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RESEARCH ARTICLE

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Pharmacodynamic effects of early aspirin withdrawal after percutaneous coronary intervention in patients with atrial fibrillation treated with ticagrelor or prasugrel

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ABSTRACT

Dual antithrombotic therapy (DAT) without aspirin reduces bleeding compared with triple antithrombotic therapy (TAT) in patients with atrial fibrillation who have undergone percutaneous coronary intervention, without apparently increasing ischemic events. A prospective pharmacodynamic study was performed to investigate the impact of aspirin on bleeding time, platelet function and fibrin clot analysis in this population. Patients receiving TAT ($n = 16$), comprising aspirin, ticagrelor/prasugrel and a direct-acting oral anticoagulant (DOAC), were compared with those receiving DAT without aspirin ($n = 18$). Bleeding time was reduced with DAT compared with TAT (median 27.8 vs 30.0 minutes, $p = .005$). Assessed by light transmission aggregometry, median platelet aggregation was significantly increased with DAT compared with TAT in response to arachidonic acid (63 vs 3%, $p = .002$) and collagen (72 vs 37%, $p < .001$) but not 5-µmol/L adenosine diphosphate (25 vs 27%, $p = .966$) or thrombin-receptor-activating peptide (37 vs 24%, $p = .086$). VerifyNow $P2Y_{12}$ assay showed > 70% inhibition in all patients. Fibrin clot lysis time and maximum turbidity were similar between groups. Using $P2Y_{12}$ inhibitors of consistent potency, DAT improves hemostasis through sparing cyclooxygenase-1-mediated platelet activation but has a comparable effect to TAT on other pathways and fibrin clot properties. DAT with ticagrelor/prasugrel and DOAC may provide sufficient antithrombotic effect without excessive anti-hemostatic effect.

Plain Language Summary

What is the context?

- Patients with atrial fibrillation (AF) on long-term anticoagulation therapy who undergo a heart procedure known as percutaneous coronary intervention (PCI) for coronary artery disease are often also commenced on antiplatelet medications to prevent clots, especially if stents are inserted.
- The recommended initial post-procedure combination is termed triple antithrombotic therapy (TAT), including two antiplatelet agents (aspirin and a P2Y12 inhibitor) and a direct-acting oral anticoagulant (DOAC).
- However, TAT increases the risk of bleeding, so contemporary research is investigating whether dual antithrombotic therapy (DAT), which removes aspirin, is a safer alternative after PCI.

KEYWORDS
Acute coronary syndrome, aspirin, atrial fibrillation, dual antithrombotic therapy, ticagrelor, triple antithrombotic therapy

History

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What is new?

- This study compared TAT and DAT without aspirin in terms of their effects on bleeding time, platelet function, and fibrin clot properties (consistency and ability to form or break down blood clots). A potent P2Y12 inhibitor (ticagrelor or prasugrel) was used in all patients.
- The research found that patients on DAT had shorter bleeding times. Platelet activity was higher in the DAT group for certain clotting pathways, but similar to the TAT group for others, while fibrin clot properties were also similar.

What is the impact?

- These findings suggest that DAT with ticagrelor or prasugrel and a DOAC may provide enough protection against clotting while reducing the risk of excessive bleeding compared to TAT.
- This could lead to safer treatment strategies for patients with AF who need additional antithrombotic therapy after PCI.

Introduction

The management of coronary artery disease often involves dual antiplatelet therapy (DAPT), especially after treatment with percutaneous coronary intervention (PCI), while the management of atrial fibrillation (AF) often involves long-term administration of an oral anticoagulant (OAC).[1,2] Antiplatelet agents and OACs are therefore commonly prescribed in combination when patients with AF undergo PCI, which raises concern about increased bleeding and the need to balance this against thrombotic risk.[3] Current recommendations are based on randomized controlled trials comparing the safety and efficacy of triple antithrombotic therapy (TAT), consisting of aspirin, a $P2Y_{12}$ inhibitor and OAC, versus dual antithrombotic therapy (DAT) without aspirin.[4–8] However, differences in OAC between trial arms, limited use of more pharmacologically-consistent $P2Y_{12}$ inhibitors (ticagrelor and prasugrel), uncertainty about the stent thrombosis risk with DAT, and sparse evidence for the week-long period between PCI and randomization reduce clarity about the optimal antithrombotic strategy for these patients.

Two of the main lingering questions are about the type of $P2Y_{12}$ inhibitor used and the duration of aspirin, if at all, after PCI. Despite clopidogrel being the current recommended $P2Y_{12}$ inhibitor in patients with AF on OAC, it is associated with a high degree of inter-individual variability in the formation of active metabolite, meaning that pharmacodynamic response is unpredictable.[9,10] A DAT regimen consisting of OAC and either ticagrelor or prasugrel could be a suitable alternative strategy that provides consistent antiplatelet activity and negates the need for aspirin. There are, however, limited data to support use of this regimen at the moment.[1] The pharmacodynamic effects of $P2Y_{12}$ inhibitors have been extensively studied but not in the context of antithrombotic therapy for patients with AF. We sought to investigate the pharmacodynamics of TAT and DAT without aspirin using $P2Y_{12}$ inhibitors of similar pharmacological consistency.

Methods

Study population and design

This was an observational pilot study on patients with AF undergoing PCI with stenting for acute coronary syndrome (ACS) at South Yorkshire Cardiothoracic Centre, Northern General Hospital, Sheffield, United Kingdom. Patients were grouped according to whether they were commenced on TAT or DAT straight after PCI, maintained until study procedures were undertaken. The decision to commence TAT or DAT (i.e. inclusion or not of aspirin), duration of therapy and choice of $P2Y_{12}$ inhibitor was determined solely by the operator at PCI and not subject to any influence by enrollment in this study. The study specifically included patients with ACS over chronic coronary syndrome (CCS) for 2 reasons: the opportunity to investigate a potentially heightened thrombotic milieu and the increased use of ticagrelor or prasugrel. Adherence to the intended regimen was confirmed at hospital discharge and subsequent study visits. Patients were enrolled between April 2023 and May 2024. The inclusion criteria were: 1.) 18 years of age or greater, 2.) undergone successful PCI with stenting of one or more coronary arteries within 2 weeks of a diagnosis of ACS, 3.) non-valvular AF diagnosed before or at the time of PCI and 4.) patient commenced on an antithrombotic regimen before or at the earliest opportunity after PCI fulfilling either of the following criteria: a.) aspirin, ticagrelor/prasugrel and OAC (TAT) or b.) ticagrelor/prasugrel and OAC (DAT). The exclusion criteria were: 1.) PCI for CCS, 2.) type 2 myocardial infarction, 3.) PCI treated with balloon angioplasty and/or drug-eluting balloons only, 4.) within 24 hours of infusion of a glycoprotein (GP) IIb/IIIa inhibitor, 5.) started or restarted on warfarin following PCI, 6.) direct-acting OAC (DOAC) at a dose below that licensed for stroke prophylaxis, 7.) hemoglobin <100 g/L, 8.) woman of child-bearing potential who had confirmed or possible pregnancy, 9.) other practical reason which, in the opinion of the investigator, would not be compatible with study participation e.g. history or strong possibility of poor compliance with medication and 10.) unwilling or unable to provide informed consent.

Data confirming medical history, baseline blood results, PCI details and prescribed antithrombotic treatment were collected at enrollment. The study visit was intended to coincide with a time when all participants were either on TAT or DAT following recent PCI. Particularly for patients on TAT, this was before any de-escalation to DAT and, for patients on DAT, this was no fewer than 7 days after PCI and the immediate discontinuation of aspirin. It was also planned to take place not later than 30 days after diagnosis of ACS and not earlier than 24 hours after PCI to ensure that the effects of any parenteral heparin administered during PCI would be negligible after > 5 elimination half-lives.[11] Participants were instructed to take the prescribed maintenance doses of all their antithrombotic medications together and measurement of bleeding time and venous blood sampling was performed 2–4 hours afterward to coincide with the peak effect of most antiplatelets and DOACs.[12–18] The study visit involved assessing medication compliance, measuring bleeding time and taking a venous blood sample for platelet function testing and assessment of fibrin clot properties.

Bleeding time

Bleeding time testing was performed by adhering to a standard operating procedure utilizing the modified Ivy method.[19]

A sphygmomanometer cuff was placed around the arm and inflated to 40 millimeters of mercury. This level was sustained throughout the test in order to maintain standardization of venous pressure between participants. The ventral aspect of the ipsilateral forearm was cleaned with an alcohol wipe and an area devoid of visible veins was identified at or just inferior to the antecubital fossa. A puncture was made on the skin in this area using a standard spring-action safety lancet (Haemolance Plus Max Flow with a 1.6 mm blade depth) and a stopwatch was simultaneously started. This was repeated at 2 locations more medially, one at 10 seconds and another at 20 seconds from the first puncture. Filter paper (Whatman Grade 1) was gently placed at the edge of each blood droplet 30 seconds after the puncture that produced it and every 30 seconds after that. The bleeding time was measured as the mean time from puncture to cessation of bleeding of the 3 punctures. The test ended if bleeding continued for 30 minutes and this time was recorded for any punctures that had not stopped by then.

Platelet function testing

Platelet function was assessed with light transmittance aggregometry (LTA), VerifyNow assay, vasodilator-stimulated phosphoprotein (VASP) phosphorylation analysis with flow cytometry, and serum thromboxane B_2 (TXB_2) assay. This not only enabled investigation of various mechanisms of platelet activation but also investigation of the same mechanism via different assays for improved reliability. All platelet function tests except the TXB_2 assay were performed immediately after obtaining venous blood samples at the study visit by individuals blinded to participants' treatment and adhering to a standard operating procedure.

LTA is the gold standard for monitoring platelet function in the presence of antiplatelet treatment.[20] Various agonists were utilized: arachidonic acid (AA; 1 mmol/L) and collagen (4 µg/mL) were from Hyphen Biomed (Neuville-sur-Oise, France), adenosine diphosphate (ADP; 5 and 20 µmol/L), 5-hydroxytryptamine (5-HT; 1 µmol/L) and adrenaline (10 µmol/L) were from Merck Life Sciences UK Ltd (Gillingham, UK), and thrombin-receptor-activating peptide-6 (TRAP; 8 µmol/L) was from Roche Diagnostics, (Burgess Hill, UK). 10 µL of each agonist (6 in total including the different concentrations) was added to separate test tubes containing a stir bar and 240 µL of platelet-rich plasma in a Platelet Aggregation Profiler (PAP)-8 machine (Bio/Data Corporation, Horsham, Pennsylvania, USA). At the same time, 10 µL of 0.9% saline was added to each of 6 test tubes containing 240 µL of platelet-poor plasma in the PAP-8 machine to act as controls. For each agonist, maximum aggregation (adjusted for baseline aggregation) was recorded as a percentage.

VerifyNow $P2Y_{12}$ cartridges (Werfen Ltd, Warrington, UK) containing ADP and prostaglandin E_1 (PGE_1) were used with a VerifyNow analyzer and the degree of platelet reactivity in a participant's sample was quantified in $P2Y_{12}$ reaction units (PRU) after automatic analysis.[21] Percentage inhibition was also estimated by the analyzer by comparing PRU with the response in a separate channel containing TRAP.

The effect of ADP on vasodilator-stimulated phosphoprotein (VASP) phosphorylation is specifically mediated by the $P2Y_{12}$ receptor and was measured by median fluorescence intensity (MFI) using VASP kit reagents (BioCytex, Marseille, France) and an Accuri C6 flow cytometer (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA).[22] The degree of $P2Y_{12}$ inhibition was estimated by comparing the MFI achieved with PGE_1 compared with that obtained with PGE_1 plus ADP. The platelet reactivity index (PRI) was calculated by the formula: $PRI = ((MFI_{PGE1} - MFI_{PGE1+ADP})/MFI_{PGE1}) \times 100$.

Thromboxane A_2 (TXA_2) produced from AA is hydrolyzed to the more stable metabolite TXB_2, which can be measured in serum as a marker of cyclo-oxygenase 1 (COX1) activity.[23] Serum obtained from participants' blood was frozen at −80°C and stored prior to analysis. Samples were analyzed with an enzyme-linked immunosorbent assay (EXPRESS ELISA) kit (Cayman Chemical, Ann Arbor, Michigan, USA) in a Multiskan FC plate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with absorbance set at a wavelength of 405 nm. The TXB_2 concentration was determined from a standard curve based on percentage sample absorbance over control absorbance. Results were reported in pg/mL but subsequently converted to ng/mL.

Fibrin clot assessment

The method used for assessment of fibrin clot properties has been validated in previous studies.[24–26] Plasma obtained from participants' blood was frozen at −80°C and stored prior to analysis. In brief, 25 µl plasma samples were added with lysis mix (167 µl 10 µg/mL tissue plasminogen activator, 9.833 ml permeation buffer) and activation mix (27 µl 20 U/mL thrombin, 135 µl 1 M calcium chloride, 5.838 ml permeation buffer). Absorbance was measured in a Multiskan FC plate reader and the fibrin clot parameters recorded were lag time, maximum absorbance and lysis time. Duplicate measurements were performed and results accepted if < 20% difference (lysis time) for the duplicate values. Lag time refers to the time from initiation to exponential increase in absorbance. Maximum absorbance/turbidity is the optical density at which 3 consecutive readings were obtained, corrected for absorbance during lag time. It is a representation of fibrin clot density or turbidity and measured in absorbance units (AU). Lysis time was taken as the time from maximum absorbance until absorbance dropped by 50%.

Statistical analysis

Continuous variables are presented as mean ± standard deviation or median and interquartile range (IQR), as appropriate. Categorical variables are presented as total numbers (proportions) and compared using the chi-square test or Fisher's exact test. The Mann-Whitney U test was used to compare continuous variables. All calculations were performed using IBM SPSS Statistics 29 (SPSS Statistics for Macintosh, Version 29.0, Armonk, New York, USA: IBM Corp) and GraphPad Prism 10 (GraphPad Prism version 10.0.0 for Macintosh, GraphPad Software, Boston, Massachusetts, USA). A P-value <.05 was considered statistically significant. There were no prior data comparing pharmacodynamics of DAT and TAT therefore no sample size calculation was performed. No adjustment was made for multiple testing and so the results are considered exploratory.

Ethical approval

This study obtained ethical approval by the Yorkshire and the Humber – Sheffield Research Ethics Committee (reference no. 20/YH/0106) and the Health Research Authority (Integrated Research Application System project identification: 278733).

Results

Patient characteristics

Thirty-four patients were enrolled in this study; 16 commenced on TAT and 18 commenced on DAT after PCI and throughout the study period. All patients received aspirin before PCI but only patients in the TAT group continued it. In the TAT group, 81% of patients received ticagrelor 90 mg twice daily ($n = 13$) and 19% received prasugrel 10 mg once daily ($n = 3$) while all patients in the DAT group received ticagrelor. The baseline characteristics of patients in the groups were

Table I. Baseline characteristics of the included patients with atrial fibrillation based on antithrombotic therapy after percutaneous coronary intervention.

	DAT (n = 18)	TAT (n = 16)	SMD	P-value
Age at PCI (years)	75 (72–78)	70 (62–78)	0.616	0.126
Male sex, n (%)	17 (94)	15 (94)	0.000	1.000
BMI, kg/m^2	27 (24–30)	29 (27–34)	0.493	0.081
Hypertension, n (%)	13 (72)	10 (63)	0.194	0.545
Diabetes mellitus, n (%)	7 (39)	4 (25)	0.296	0.388
Heart failure, n (%)	5 (28)	5 (31)	0.065	1.000
CKD (eGFR <60 mL/min/1.73 m^2), n (%)	3 (17)	4 (25)	0.204	0.681
Current smoker, n (%)	3 (17)	4 (25)	0.204	0.681
CHA_2DS_2-VASc score	4 (3–6)	3 (2–5)	0.589	0.175
HAS-BLED ≥3, n (%)	3 [17)	2 (13)	0.113	1.000
PRECISE-DAPT ≥25, n (%)	8 (44)	5 (31)	0.266	0.429
Complex PCI, n (%)	4 (22)	6 (38)	0.353	0.457
Type of ACS			0.021	0.934
STEMI, n (%)	7 (39)	6 (38)		
NSTE-ACS, n (%)	11 (61)	10 (63)		
Type of AF			0.230	0.533
Paroxysmal, n (%)	6 (33)	7 (44)		
Persistent, n (%)	12 (67)	9 (56)		

ACS = acute coronary syndrome; AF = atrial fibrillation; BMI = body mass index; CKD = chronic kidney disease; DAT = dual antithrombotic therapy; eGFR = estimated glomerular filtration rate; NSTE-ACS = non-ST-elevation acute coronary syndrome; PCI = percutaneous coronary intervention; SMD = standardized mean difference; STEMI = ST-elevation myocardial infarction; TAT= triple antithrombotic therapy.

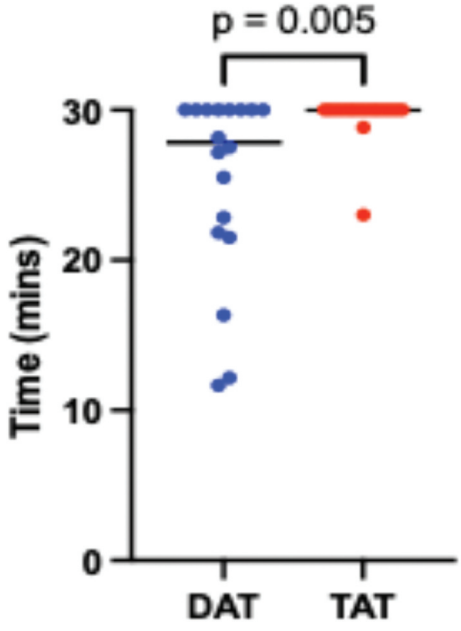

Figure 1. Bleeding time according to antithrombotic regimen group showing individual values and median (black line).

DAT = dual antithrombotic therapy; TAT = triple antithrombotic therapy.

similar although the DAT group had a higher mean age, lower body mass index and higher proportion of diabetes (Table I). Apixaban was the commonest DOAC used (78% and 100% in DAT and TAT groups, respectively), prescribed at a dose of 5 mg twice daily, or 2.5 mg twice daily if patients met licensed dose-reduction criteria (i.e. two or more of the following: age ≥ 80, body weight ≤60 kg and serum creatinine ≥133 µmol/L). Other DOACs used in the DAT group included rivaroxaban at 20 mg once daily (11%), edoxaban at 60 mg once daily (6%) and dabigatran at 150 mg twice daily (6%). No patients were treated with a vitamin K antagonist. There was no difference in DAT and TAT groups between the onset of ACS and timing of the study visit (median 11 vs 10 days, $p = .075$) or in the time between ingestion of antithrombotic therapy and study-related procedures (median 2.8 vs 3.0 hours, $p = .443$).

Bleeding time

Bleeding time was significantly shorter among patients receiving DAT compared with TAT (median 27.8 vs 30 minutes, $p = .005$) (Figure 1). Maximum bleeding time was reached in fewer patients receiving DAT than TAT (44 vs 88%, $p = .013$).

Platelet function testing

The platelet count was similar in DAT and TAT groups (median 223 vs 207×10^9/L, $p = .746$). Comparison of the maximum platelet aggregation responses to various agonists obtained with LTA is displayed in Figure 2. There were no significant differences between the DAT and TAT groups in median responses to 5 and 20 µmol/L ADP (25 vs 27%, $p = .966$; and 37 vs 34%, $p = .284$, respectively) or TRAP (37 vs 24%, $p = .086$). Median platelet aggregation responses were higher with DAT compared with TAT in response to AA (63 vs 3%, $p = .002$), collagen (72 vs 37%, $p < .001$) and 5-HT plus adrenaline (74 vs 45%, $p < .001$).

With the VerifyNow $P2Y_{12}$ assay, patients receiving DAT and TAT had similar platelet reactivity and inhibition (median PRU 12 vs 9, $p = .695$; and median inhibition 94 vs 95%, $p = .825$) (Figure 3). The platelet reactivity obtained from measuring VASP dephosphorylation was also similar between DAT and TAT groups (median PRI 3.9 vs 0.0, $p = .251$) (Figure 4). Serum TXB_2 levels were reduced in patients receiving TAT compared with DAT (median 0.8 vs 114.1 ng/mL, $p < .001$) (Figure 5).

Fibrin clot properties

There were no differences in fibrin clot lag time and lysis time between DAT and TAT groups (median 452 vs 372s, $p = .164$ and median 896 vs 893s, $p = .905$, respectively) (Figure 6A, B). There was also no difference in median clot maximum turbidity between the two groups (0.57 vs 0.58 AU, $p = .986$) (Figure 6C).

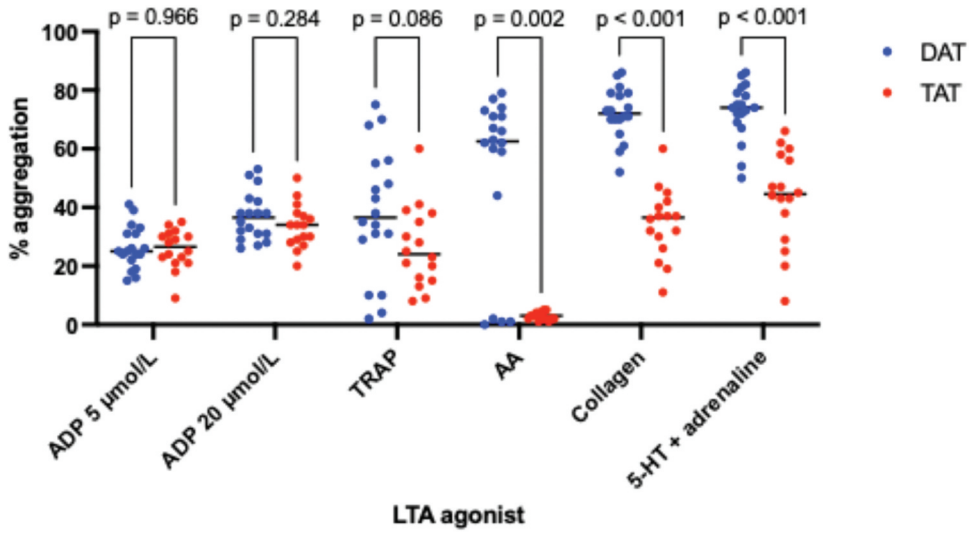

Figure 2. Summary of the maximum platelet aggregation to various agonists at light transmittance aggregometry by antithrombotic regimen group. Data are represented as individual values and median (black line).

5-HT = 5-hydroxytryptamine; AA = arachidonic acid; ADP = adenosine diphosphate; DAT = dual antithrombotic therapy; TAT = triple antithrombotic therapy; TRAP = thrombin-receptor-activating peptide-6.

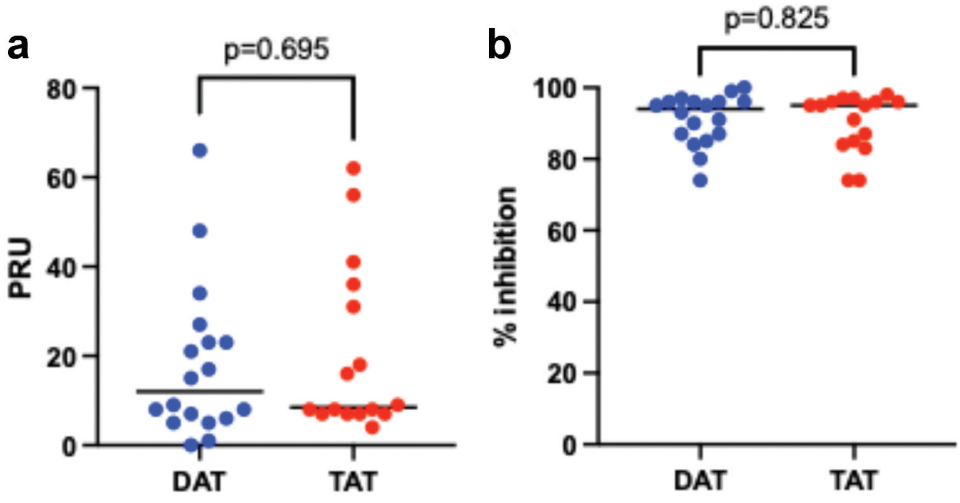

Figure 3. VerifyNow $P2Y_{12}$ assay by antithrombotic regimen group measuring platelet reactivity (a) and degree of platelet inhibition (b). Data are represented as individual values and median (black line).

DAT = dual antithrombotic therapy; PRU = $P2Y_{12}$ reaction units; TAT = triple antithrombotic therapy.

Effect of diabetes status

There was no apparent impact of diabetes status on bleeding time (Supplementary Figure S1), platelet reactivity (Supplementary Figures S2–S4) or serum TXB_2 levels (Supplementary Figure S5).

Discussion

This study highlights pharmacodynamic differences between TAT and DAT in patients with AF and recent ACS treated by stenting so as to better understand the role of aspirin in this context. The main findings in relation to DAT, i.e. the absence of aspirin, are threefold: 1.) a reduction in bleeding time compared with TAT using a $P2Y_{12}$ inhibitor of similar potency, 2.) reduced inhibition of COX1-mediated platelet activation and 3.) similar inhibition of thrombin-related pathways.

Choosing an appropriate antithrombotic regimen for patients undergoing PCI with a concomitant indication for OAC must simultaneously take into account the duration of aspirin and also the type of $P2Y_{12}$ inhibitor to prescribe. Both these factors have important implications for patients' risk of bleeding and ischemic events post-procedure. Meta-analyses of landmark RCTs demonstrated that DAT causes less bleeding than TAT and similar or increased incidence of ACS and stent thrombosis.[27–31] However, both effects may have been driven by the predominant use of clopidogrel, which is known to provide inconsistent $P2Y_{12}$ inhibition, thus potentially exposing some patients to inadequate antiplatelet cover if no longer receiving aspirin.[32–34] There has been limited investigation of DAT with ticagrelor or prasugrel alongside OAC to determine its clinical efficacy, and even less at the mechanistic level. This study utilized an array of tests targeting various thrombotic pathways for this purpose.

$P2Y_{12}$ inhibition was consistently at a high level and similar in both DAT and TAT groups as confirmed by LTA with ADP and

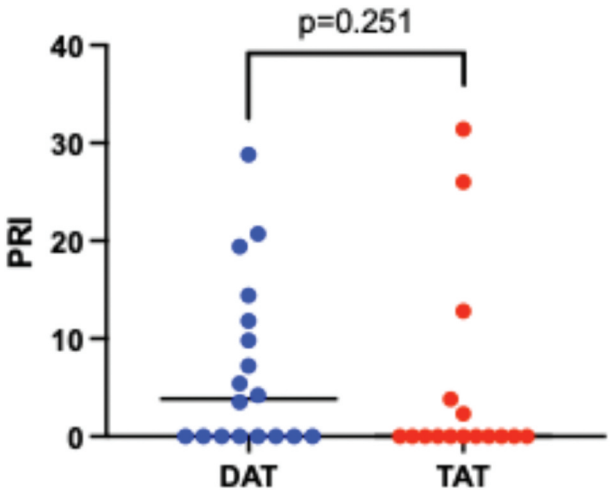

Figure 4. Platelet reactivity on flow cytometry measuring vasodilator-stimulated phosphoprotein (VASP) dephosphorylation by antithrombotic regimen group. Data are represented as individual values and median (black line).

DAT = dual antithrombotic therapy; PRI = platelet reactivity index; TAT = triple antithrombotic therapy.

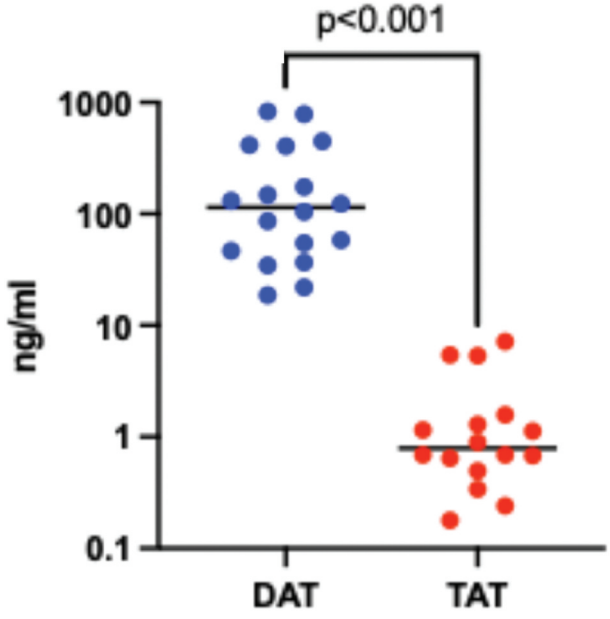

Figure 5. Serum thromboxane B_2 levels in ng/mL by antithrombotic regimen group. Data are represented as individual values and median (black line).

DAT = dual antithrombotic therapy; TAT = triple antithrombotic therapy.

the VerifyNow and VASP assays. This was expected due to the exclusive use of ticagrelor and prasugrel in the study. It is important to note that TAT with ticagrelor or prasugrel is chosen by some PCI operators at our center in the ACS setting despite not being recommended by international guidelines due to increased bleeding risk, albeit for no longer than 1 week. The consistent $P2Y_{12}$ inhibition observed with ticagrelor is supported by larger pharmacodynamic studies.[32,33,35]

The main differences in platelet reactivity between DAT and TAT were observed in AA- and collagen-induced platelet aggregation via LTA and thus reflective of the absence or presence of aspirin. A greater response to 5-HT plus adrenaline was similarly observed in the DAT group, which may be explained by the use of citrate anticoagulation since this lowers divalent cation levels and is known to cause artefactual TXA_2 release in response to stimulation with adrenaline.[36,37] Aspirin irreversibly binds to COX1 and inhibits the conversion of AA to TXA_2, a potent platelet agonist which, under normal conditions, is itself metabolized to the more assayable TXB_2.[23] Aspirin is clearly a strong inhibitor of the AA-TXA_2 pathway but assessment of maximum platelet aggregation by LTA in these samples must also take into account the potential confounding effects of other agonists such as ADP released from platelet dense granules and the presence of their inhibitors.[38] Measurement of serum TXB_2 levels represents the most reliable way to assess the specific effect of aspirin. 4 patients in the DAT group demonstrated negligible AA-induced platelet aggregation by LTA, which may at least partly relate to inhibition of $P2Y_{12}$-mediated amplification of the response to TXA_2 but has also been reported in other studies on healthy donors not receiving aspirin or other non-steroidal anti-inflammatory agents.[39–41] All patients in the DAT group, however, had high levels of serum TXB_2, confirming the true absence of aspirin activity at the time of sampling.

Collagen in the subendothelium acts as both a substrate for platelet adhesion and as a potent agonist for platelet activation via the GP VI receptor.[42–44] At relatively low collagen concentrations, a substantial proportion of the induced platelet activation is mediated by the release of AA and the inhibitory effect of aspirin is clearly demonstrated despite the presence of a $P2Y_{12}$ inhibitor and a DOAC in this study.[45] Despite some evidence suggesting that DOACs may have an additive inhibitory effect on GP VI-mediated platelet activation and TXB_2 levels, this study's results emphasize that aspirin is still the predominant inhibitor of this pathway.[46–48] Thrombin is also a potent platelet agonist that activates protease-activated receptor (PAR)-1 by cleaving the amino-terminal extracellular domain to create a new terminus that acts as a tethered ligand for transmembrane signaling.[49] TRAP somewhat simulates these effects by mimicking the tethered ligand itself for PAR-1 activation and so, while it can be considered reflective of thrombin-mediated platelet activation, it is not a direct substitute for thrombin.[50] Therefore, TRAP-induced platelet aggregation cannot reliably assess the antiplatelet effect of DOACs and mixed results have been observed in other studies.[48,51–53] This study demonstrated no difference in TRAP-induced platelet aggregation between DAT and TAT groups, suggesting the lack of a significant inhibitory effect of aspirin.

Viewed together, these platelet function test results exhibit similarities to those obtained by other studies investigating the pharmacodynamics of $P2Y_{12}$ monotherapy versus DAPT i.e. without OAC. The GLOBAL LEADERS and TWILIGHT platelet sub-studies and the TEMPLATE study all reported significantly increased AA- and collagen-induced platelet aggregation with ticagrelor monotherapy compared with DAPT but no change in response to ADP and TRAP.[54–56] Galli et al conducted two small pharmacodynamic studies on antiplatelet and OAC combinations, one comparing DAPT (aspirin and clopidogrel), aspirin plus very-low-dose rivaroxaban and DAPT plus very-low-dose rivaroxaban, and another comparing DAPT with $P2Y_{12}$ inhibitor plus very-low-dose rivaroxaban.[57,58] AA- and collagen-induced platelet aggregation were once again increased in regimens without aspirin. However, TRAP-induced platelet aggregation was increased in the absence of clopidogrel in the first study and in the absence of aspirin in the second. It is unclear whether these results contrast with our study due to the lower dose of rivaroxaban, the higher concentration of TRAP or other factors. So far, there has been one platelet study investigating DAT and TAT after PCI, derived from a Greek nationwide registry that looked at platelet reactivity in response to ADP using VerifyNow and

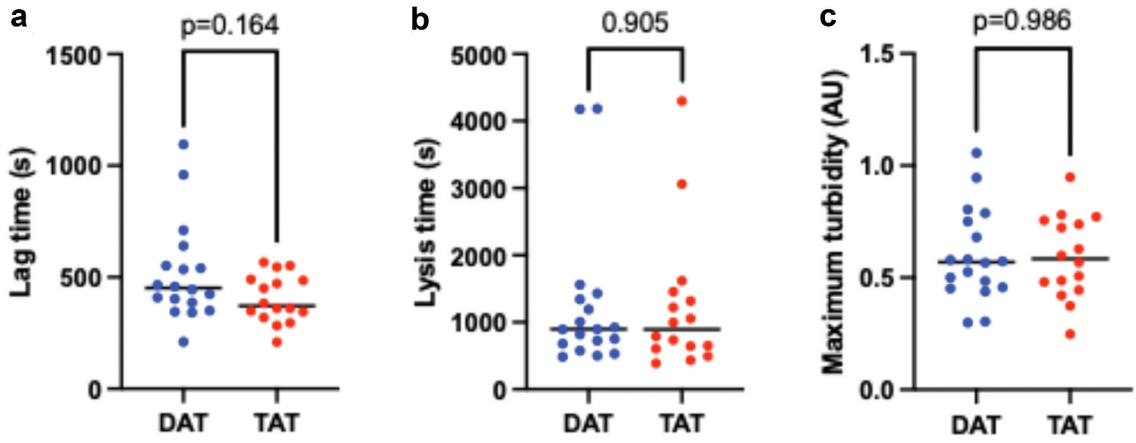

Figure 6. Fibrin clot properties by antithrombotic regimen group: clot lag time (a), clot lysis time (b) and clot maximum turbidity (c). Data are represented as individual values and median (black line).

AU = absorbance units; DAT = dual antithrombotic therapy; TAT = triple antithrombotic therapy.

found no difference between DAT and TAT or between OAC agents when using the same $P2Y_{12}$ inhibitor.[35]

In order to provide a more comprehensive antithrombotic assessment not limited to platelet function testing, fibrin clot parameters were also measured. Impaired fibrin network architecture and impaired clot breakdown (hypofibrinolysis) are known to be associated with adverse cardiovascular outcomes.[24,59,60] Furthermore, aspirin, vitamin K antagonists and DOACs increase clot porosity and lysis time when investigated separately.[61–64] On a background of DAPT, clot lag time was increased with the addition of dabigatran or very-low-dose rivaroxaban in separate studies.[57,65] In this study, which is the first to compare fibrin clot parameters between DAT and TAT, no difference was observed in clot lag time, clot lysis time or clot turbidity. This would suggest minimal contribution from aspirin in the presence of a $P2Y_{12}$ inhibitor and a DOAC and complements the data from Galli et al demonstrating reduced thrombin generation (including increased lag time) with a $P2Y_{12}$ inhibitor and very-low-dose rivaroxaban compared with the same $P2Y_{12}$ inhibitor and aspirin.[58]

Bleeding time in patients receiving DAT was reduced compared with patients receiving TAT. The test was stopped at 30 minutes for pragmatic reasons but the majority of patients in the TAT group continued to bleed up until this point and possibly would have carried on for a lot longer. Limited quantitative difference between groups can therefore be inferred from the test in this study, and correlation with other platelet function tests or fibrin clot properties cannot be performed appropriately. Nevertheless, the results obtained are indicative of an additional anti-hemostatic effect of aspirin on top of $P2Y_{12}$ inhibition and OAC, which could be attributed to the aforementioned platelet activation pathways involving collagen and AA.

This study identified thrombotic pathways in which aspirin is involved to a greater or lesser extent. The avoidance or withdrawal of aspirin straight after PCI for ACS in patients with AF appears to reduce bleeding by largely sparing the TXA_2 pathway of platelet activation. However, in patients receiving DAT comprising of ticagrelor or prasugrel and a DOAC, consistent $P2Y_{12}$ inhibition is achieved and the inhibitory effects on fibrin clot generation and sustainability are no different than with aspirin also on board. From a hemostatic perspective, DAT with ticagrelor may, on average, carry similar bleeding risks to DAPT with ticagrelor and aspirin while TAT with ticagrelor likely carries excessive risk of bleeding. From an ischemic perspective, targeting both platelet and protein pathways with a DAT regimen that excludes aspirin but includes potent, consistent $P2Y_{12}$ inhibition may provide sufficient antithrombotic cover for important events in the precarious post-ACS period such as stroke and stent thrombosis, especially in the presence of modern drug-eluting stents. Pharmacodynamic studies like this one lay the groundwork for further investigation of DAT with ticagrelor or prasugrel as a potentially effective and relatively safe regimen to be used straight after coronary stenting for ACS.

Several limitations were present in this study. First, despite its prospective nature, the antithrombotic regimen was not randomized but instead decided by the patients' clinical team. This led to the study being more representative of contemporary practice at our center than of conventional, guideline-recommended practice of TAT with clopidogrel. Nevertheless, the main aim of the study was to investigate pharmacodynamics of aspirin removal and so maintaining relatively consistent $P2Y_{12}$ inhibition throughout allowed for fairer comparison of DAT and TAT in this regard. Most patients were also on apixaban and so differences between DOACs could not be assessed. There was a higher proportion of patients in the DAT group with diabetes mellitus, which is associated with increased platelet turnover and could have affected results.[66] However, we measured platelet function at peak rather than trough effect to reduce the impact of diabetes. Furthermore, we have previously found that diabetes status does not impact on the consistent antiplatelet effect of ticagrelor and, in the present work, found similar evidence of lack of impact of diabetes on platelet reactivity.[67] With regards to the bleeding time test, the limitations arising from capping at 30 minutes have already been discussed, mainly that the observed difference in bleeding between DAT and TAT could not be fully appreciated quantitatively. The number of patients who reached maximum bleeding time was analyzed to provide a semi-quantitative method of assessment. In addition, the test could have been subject to performance bias; however, it was carried out in front of an independent witness who was blinded to the patients' treatment. Finally, this was a pharmacodynamic study on a small number of patients and so the results are merely intended to provide pilot data for future research.

Conclusions

The use of DAT without aspirin improves hemostasis through sparing of COX1-mediated platelet activation but has a similar effect on other pathways and fibrin clot properties compared with TAT using $P2Y_{12}$ inhibitors of consistent potency. DAT with ticagrelor or prasugrel, combined with a DOAC, may provide

sufficient antithrombotic effect without excessive anti-hemostatic effect but this merits further investigation in larger studies.

Disclosure statement

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Supplementary material

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