Changes in platelet function independent of pharmacotherapy following coronary intervention in non-ST-elevation myocardial infarction patients

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Tables 2, Figures 6 included as separate files

HIGHLIGHTS

- NSTEMI patients treated with chronic clopidogrel (at least 5 days) with no changes to pharmacotherapy
- Predictable & large reduction in ADP aggregation 24hrs post-PCI using bedside platelet function test (PFT)
- 12-HETE, soluble and platelet bound p-selectin elevated at 24hrs post-PCI
- Low ex-vivo ADP signal likely indicates dysfunctional "spent" platelets
- Suggests PFT should not be used at 24 hours post-PCI to inform changes in therapy
- Majority of negative tailored therapy trials use post-PCI testing in protocols

ABSTRACT

Background

High on treatment platelet reactivity (HTPR) is common in patients receiving clopidogrel following an acute coronary syndrome (ACS); it's also associated with increased morbidity and mortality. More potent and predictable antiplatelet drugs have addressed this issue at the expense of increased bleeding. Identification of HTPR and the targeted use of more potent antiplatelet drugs has, so far, broadly failed. We investigate this approach in terms of the timing of platelet function testing and how this can impact on the ability of these bedside tests to predict HTPR around the time of coronary intervention.

Methods

High risk ACS patients treated with 5 days of clopidogrel had platelet function assessed using the multiple electrode aggregometry system (MEA) pre, post and 24 hours following percutaneous coronary intervention (PCI). Simultaneous detailed analysis of platelet status was undertaken with quantification of platelet bound and soluble p-selectin and mass spectrometry quantification of the eicosanoid 12-HETE.

Results

As assessed by MEA 40.5% of patients had HTPR pre-PCI; mean aggregation units (AU) in response to ADP were 499.1±46.3 pre-PCI, 407.6±37.7 post-PCI and 269.1±24.6 AU 24 hrs post-PCI (pre to post PCI $p>0.05$, pre to 24 hrs post-PCI $p=0.0002$). This highly significant drop in platelet reactivity was contrasted with on-going expression of platelet bound p-selectin, increased soluble p-selectin and rising 12-HETE concentrations.

Conclusions

This study outlines significant changes in *ex-vivo* platelet aggregation that occur within 24 hours of PCI in high risk NSTEMI patients using bedside PFT. Whilst there were no changes in antiplatelet therapy during the study period its clear that timing is crucial when assessing high on treatment residual platelet activity.

Key words:

Platelet function testing, residual platelet reactivity, acute coronary syndrome, clopidogrel

Abbreviations:

NSTEMI - Non ST Elevation Myocardial Infarction

PCI - Percutaneous Coronary Intervention

HTPR - High on treatment platelet reactivity

PFT - Platelet function testing

INTRODUCTION

Antiplatelet therapy with clopidogrel has been shown to confer a significant morbidity and/or mortality benefit in the prevention of ischaemic complications in patients with Non-ST Elevation Myocardial Infarction (NSTEMI)(1) and/or after percutaneous coronary intervention (PCI). (2)

High on treatment platelet reactivity (HTPR) in patients receiving clopidogrel has been identified as a risk factor for stent thrombosis and myocardial infarction and is inversely related to bleeding risk.(3, 4) More potent and predictable platelet inhibition using newer antiplatelet agents (prasugrel and ticagrelor) in NSTEMI patients have shown clinical benefit at the expense of increased rate of bleeding. (5, 6) Therefore, clopidogrel therapy remains a valid alternative in NSTEMI patients with a high bleeding risk. Identifying patients with HTPR on clopidogrel may, therefore, allow enhanced tailored therapy and offer a potential solution to the issue of balancing increased platelet inhibition, and its associated complications, against increased bleeding risk.

Nevertheless, large multi-centre double-blind randomised controlled trials have, to date, failed to show a benefit of identifying these patients and adjusting anti-platelet therapy, (7-10)the “tailored therapy” approach. However, a number of smaller trials have indicated that with alternate trial designs tailored therapy can reduce stent thrombosis and re-infarction as well as bleeding complications.(11-13) Notably, there appear to be significant methodological heterogeneity in those trials, including patient cohort selection (in terms of cardiovascular risk), timing of platelet function testing (PFT), cut-off values used to define HTPR, drug regimen combinations, and follow-up. The inclusion of low risk and stable patients seen in TRIGGER-PCI limited the effectiveness of PFT and switching to high-potency antiplatelet agents, primarily due to the extremely low event rates seen in these cohorts. (8, 14) Furthermore, use of higher fixed doses of clopidogrel and lack of follow-up PFT may suggest that a potentially significant proportion of patients in the “tailored arm” (~ 40%) retain a HTPR but remain unidentifiable, confounding follow-up comparisons. (15,

16)Extended testing or a switch to prasugrel or ticagrelor may be required to ensure such patients are fully treated.

All of the above trials have been inconsistent with regards to the timing of PFT relative to patient management and/or follow-up. GRAVITAS, (7) TRIGGER-PCI(8) and ADAPT-DES(3) trials all used post procedural PFT within – and usually at – 24 hours. ARCTIC(9) and MADONNA(11) trials both principally used pre-procedural PFT. However, there are no studies to date that assessed the impact of Percutaneous Coronary Intervention (PCI) on PFT independently of any changes in pharmacotherapy. The timing of PFT relative to PCI may have a profound impact on the measurement of HTPR, confounding the investigated effect of pharmacotherapy and rendering interpretation challenging.

We believe that one of the major limitations to many of the previous tailored therapy trials is the lack of attention to the timing of platelet function testing. In the context of a single measure of platelet reactivity this can mislead clinicians away from the true *in-vivo* status of circulating platelets and lead to false assumptions and treatment decisions.
We hypothesised that platelet function - as measured using bedside PFT - changes independently of pharmacotherapy in the 24-hour period after PCI and by analysing other detailed markers of platelet status we will highlight the dangers of making interpretations of bedside platelet function tests in this period.

MATERIALS AND METHODS

We conducted a prospective observational study on high-risk NSTEMI patients who received clopidogrel at diagnosis and for a minimum period of 5 days before PCI was conducted (see below). Various aspects of platelet function were assessed using Multiple Electrode Aggregometry (MEA), Electrochemiluminescent (ECL) assay, Flow-cytometry and Mass Spectrometry.

Statistical methods

Confidence intervals of 95% with an power of 0.8 were used throughout. Each dataset was tested for normal distribution using the Shapiro-Wilk normality test. Continuous variables with normal distribution are presented in tabular form as mean ± SD, whereas non-normally distributed variables are presented as median with interquartile range. Multiple comparisons

of normally distributed data were made with a one-way paired ANOVA with Tukey's correction. For single comparisons a paired two-tailed student t-test was used. When data sets were not normally distributed multiple comparisons were tested with Fieldman's test with Dunn's correction, for single comparisons the Wilcox test was used for paired data and the Mann-Whitney test for unpaired analysis.

We had pilot data for ADP induced aggregation only; sample size was calculated to show a clinically relevant change in ADP induced aggregation between pre-PCI and 24 hours post-PCI. The threshold between those patients with and without HTPR was taken as 500 AU therefor a power calculation enabling detection of a mean difference of 100 AU between timepoints would capture significant numbers traversing this clinically relevant threshold. Using a 2-sided alpha of 0.05 and 80% power and an observed SD of 149; 35 patients were required to detect this difference.

Study Patients

Inclusion criteria were patients with an index admission following an NSTEMI (troponin positive and / or typical ECG changes with consistent clinical history), and a GRACE score >140 who subsequently went on to have significant disease treated by PCI and stent implantation. Exclusion criteria included any patient receiving intravenous antiplatelet agents (glycoprotein IIb/IIIa inhibitors (GPI) or other) and abnormal renal function (eGFR <70mls/min). Local research ethics approval was granted and written informed consent was obtained from each patient.

All patients received a loading dose of aspirin (300mg) and a maintenance dose of 75mg once daily initiated at the same time as the clopidogrel unless this was already taken prior to their hospital admission. To exclude any influence of variable clopidogrel loading only patients that had received a 600mg loading-dose followed by a minimum of 5 days of maintenance therapy (75mg OD) were recruited. (17) Timing of dose was also corrected for by ensuring the third sampling point was exactly (+/- 1hr) 24 hours after the baseline measurement and drugs received at the same time each day.

All patients received a weight-adjusted dose of intravenous unfractionated heparin 70 – 100 Units/kg bolus pre-procedurally. All blood samples were taken from the same large calibre venous sheath in the antecubital fossa and 3 samples collected in the 24-hour period (immediately pre- and post-PCI and 24hrs post-PCI). Samples were collected into a depressurised vacutainer (hirudin for MEA, citrate for flow cytometry, EDTA for mass

spectrometry and ELISA). The 24 hour sample was taken exactly at 24 hours (+/- 1 hour) and the morning dose of clopidogrel was administered at 8am on both days to ensure there a was no influence of dose timing on test results. The PCI itself was carried out with no restrictions and as per the operator preference. Operators were blinded to the results of the platelet function testing.

Impedance aggregometry

Whole blood aggregation was determined using Multiple Electrode Aggregometry (MEA), Multiplate Analyzer (Roche Diagnostics), which detects the change in electrical impedance due to the adhesion and aggregation of platelets on two independent electrode-set surfaces in the test cuvette. As per the manufacturer's recommendations, hirudin and Adenosine Diphosphate (ADP) were used as the sample anticoagulant and agonist respectively. A 1:2 dilution of whole blood and 0.9% NaCl was stirred at 37 °C for 3 min in the test cuvettes, ADP was added (final concentration 6.5 μM) and the increase in impedance was recorded continuously for 6 min. The process was repeated using Thrombin Receptor Activating Peptide 6 (TRAP-6), (final concentration 32 μM), in order to assess platelet aggregation independently of clopidogrel. Each test cell generates two independent values, which are averaged and expressed as the area under the curve of the aggregation tracing (AUC). Consequently, manufacturer's software performs a Pearson's correlation coefficient on the two measurements and the result is rejected if the coefficient is <98% or the difference to the mean curve is >20%. AUC was reported as arbitrary units and a cutoff of 500 U was defined as the threshold above which a patient was characterised as having HTPR ("non-responder"), according to the mean of two previously proposed cut-offs. (4, 18)Following a resting period of 20 minutes trained laboratory technicians processed the samples.

Flow cytometry assessment of Platelet bound P-selectin

Sample preparation:

20μl of whole blood was initially diluted in 50μl Tyrodes buffer (137mM NaCl, 2.68mM KCl, 1mM $MgCl_2$, 1.8mM $CaCl_2$, 0.2mM Na_2HPO_4, 12mM $NaHCO_3$, pH7.4) and subsequently incubated in 10μl fluoresceine-isothiocyanate-(FITC)-mount-Anti-Human CD61, 20μl of allophycocyanin-(APC)-Mount-Anti-Human CD62P antibody or (APC)-Mount-IgG1 isotype control (BD Biosciences, Pharmigen™) and incubated at room

temperature for 15 min. Incubation was terminated by fixation of 20µl of the reaction mixture to 1% paraformaldehyde. Samples were diluted four-fold in Tyrodes buffer prior to analysis. The Anti-CD61 antibody is targeted against the constitutively expressed IIIa domain of the GpIIb/IIIa receptor and identifies resting and activated platelets (19, 20).

Sample analysis:

The samples were analysed by CyAn™ ADP-Analyzer (Beckman Coulter) on the same or the following day. Platelets were initially identified and gated at 50,000 events according to their side and forward scatter characteristics and consequently gated for CD61 positivity. Samples containing non-specific IgG1-APC antibodies were used as a negative control and to set an analysis marker so that 99.9% of the total population of platelets to be defined as negative. Platelets with fluorescence intensity higher that this value for anti-CD62P-APC antibody were defined as P-selectin positive and were expressed as a percentage of positive platelets compared to the total CD61 positive population. To correct for fluctuations of total platelet count at the different time-points, the ratio of platelet to total cells in the pre-PCI sample was used to correct the number of CD61 positive platelets in the subsequent time-point measurements.

Soluble P-selectin quantification

MSD Human Ultra-Sensitive P-selectin ECL assay (MSD: Meso Scale Discovery, Gaithersburg, MD) was used as per manufacturers recommendations. A human anti-P-selectin coated MULTI-ARRAY 96-well small spot plates was initially blocked with bovine albumin followed by addition of serum samples and detection antibody. P-selectin calibration standards were run in duplicate on the same plate under the same conditions. Following incubation and washing the Read buffer was added and the 96 well plate was analysed on an ImageSector 6000. Results were analysed using MSD workbench software. Samples were run as duplicates, and measurements with coefficient of variation less than 15% were considered acceptable.

Assessment of 12-HETE by lipid extraction and reverse phase LC/MS/MS

Lipid Extraction

For analysis of lipids in human plasma, 5 ng of 12-HETE-d8 were added to the samples before extraction, as an internal standard. Lipids were extracted by adding a solvent mixture (1 mol/L acetic acid, isopropyl alcohol, hexane (2:20:30, v/v/v)) to the sample at a ratio of 2.5

ml to 1 ml sample, vortexing, and then adding 2.5 ml of hexane. (21) After vortexing and centrifugation, lipids were recovered from the upper hexane layer. The samples were then re-extracted by addition of an equal volume of hexane. The combined hexane layers were dried and the aqueous layer was re-extracted by adding 3.75 ml of a chloroform and methanol mixture (1:2, v/v), vortexing, then adding 1.25 ml chloroform. After vortexing, 1.25 ml water was added and the mixture was vortexed again and centrifuged. Lipids were recovered in the chloroform layer,(22) and dried; the combined lipid extracts were consequently analysed for free 12-HETE using LC-MS/MS as described below.

Reversed phase LC/MS/MS of free HETE.

For 12-HETE quantitation lipids were separated on a C18 Spherisorb ODS2, 5 μm^{-1}, 150 x 4.6 mm column (Waters, Hertfordshire, UK). The mobile phase was composed of water: acetonitrile: acetic acid (75:35:0.1, solvent A) and methanol: acetonitrile: acetic acid (60:40:0.1, solvent B), with flow rate 1 $ml.min^{-1}$. Solvent B was increased from 50 % to 90 % over 10 min, held for 20 min, then returned to 50 %.(21) MS was performed using a Sciex 4000 Q-Trap, using DP -85V, CE -20V and a dynamic fill time monitoring the parent to daughter m/z (m/z 319.2 to 179.1, m/z 327.2 to 184.1 for 12-HETE or 12-HETE-d8, respectively). 12-HETE was identified and quantified using 12-HETE and 12-HETE-d8 standards run in parallel under the same conditions.

RESULTS

A total of 74 ACS patients were consented for the study; however 30 were excluded as they did not undergo PCI following coronary angiography. Two patients were further excluded due to peri-procedural glycoprotein IIb/IIIa inhibitor (GPI) use. The remaining 42 patients completed the study in full with no peri-procedural major complications. Baseline characteristics of the study group (including pre and 24 hr post-PCI Hb and platelet count) can be seen in table 1.

Overall, there was a consistent and significant decrease in platelet reactivity in the first 24 hours after PCI (Figure 1 and Table 2). The mean value of platelet aggregation pre-PCI was 499.1 AU (± 46.3), with 40.5% (17 patients) exhibiting HTPR. Immediately post PCI, this value decreased to 407.6 AU (±37.7) translating to 26.2% (11 patients) with HTPR ($p > 0.05$). At 24 hours post-procedure, platelet aggregation decreased to 269.1 AU (±24.6) further reducing the number of patients who appeared to have HTPR to 4.7% (2 patients). The mean change in platelet aggregation from Pre-PCI to 24 hrs Post-PCI was 230.0 AU (CI 104.5 to 355.6, p=0. 0002^{ANOVA}). (Figure 1 and Table 2)

In patients with HTPR the mean reduction in ADP induced aggregation from Pre-PCI to 24 hrs Post-PCI was 379.1 AU (52%) (CI 255.9 to 502.2, p=0.0001). In comparison, patients without HTPR had a proportionately smaller reduction of 98.6 AU (33.5%) over 24hours (CI 23.04 to 174.1, p=0.0028).

Baseline pre-PCI samples were collected before heparin administration in 32 out of the 42 patients. There was no significant difference between pre-PCI measurements collected before and after heparin administration; thereby demonstrating that heparin did not confound ADP-induced platelet aggregation. (Pre heparin mean 476.2 AU SEM 68.08, Post heparin mean 520.3 AU SEM 66.49. p=0.1795 CI -22.6 to 110.1).

The changes in ADP-induced platelet aggregation illustrated in Figure 1 were reflected to a lesser extent with TRAP as the agonist for aggregation. The mean value of aggregation pre-PCI was 1000 AU (±51.6) reducing to 843.8 AU (±42.1) post-PCI ($p < 0.05$). At 24 hours post-PCI, aggregation further decreased to 792.0 (±41.0) equating to a mean reduction from Pre-PCI to 24 hrs Post-PCI of 208.4 AU (20.8%) (CI 114.1 to 302.6, p=0.0002 ^{ANOVA}). (Figure 2 and Table 2)

Platelet aggregation pre- and post-PCI changed to a greater extend in patients with HTPR compared to those without HTPR (Figure 1,2 and Table 2). Notably, in patients with HTPR, TRAP-induced aggregation had a similar mean numerical reduction as in patients without HTPR (214.1 AU vs 203.9 AU respectively). Individually these reductions were both

statistically significant. However, between groups, comparisons did not differ significantly in relation to the initial absolute pre-PCI value.

Despite a significant reduction in both ADP and TRAP induced aggregation we saw a consistent and significant rise in soluble P-selectin (4 ng/ml increase, CI 0.82 to 10.0, p=0.0225) between pre-PCI and 24hrs post-PCI (Table 2), mainly in the HTPR group (Figure 3). Overall the consistent levels of P-selectin found across all time-points suggest ongoing platelet activation in the face of a reduction in ADP and TRAP induced aggregation ex-vivo. In addition, ADP-induced platelet aggregation pre-PCI positively correlated with 24 hours post-PCI P-selectin levels (Pearson r 0.391, CI 0.071 to 0.64, p=0.0185), (Figure 6), suggesting that high baseline platelet reactivity pre-procedurally predicts aggregation after 24 hours. There is no correlation when we compare 24hr post-PCI ADP induced aggregation to the same 24 hr post-PCI P-selectin concentration.

Since soluble P-selectin in plasma may be derived from endothelial cells as well as platelets, (23) we measured platelet bound P-selectin in a random population of the study's cohort (n=16). An overall increase in platelet bound P-selectin between pre-PCI and 24 hours post-PCI suggests that the soluble P-selectin measured may originate from platelets (Figure 3 and 4). Overall, pre-PCI, 3.3% (±0.6) of platelets express P-selectin rising to 4.5% (±1.6) immediately post-PCI. At 24 hours post PCI, 4.8% (± 1.4) of platelets continue to express P-selectin. Whilst these numerical changes do not reach significance the importance of ongoing expression of platelet bound P-selectin in this context should be noted.

Specifically in patients with HTPR, platelet bound P-selectin changed non-significantly during the 24 hours period (4.6% pre-PCI and 5.3% 24 hours post-PCI), further suggesting on-going platelet activation during that time. (Table 2, Figure 4)

12-Hydroxyeicosatetraenoic Acid (12-HETE) is produced by platelet 12-lipoxygenase metabolism of arachidonic acid and appears to be both a marker and mediator of platelet aggregation. (24) In patients with HTPR, levels of 12-HETE increased by 0.55 ng/ml over the 24 hour period post-PCI (CI 0.08 to 1.0, p=0.0248). On the other hand, in patients without HTPR, levels of 12-HETE decreased by 0.1 ng/ml over the same period (CI -0.19 to -0.01, p=0.0302) (Figure 5)

CONCLUSION

This study outlines significant changes in *ex-vivo* platelet aggregation that occur within 24 hours of PCI in high risk NSTEMI patients using bedside PFT. Whilst there were no changes in antiplatelet therapy during the study period its clear that timing is crucial when assessing high on treatment residual platelet activity.

Along with this reduction in *ex-vivo* platelet aggregation we have presented evidence of ongoing platelet activation with increased serum P-selectin indicating α-granule secretion and expression of P-selectin on the platelet membrane, which is consequently cleaved and released in plasma as a free molecule. (25)Ongoing activation of platelets is also confirmed by the continued expression of platelet bound P-selectin and significant elevation of the eicosanoid 12-HETE, which is both secreted by and activates platelets per se.(24, 26) These markers of platelet activation are seen to greatest extent in those patients defined as having HTPR at baseline (pre-PCI) using ADP induced aggregation.

This ongoing platelet activation and loss of ADP induced aggregation is well documented in patients during and following cardiopulmonary bypass (CPB), where this ADP aggregation defect is seen simultaneously with increased expression of p-selectin measured by flow cytometry. (27) A median of 89 minutes of CPB is clearly more traumatic to platelets than PCI and this is reflected in the very large increases in platelet bound p-selectin (7% at baseline to 29% at the end of CPB). We believe our observed aggregation defect is a smaller version of that seen during CPB and is secondary to the procedure itself and potentially activation by the implanted stent(s). This is the first study that raises the possibility of this phenomenon being present post coronary stenting as a potential stimulus for ongoing platelet activation, which may reflect their "near-maximal" activation *in situ* and consequent inability to contribute further to the *ex vivo* aggregation required by PFT assays.

Unfortunately we were not able to collect samples beyond 24 hours, clearly later samples would help us to understand optimal timing for post-PCI measurement of platelet function. The surgical cohort with CPB (27)exhibited recovery in the ADP aggregation defect at 18 hours with ADP aggregation approaching baseline levels (baseline 7%, 18 hrs 11%). This

surgical data suggests that recovery may be occurring back to baseline within three days however the Alfredsson study (28)suggests that ex-vivo aggregation may still be defective beyond 3 days. Clearly longer term analysis needs to be undertaken with careful follow-up testing beyond the 24 hour period in a further study or during the next wave of clinical trials.

The data presented supports the hypothesis that the observed flux in response to ADP – rather than being related to the type of bedside PFT device used – is the result of ongoing platelet activation occurring during the PCI and 24 hours following the procedure. This persistent activation is reflected by the ongoing expression of p-selectin on the platelet membrane with cleavage of this molecule forming soluble p-selectin. Indeed higher levels of pre-PCI ADP induced aggregation are a very good predictor of ongoing platelet activation at 24 hours as reflected by its positive association with soluble P-selectin levels at 24 hours post-PCI, a correlation that is lost when using 24hr Post-PCI ADP-induced aggregation. (Fig 6)

We have made attempts to exclude any confounding periprocedural drug effects in the methodology. In particular we have enrolled patients following loading and at least 5 days maintenance of clopidogrel in order to exclude cumulative clopidogrel response that occurs with acute periprocedural loading. The influence of heparin on platelet aggregation has been quantified and shown to be non-significant.

The 24 hour post-PCI ADP induced aggregation suggests that only 4.7% of patients had HTPR, if this is compared to other studies using the same bedside PFT apparent HTPR is much lower in our group. (29-31) However, it's clear that the first two use pre-PCI PFT and the degree of HTPR agrees with our data. The third by Aradi et al, does indeed use post-PCI PFT and the rates of HTPR are much higher post-PCI. However, it should be noted that the vast majority of patients (97%) received an acute loading dose of clopidogrel (600mg) just prior to the PCI and a further unspecified number also received the glycoprotein inhibitor tirofiban with further delay in PFT measurement. It's likely that these two important factors protect from the ongoing platelet activation we see at 24 hours as reflected in the low ADP signals with corresponding raised markers of platelet activation. Our pre-PCI platelet aggregation tests show the study population had a high prevalence of HTPR (40.5%), this compares to a lower value in a recent study by Alfredsson et al (28)in which 23% of patients not treated with abciximab had HTPR 6 – 8 hours after loading with 600mg of clopidogrel. The Alfredsson study was an analysis of platelet function in relation to a loading dose of

clopidogrel that did not take into account the PCI itself, a different emphasis to this study which focused on the PCI as the index event. The reasons for the lower rate of HTPR are likely to be a combination of the pharmcodynamic effects of a large loading dose (rather than chronic clopidogrel kinetics pre-PCI) and the timing of the PCI itself causing the same aggregation defect described in this study (the study does not contain data on the timing of the PCI but presumably a substantial number of patients had PCI shortly after loading). The variability in platelet function described by Alfredsson could easily (in part) be attributed to the mechanism outlined in this study.

This study demonstrates the influence of timing of peri-procedural PFT in NSTEMI patients and may indicate an important factor in the design of future tailored therapy trials. Whilst pre-PCI PFT appears more useful in the identification of patients with baseline HTPR and ongoing platelet activation, testing at 24 hours post-PCI seems highly unpredictable in terms of demonstrating ex-vivo residual platelet activity.

Therefore, post-PCI, and especially 24 hours Post PCI, bedside PFT should be used with extreme caution in terms of guiding tailored therapy either on an ad-hoc basis or in future clinical trials in NSTEMI patients. This approach is *unlikely* to differentiate between those patients without HTPR and those with HTPR who may be targeted with more potent antiplatelet agents.

Upcoming, multicentre, double blind randomised controlled trials ANTARTIC and TROPICAL-ACS (NCT01959451, NCT01538446), both employ post-PCI PFT remote from the initial PCI at 14 days and 7 days respectively, steering clear of the highly labile period during the first 24 hours post-PCI described in this paper. Whilst we have no direct evidence of this being superior to peri-procedural testing it seems a sensible strategy to test as remote as possible from this acute phase; unlike the majority of trials preceding them. These trials have also selected a high-risk patient cohort and both have focused on a novel de-escalation strategy that focuses on reduced major bleeding. The results are eagerly anticipated.

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Conflicts of interest

None of the authors have any conflicts of interests

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