrel/$P2Y_{12}$ antagonists, respectively.

Exploratory novel longitudinal definition of dipyridamole-HTPR on the multiplate ADP assay

In our laboratory, the intra-assay CV for the Aspirin test was 7.3% and for the ADP test was 7.8% ($N = 8$ assays). We assessed the potential ability of the Multiplate ADP assay to detect additional inhibition of platelet function ex-vivo following stimulation with ADP when dipyridamole was added to aspirin. 'Dipyridamole-HTPR on the Multiplate analyser' was provisionally defined as failure to decrease the AUC of the Multiplate ADP test compared with the patient's baseline AUC on aspirin monotherapy by more than twice the CV of the assay when dipyridamole was added to aspirin therapy i.e. failure to decrease the AUC by >15.6% of the patient's AUC at baseline.

2.6.4. Whole blood flow cytometry

Platelets were distinguished from red and white blood cells, as described previously [8,27,30,31]. Platelet surface CD62P and CD63 expression [8,30,31], and the percentages of circulating neutrophil-platelet, monocyte-platelet and lymphocyte-platelet complexes were quantified as markers of platelet activation, using previously described and validated methods [8,30].

2.7. Statistical methods

All statistical analyses were performed using SPSS (Version 23). Descriptive statistical calculations were performed to describe demographic and vascular risk factors, and TIA/stroke subtyping in our patient population. The maximum closure time recorded by the PFA-100 is 300 s. Therefore, we arbitrarily classified results >300 s as 301 s for the purpose of our analyses, and assumed that the data were not normally distributed. The Wilcoxon signed rank test was used for comparison of 'matched' C-EPI, C-ADP and INNOVANCE P2Y closure times, VerifyNow Aspirin and VerifyNow P2Y12 reaction units, and Multiplate-Aspirin and Multiplate-ADP units at 14d and 90d relative to baseline. The Mann-Whitney *U* test was used to compare the median % expression of platelet activation markers between patients with vs. those without dipyridamole-HTPR on the relevant platelet reactivity assays, and the chi-squared test was used to compare proportions between unmatched datasets, where appropriate. Spearman's rank correlation analysis was performed post hoc to assess the potential correlation between age and the % expression of each platelet activation marker on flow cytometry at baseline (see below).

3. Results

Sixty patients were recruited at baseline, 39 of whom had data at 14d and 31 had follow-up data at 90d. The reasons for lack of complete follow-up data in this component of the OATS study are clearly outlined in Fig. 1. The proportion of patients who discontinued dipyridamole MR in the study due to adverse effects between baseline and 14 days (10/60 [16.7%], between 14d and 90d (4/39 [10.3%]), and overall (14/60 [23.3%]) is in keeping with published data (16.4% in the PRoFESS trial [6], 15–20% in the EARLY trial [5], and lower than that reported in two other clinical trials (29% in ESPS-2 [1]; 34% in ESPRIT [4]). The main side effects leading to discontinuation of dipyridamole were headache (9/60 [15%]) and GI upset (5/60 [8.3%]). Furthermore, every laboratory assay could not be performed at each time point if, for example, certain devices were being serviced or temporarily malfunctioned when patients re-attended for follow-up.

The baseline demographic and vascular risk factor profiles (Table 1), and the TIA/stroke aetiological subtypes in CVD patients at enrolment according to the TOAST (Table 2) and ASCOD (Table 3) classification systems are outlined below. Four patients had a cerebral ischaemic stroke, one had a thoracic spinal cord infarct, and the remainder had TIAs at enrolment. The median daily dose of aspirin was 150 mg at baseline, and 75 mg at 14d and 90d. All patients were on dipyridamole MR 200 mg BD at 14d and 90d. Only one patient who was classified as having had a 'cryptogenic TIA / TIA of undetermined aetiology' at initial presentation in association with a PFO and an inter-atrial septal aneurysm had a recurrent TIA on 300 mg of aspirin daily and 200 mg of dipyridamole MR once daily, but dipyridamole MR had only been started 24 h earlier. The patient underwent an immediate change in antithrombotic treatment on the advice of the treating physician and was excluded from follow-up in this arm of the study. No other patients had recurrent cerebrovascular, cardiovascular, venous thrombo-embolic or haemorrhagic outcomes during follow up.

3.1. Platelet function/reactivity after the addition of dipyridamole to aspirin

In the following sections, follow-up matched biomarker data are presented first and baseline data are presented second for comparative purposes in the text, unless otherwise specified. The PFA-100 C-ADP, Verify Now P2Y12 and Multiplate ADP assay results are presented in detail in the text and tables because these assays were considered a priori to be most likely to detect the additional antiplatelet effects of dipyridamole. Data from other simultaneously-conducted assays on the PFA-100 (C-EPI, INNOVANCE P2Y closure times), VerifyNow Aspirin (ARU), and Multiplate Aspirin (U) at each timepoint, which were not specifically designed or felt likely to detect inhibition of platelet function with dipyridamole, are tabulated for descriptive purposes only.

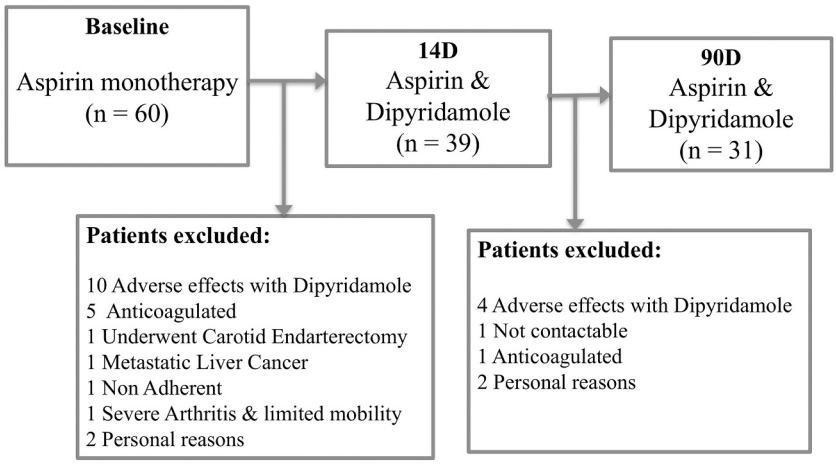

Fig. 1. Flow diagram of patients who were initially included at baseline on aspirin monotherapy and those who were followed up or excluded over time after adding dipyridamole to aspirin.

Table 1
Demographic and vascular risk factor profiles in patients at enrolment. TIA = Transient Ischaemic Attack; IHD = Ischaemic Heart Disease; DVT = Deep Venous Thrombosis; PE = Pulmonary Embolism.

Characteristic	Number	(%)
Mean age in years (SD)	60.2 (12.7)	
Gender (M/F)	32/28	53.3%
Ischaemic stroke at presentation	Cerebral (4); Spinal cord (1)	8.3%
TIA at presentation	55	91.7%
Thrombolysed with IV TPA	2	3.3%
Prior stroke/TIA	4	6.7%
IHD	3	5%
Hypertension	18	30%
Diabetes mellitus	2	3.3%
Atrial fibrillation at enrolment	0	0
Family history of stroke	13	21.7%
Prior DVT/PE	0	0
Peripheral vascular disease	2	3.3%
Hyperlipidaemia	14	23%
Migraine (with or without aura)	11	18.3%
Current smoker	12	20%
Ex-smoker	20	33.3%
Never smoker	26	43.3%
Statin therapy	31	51.7%
Index event on antiplatelet therapy	9	15%

Table 2
TOAST classification in patients at enrolment (Total N = 60).

Stroke / TIA subtype	Number (%)
Large artery atherosclerotic	4 (6.7%)
Small vessel disease	5 (8.3%)
Cardioembolic	6 (10%)
Other determined	0
Undetermined aetiology	45 (75%)

3.1.1. PFA-100 C-ADP data

There was no statistically significant increase in median C-ADP closure times after adding dipyridamole to aspirin monotherapy at 14d [87.5 s (inter-quartile range: 78–97 s) vs. 88.5 s (range: 84–111 s), $P = 0.2$; $N = 35$], or 90d [92 s (range: 80.5–114.5 s) vs. 90.5 s (range: 86–114 s), $P = 0.6$; $N = 27$] (Table 4). However, using our longitudinal definition, the prevalence of dipyridamole-HTPR on the PFA-100 C-ADP assay was 75% (27/36) at 14d and 71.4% (20/28) at 90d. Six patients with dipyridamole-HTPR at 14d did not have persistent dipyridamole-HTPR at 90d. Four patients who had dipyridamole-HTPR at 14d had no data available for analysis at 90d because the patients either dropped out of this arm of the study or the device was not functioning at the 90d follow-up visit. Two patients with dipyridamole-HTPR at 90d had not had dipyridamole-HTPR at 14d, and a further two patients with dipyridamole-HTPR at 90d had no 14d data available. Overall, amongst patients who had matched data at both 14d and 90d ($N = 27$), 18/27 (66.7%) retained the same dipyridamole-HTPR status during follow-up.

Table 3
ASCOD classification in patients at enrolment (Total N = 60).

ASCOD phenotype	Disease present (ASCOD 1,2,3)	Disease absent (ASCOD 0)	Insufficient investigation (ASCOD 9)
A [Number (%)]	28 (46.7%)	32 (53.3%)	0
S [Number (%)]	36 (60%)	24 (40%)	0
C [Number (%)]	30 (50%)	30 (50%)	0
O [Number (%)]	17 (28%)	43 (72%)	0
D [Number (%)]	0 (0%)	60 (100%)	0

A: Atherosclerosis; S: Small vessel disease; C: Cardiac source; O: Other cause; D: Dissection.
1: 'Definitely a potential cause of the index stroke';
2: 'Causality uncertain';
3: 'Unlikely a direct cause of the index stroke, but disease is present';
0: 'Disease absent'.

3.1.2. PFA-100C-EPI and INNOVANCE P2Y data

Compared with baseline, there was no significant change in median C-EPI closure times or INNOVANCE P2Y closure times during follow-up after adding dipyridamole to aspirin monotherapy (Table 4).

3.1.3. VerifyNow P2Y12 data

There was no significant change in median PRU values after adding dipyridamole to aspirin monotherapy at 14d [284 (range: 240–320) vs. 278.5 (range: 235–330), $P = 1.0$; $N = 36$], or 90d [292.5 (range: 232–323) vs. 278.5 (range: 229–320), $P = 0.13$; $N = 30$) (Table 4). However, using our novel longitudinal definition, the prevalence of dipyridamole-HTPR on the VerifyNow P2Y12 assay was 86.8% at 14d (33/38) and 83.9% at 90d (26/31). Amongst patients who had matched data at both 14d and 90d ($N = 28$), 21/28 (75%) retained the same 'dipyridamole-HTPR status' during follow-up.

Table 4
Median values (range: 25th–75th percentiles) from different platelet function/reactivity assays at each time point. *P* values refer to comparisons between data at baseline vs. 14d and 90d, respectively. Values in different cells refer to median values of all available data at each timepoint, so values in text may differ slightly from the tabulated data for comparisons of matched data at different time points.

Time point	Baseline	14d	90d
Median daily dose of: Aspirin	150 mg daily	75 mg daily	75 mg daily
Dipyridamole MR	0 mg	200 mg BD	200 mg BD
PFA-100			
C-EPI (S)	301 (182–301)	301 (146–301)	292 (232–301)
P value		0.36	0.1
C-ADP (S)	87 (75–102)	87.5 (78–97)	92 (80.5–114.5)
P value		0.2	0.6
INNOVANCE P2Y (S)	71 (61–78)	67 (60–78)	74.5 (65–89)
P value		0.3	0.1
VerifyNow			
Aspirin (ARU)	414 (399–448)	431.5 (407–462)	455 (422.5–522)
P value		0.5	0.008
P2Y12 (PRU)	271 (236.5–317.5)	284 (240–320)	292.5 (232–323)
P value		0.1	0.1
Multiplate			
ASPIRIN (U)	25 (16–31)	24 (17.5–33.5)	27 (21–40)
P value		0.5	0.3
ADP (U)	92 (82–106)	89 (82–106)	83 (76–105.5)
P value		0.5	0.9

3.1.4. VerifyNow Aspirin data

There was no significant change in Aspirin Reaction Units (ARU) after adding dipyridamole to aspirin at 14d [$P = 0.5$; $N = 38$]. However, the median ARU was significantly higher at 90d vs. baseline [455 (range: 422.5–522) vs. 409 (range: 400–451.5), $P = 0.008$; $N = 32$] (Table 4).

3.1.5. Multiplate ADP assay

There was no significant change in Multiplate ADP units after adding dipyridamole at 14d [89 U (range: 82–106) vs. 93 U (range: 79.5–106.5); $P = 0.5$, $N = 35$], or at 90d [83 U (range: 76–105.5) vs. 95.5 U (range: 77.5–106); $P = 0.9$, $N = 27$] (Table 4). However, using our exploratory, longitudinal definition of dipyridamole-HTPR on the Multiplate ADP assay, the prevalence of dipyridamole-HTPR was 83.3% (30/36) at 14d and 81.5% (22/27) at 90d on this device. Two patients had dipyridamole-HTPR at 14d, but did not exhibit dipyridamole-HTPR at 90d. However, all patients who had dipyridamole-HTPR at 90d also had preceding 'dipyridamole-HTPR' at 14d. Overall, amongst patients who had matched data at both 14d and 90d (N = 27), 24 (88.9%) retained the same 'dipyridamole-HTPR status' during follow-up.

3.1.6. Multiplate Aspirin data

There was no significant change in Multiplate Aspirin units after commencing dipyridamole at 14d [$P = 0.5$, N = 35] or at 90d [$P = 0.3$, N = 27] (Table 4).

3.2. Platelet activation status after the addition of dipyridamole to aspirin (Table 5)

There were no statistically significant changes in platelet surface CD62P or CD63 expression at 14d or 90d vs. baseline after commencing dipyridamole in the overall study population. Compared with baseline values, the % circulating neutrophil-platelet complexes did not change at 14 days, but did significantly increase at 90d ($P = 0.01$). The % monocyte-platelet complexes initially increased at 14d ($P = 0.02$), but this increase did not reach statistical significance at 90d after commencing dipyridamole ($P = 0.06$).

3.3. Comparison of platelet activation markers according to dipyridamole-HTPR status on the PFA-100 C-ADP, VerifyNow P2Y12 and Multiplate ADP assays

3.3.1. PFA-100 C-ADP assay

The median %CD62P expression was significantly lower overall in the subgroup of patients with dipyridamole-HTPR than in those without dipyridamole-HTPR at 14d ($P = 0.03$), but not at 90d ($P = 0.07$) (Table 6). There were no significant differences in the expression of any other platelet surface activation markers between those with vs. those without dipyridamole-HTPR at any timepoint (Table 6). However, the % monocyte-platelet complexes significantly increased over time only in the subgroup of patients with dipyridamole-HTPR at 14d ($P = 0.02$; Fig. 2) and at 90d ($P = 0.038$; Fig. 3), but not in those without dipyridamole-HTPR at 14d ($P = 0.61$) or at 90d ($P = 0.78$) compared

Table 5
Platelet activation markers after adding dipyridamole to aspirin. Values are medians (range: 25th–75th percentiles). Significant *P* values highlighted in bold.

	Baseline	14 days	90 days
	N = 47	N = 30	*N* = 23
Platelet surface markers:			
CD62P %	4.0 (2.15–6.07)	3.14 (1.58–6.35)	3.3 (2.6–5.47)
P value		0.37	0.18
CD63%	13.5 (9.56–17.7)	12.8 (8.53–19)	12.5 (10.5–20.8)
P value		0.18	0.32
Leucocyte-platelet complexes:			
Neutrophil-platelet complexes (%)	2.63 (2.22–3.29)	2.69 (2.3–3.79)	3.0 (2.49–3.83)
P value		0.59	**0.01**
Monocyte-platelet complexes (%)	6.0 (4.3–6.75)	6.1 (4.7–7.55)	6.5 (4.83–7.2)
P value		**0.02**	0.06
Lymphocyte-platelet complexes (%)	2.0 (1.58–2.63)	2.2 (1.73–3.02)	2.42 (1.69–2.70)
P value		0.25	0.18

Table 6
Comparison of platelet activation markers between those with vs. those without dipyridamole-HTPR on the PFA-100 C-ADP assay. Values represent medians (25th -75th percentiles). Patients without dipyridamole-HTPR are designated as 'No HTPR' in the following 2 tables. Significant P values are highlighted in bold.

Marker	14 days	90 days
% CD62P expression		
HTPR	3.01 (1.68–3.54)	2.85 (1.95–4.01)
No HTPR	6.17 (5.36–7.62)	4.56 (2.98–6.13)
P Value	**0.03**	0.07
% CD63 expression		
HTPR	13.7 (10.5–19.2)	15.0 (10.55–19.25)
No HTPR	11.4 (6.70–15.3)	15.2 (11.75–25.7)
P Value	0.32	0.39
% Neutrophil-platelet complexes		
HTPR	3.12 (2.32–3.88)	3.01 (2.29–3.83)
No HTPR	2.65 (2.41–3.39)	2.99 (2.84–3.46)
P Value	0.69	0.93
% Monocyte-platelet complexes		
HTPR	5.88 (4.65–6.64)	6.60 (4.83–7.50)
No HTPR	7.5 (7.35–8.45)	6.05 (3.99–7.08)
P Value	0.08	0.73
% Lymphocyte-platelet complexes		
HTPR	2.15 (1.73–2.87)	2.42 (1.78–2.70)
No HTPR	2.06 (1.50–2.41)	2.24 (1.46–2.50)
P Value	0.5	0.64

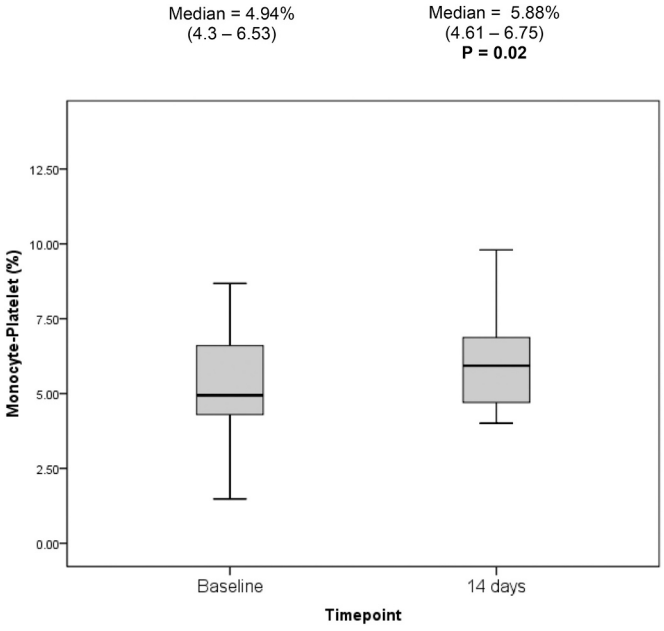

Fig. 2. Boxplot of % monocyte-platelet complexes at baseline vs. 14d in patients with dipyridamole-HTPR on the PFA-100 C-ADP assay. Values above boxplots are medians (range: 25th–75th percentiles).

with baseline.

3.3.2. VerifyNow P2Y12 assay

There were no significant differences in the expression of any platelet activation markers or leucocyte-platelet complexes between those with vs. those without dipyridamole-HTPR on the VerifyNow at any timepoint ($P \geq 0.07$) (Table 7). There was a significant increase in the % monocyte-platelet complexes at 14d vs. baseline in the subgroup of patients with dipyridamole-HTPR ($P = 0.04$) on the VerifyNow, but the increase at 90d vs. baseline was not statistically significant ($P = 0.07$). There was no significant change in the % monocyte-platelet complexes at 14d or 90d vs. baseline in the subgroup of patients without dipyridamole-HTPR on the VerifyNow ($P \geq 0.69$).

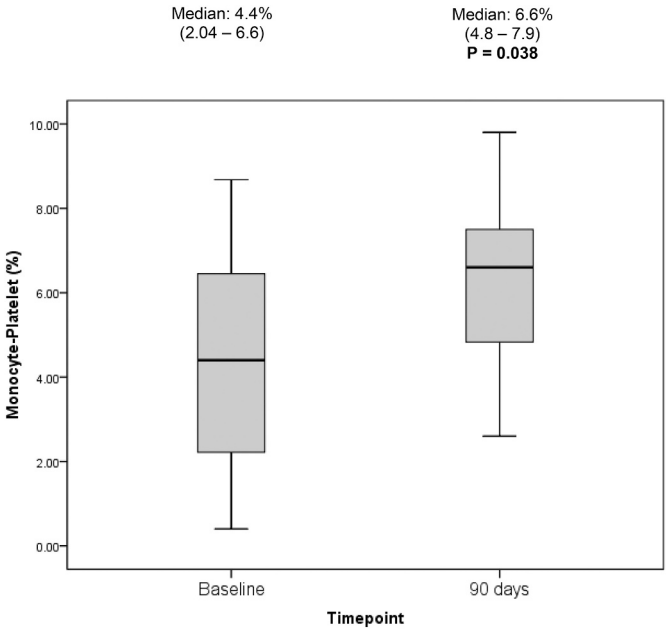

Fig. 3. Boxplot of % monocyte-platelet complexes at baseline vs. 90d in patients with dipyridamole-HTPR on the PFA-100 C-ADP assay. Values above boxplots are medians (range: 25th–75th percentiles).

Table 7
Comparison of platelet activation markers between those with vs. those without dipyridamole-HTPR on the VerifyNow® P2Y12 assay. Values represent medians (25th–75th percentiles).

Marker	14 days	90 days
% CD62P expression		
HTPR	3.16 (2.0–6.35)	3.43 (2.57–5.16)
No HTPR	2.41 (1.95–3.67)	2.78 (1.87–3.16)
P Value	0.55	0.3
% CD63 expression		
HTPR	12.9 (9.43–20)	12.8 (10.5–21.6)
No HTPR	10.2 (9.45–14.1)	15 (11.8–16.6)
P Value	0.6	0.7
% Neutrophil-platelet complexes		
HTPR	3.00 (2.33–3.83)	3.01 (2.49–3.94)
No HTPR	2.95 (2.02–3.43)	3.07 (2.93–3.45)
P Value	0.8	0.7
% Monocyte-platelet complexes		
HTPR	6.3 (4.70–7.6)	6.4 (4.39–7.2)
No HTPR	4.01 (2.55–5.06)	6.5 (5.51–6.8)
P Value	0.07	0.9
% Lymphocyte-platelet complexes		
HTPR	2.15 (1.64–2.95)	2.27 (1.77–3.14)
No HTPR	2.77 (1.95–3.04)	2.42 (1.57–2.46)
P Value	0.9	0.58

3.3.3. Multiplate ADP assay

The median % neutrophil-platelet complexes was significantly lower overall in the subgroup of patients with dipyridamole-HTPR than in those without dipyridamole-HTPR at 90d (2.91 vs. 4.59%; $P = 0.02$) (Table 8). There were no other significant differences in the expression of other platelet activation markers between those with vs. those without dipyridamole-HTPR at any timepoint (Table 8). There was no significant change in the % monocyte-platelet complexes at 14d or 90d vs. baseline in either the subgroup of patients with dipyridamole-HTPR ($P \geq 0.09$) or in those without dipyridamole-HTPR on the Multiplate ADP assay ($P \geq 0.07$).

Table 8
Comparison of platelet activation markers between those with vs. those without dipyridamole-HTPR on the Multiplate ADP assay. Values represent medians (25th–75th percentiles). Significant P values are highlighted in bold.

Marker	14 days	90 days
% CD62P expression		
HTPR	3.34 (2.41–6.53)	3.17 (2.54–5.47)
No HTPR	1.8 (1.07–3.50)	3.77 (3.02–8.75)
P Value	0.12	0.54
% CD63 expression		
HTPR	12.9 (10.2–19.2)	11.95 (10.5–20.8)
No HTPR	8.94 (5.90–16.75)	21.25 (10.79–25.65)
P Value	0.31	0.48
% Neutrophil-platelet complexes		
HTPR	3.36 (2.40–3.88)	2.91 (2.33–3.69)
No HTPR	2.51 (1.55–3.01)	4.59 (3.90–4.95)
P Value	0.13	**0.02**
% Monocyte-platelet complexes		
HTPR	6.10 (4.70–7.55)	6.05 (3.97–7.10)
No HTPR	6.54 (4.55–8.94)	6.85 (5.60–8.55)
P Value	0.87	0.39
% Lymphocyte-platelet complexes		
HTPR	2.16 (1.58–2.87)	2.24 (1.57–2.54)
No HTPR	2.63 (2.15–5.93)	3.80 (2.13–5.91)
P Value	0.24	0.2

3.4. Post-hoc analysis to investigate whether any key clinical variables might have influenced dipyridamole HTPR status or the expression of any platelet activation markers

We performed post hoc analyses to investigate whether any key clinical variables (age, sex, prior TIA/stroke, hypertension, hyperlipidaemia, statin therapy, diabetes mellitus, ischaemic heart disease (IHD), peripheral vascular disease (PVD), migraine, smoking status, or a family history of stroke) might have influenced dipyridamole HTPR status at 14d or 90d, and the expression of any platelet activation markers at baseline. There was no significant difference in the median age or in the proportion of patients who were male, had prior TIA/stroke, hypertension, hyperlipidaemia, were on statin therapy, had diabetes mellitus, IHD, PVD, migraine, were current or ex-smokers, or had a family history of stroke between those with vs. those without dipyridamole-HTPR at 14d or 90d on any of the 3 platelet reactivity assays ($P > 0.05$). Furthermore, there was no significant correlation between age and the expression of any of the platelet activation markers on whole blood flow cytometry at baseline ($P > 0.05$). The median expression of each platelet activation marker at baseline was similar in males vs. females, in those with vs. those without hypertension, in those with vs. those without hyperlipidemia, in those who were taking vs. not taking a statin, in those with vs. those without migraine, in current and ex-smokers vs. never smokers, and in those with vs. those without a family history of stroke, ($P > 0.05$). Therefore, we have no evidence that any of these factors had any significant confounding influence on the results obtained. The number of patients with prior TIA/stroke, diabetes mellitus, IHD or PVD was considered too few (≤4 cases each) to reliably assess the association between these variables with expression of platelet activation markers in this study.

4. Discussion

This comprehensive, pilot longitudinal study has confirmed the interesting finding that one may detect additional inhibition of platelet adhesion and/or aggregation with the high shear stress PFA-100 C-ADP assay in at least 25–28.6% of CVD patients after starting dipyridamole [8], and revealed the novel finding that one may detect additional inhibition of platelet aggregation in a clinically important proportion of patients with the low shear stress VerifyNow P2Y12 (13.2–16.1%) and Multiplate ADP (16.7–18.5%) assays. Therefore, the VerifyNow and Multiplate analysers can be added to the small list of devices which are capable of detecting additional antiplatelet effects of dipyridamole ex vivo if our innovative proposed longitudinal definitions of dipyridamole-HTPR are employed.

We did not detect any statistically significant changes in median C-EPI, C-ADP or INNOVANCE P2Y closure times on the PFA-100 after adding dipyridamole to aspirin. The C-EPI assay findings are in keeping with prior findings by our research group which revealed that this assay does not detect additional inhibition of platelet function with dipyridamole [8]. Although there was a non-significant trend, we did not confirm prior findings of a statistically significant prolongation of median C-ADP closure times at 14d or 90d compared with baseline after adding dipyridamole to aspirin [8]. Although there was a lower proportion of stroke patients and a higher proportion of TIA patients in this study compared with the prior study by Tobin et al., this does not explain the differences in findings between the studies. These differences may well, in part, reflect a type II error with a smaller number of patients with follow-up C-ADP data at both 14d and 90d in the OATS than in the prior TRAP study [8]. In addition, because there was a trend towards prolonged C-ADP closure times after the addition of dipyridamole to aspirin in this study, we think it is unlikely that the findings in the prior TRAP study represented a type I error [8]. The prevalence of dipyridamole-HTPR on the PFA-100 ADP assay was also numerically higher in this study (71.4–75%) than in the TRAP study (56–59%) [8], but the proportions of patients with dipyridamole-HTPR was not actually significantly different between the two studies at 14d ($P = 0.169$) or at 90d ($P = 0.228$; chi-squared testing). We do not think it likely that any difference in the degree of adherence to dipyridamole prior to retesting explained the slight disparity in the prevalence of dipyridamole-HTPR between these studies because each study employed a similar methodology of phoning patients before their planned reassessment to verbally confirm adherence to their treatment regimen.

The higher median ARU values on the VerifyNow Aspirin assay at 90d vs. baseline ($p = 0.008$) most likely reflect less marked inhibition of platelet function on the VerifyNow Aspirin assay due to a reduction in the median daily prescribed dose of aspirin during follow up (150 mg daily at baseline vs. 75 mg daily at 14d and at 90d, respectively; Table 4).

CD62P and CD63 expression did not change after adding dipyridamole to aspirin, in keeping with the findings from an earlier study by our research group [8]. These data confirm that one cannot use these 'unstimulated whole blood flow cytometry assays' to reliably detect additional inhibition of platelet activation ex vivo when dipyridamole is added to aspirin. Prior studies have found an increase in leucocyte-platelet complexes in the early and late phases after TIA or ischaemic stroke compared with controls, indicating excessive platelet activation in CVD patients [30,34]. There was a significant increase in the % circulating monocyte-platelet complexes at 14d on aspirin-dipyridamole combination therapy compared with baseline levels on aspirin monotherapy overall. However, the difference in the % monocyte-platelet complexes at 90d vs. baseline did not reach statistical significance ($P = 0.06$), possibly reflective of a type II error due to the smaller number of subjects who were followed up to 90d. Our group previously found increased monocyte-platelet complexes at both 14d and 90d after the addition of dipyridamole to aspirin after TIA or ischaemic stroke, but this rise in monocyte-platelet complexes over time was driven by data from the patient subgroup with dipyridamole-HTPR on the PFA-100 C-ADP assay [8]. Marquardt et al. reported an increased % monocyte-platelet complexes on day 2 following acute ischaemic stroke compared with age- and sex-matched controls, but the levels were not increased at any other timepoint during follow-up to 90 days after symptom onset [35]. However, precise details regarding prescribed antiplatelet regimens were not described, and that study was not designed to assess dipyridamole-HTPR status at variable levels of shear stress [35]. The results of this component of the OATS study independently validate prior findings that monocyte-platelet complexes only significantly increase over time in the patient subgroup with

dipyridamole-HTPR on the PFA-100 C-ADP assay [8], with the novel finding of a significant increase in monocyte-platelet complexes also at 14d but not at 90d vs. baseline in those with dipyridamole-HTPR on the VerifyNow. Although these was no change in monocyte-platelet complexes during follow-up in the subgroup of patients with dipyridamole-HTPR on the Multiplate ADP assay, these data support the hypothesis that elevated monocyte-platelet complexes contribute to the molecular mechanisms responsible for dipyridamole-HTPR at high shear stress, with some preliminary evidence that they may contribute to dipyridamole-HTPR at low stress on the VerifyNow at least early after TIA/stroke onset. Larger prospective studies are warranted to confirm or refute these findings under low shear stress conditions, and to determine whether one could optimise secondary prevention by targeting individual patients with dipyridamole-HTPR, especially those with increasing monocyte-platelet complexes, with an alternative antiplatelet regimen if it is shown that the presence of dipyridamole-HTPR predicts the risk of recurrent vascular events after TIA/stroke.

We observed a significant increase in the % neutrophil-platelet complexes at 90d but not at 14d in the overall patient population on aspirin-dipyridamole combination therapy compared with baseline values on aspirin monotherapy. This may reflect a type II error at 14d or potentially a type I error at 90d, so assessment of the profile of neutrophil-platelet complexes in patients commencing dipyridamole deserves further study. Furthermore, compared with patients without dipyridamole-HTPR on the respective devices, patients with dipyridamole-HTPR on the PFA-100 C-ADP assay at 14d had lower CD62P platelet surface expression, and those with dipyridamole-HTPR on the Multiplate ADP assay at 90d had a lower % neutrophil-platelet complexes. The lower CD62P platelet surface expression in patients with dipyridamole-HTPR on the PFA-100 could potentially have been influenced by 'CD62P consumption' during monocyte-platelet complex formation, but it is possible that these findings also reflect a type I error because differences between groups were not consistent at each time point during follow up.

This study was conducted before the emergence of the COVID-19 pandemic in 2020, but data on dipyridamole-HTPR may have even more relevance in the current COVID-19 era. There is an increased risk of TIA/stroke associated with SARS-CoV-2 infection, especially in those <50 years old [36–38]. The proposed pathophysiological mechanisms responsible for stroke with COVID-19 include activation of the coagulation system [39–42] and endothelium [43–47], with more recent evidence of enhanced platelet activation and aggregation [48–50]. Some of the proposed pathways by which SARS-CoV-2 may interact with platelets have been reviewed very recently [51]. Dipyridamole has also been shown to inhibit SARS-CoV-2 viral replication in vitro in a pilot study in China, and treatment with dipyridamole was associated with significantly improved clinical outcomes in hospitalized patients [52]. Furthermore, there is evolving interest in using dipyridamole as part of a treatment regimen for patients with severe COVID-19 [53,54], and a larger study assessing the effect of dipyridamole in patients with COVID-19 is ongoing (ChiCTR2000030055) [54]. The published data provide a clear rationale for the use of an antiplatelet agent with antiplatelet, and potential additional 'anti-endothelial' [11] and 'anticoagulant effects' [13] in selected patients with TIA or stroke associated with COVID-19, with an opportunity to determine whether assessment of dipyridamole-HTPR status may predict outcomes in such individuals [8].

This study had some limitations. Only a small proportion of patients with large artery atherosclerotic TIA/ischaemic stroke were recruited to this aspect of the OATS study because most of these patients underwent urgent carotid revascularisation, mainly with carotid endarterectomy at our centre. Because 'recent surgery' was an exclusion criterion, the majority of these patients could not undergo repeat platelet function/reactivity testing 14d after changing antiplatelet therapy. Only one patient had a recurrent TIA/stroke during comprehensive clinical study follow-up. This patient did not have data on dipyridamole-HTPR status because his antithrombotic treatment regimen was changed by his treating physician prior to his scheduled 14d follow-up visit, and he was thus excluded from further follow-up thereafter. Therefore, we cannot make any reliable comment about the value of dipyridamole-HTPR status at predicting the risk of recurrent vascular events from this phase of the OATS study; much larger studies would be needed to address this issue. We did not systematically study inhibition of platelet function/activation with our comprehensive suite of assays in patients who were being changed from no medication to dipyridamole alone because the numbers of patients who are prescribed dipyridamole monotherapy in clinical practice is very small. This pilot study may have been prone to type II errors and occasional type I errors, as clearly acknowledged above, warranting future multicentre studies in this field on the concept of dipyridamole-HTPR to confirm our novel observations.

5. Conclusions

One can detect additional antiplatelet effects of dipyridamole with user-friendly tests of platelet reactivity under both high and low shear stress conditions ex vivo. Increasing circulating monocyte-platelet complexes over time are associated with dipyridamole-HTPR, especially under conditions of moderately high shear stress, thus improving our understanding of the cellular mechanisms responsible for dipyridamole-HTPR in patients with TIA/ischaemic stroke. Larger longitudinal studies are warranted to determine whether assessment of dipyridamole-HTPR status improves our ability to predict the risk of recurrent vascular events to enable personalisation of secondary preventive treatment following TIA or ischaemic stroke.

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