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# Clopidogrel discontinuation and platelet reactivity following coronary stenting.

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## **Original Article**

### **Title**

Clopidogrel Discontinuation and Platelet Reactivity following Coronary Stenting

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## **Abstract**

### **Aims**

Antiplatelet therapy with aspirin and clopidogrel are recommended for 1 year after drug-eluting stent (DES) implantation or myocardial infarction. However, the discontinuation of antiplatelet therapy has become an important issue as recent studies have suggested a clustering of ischaemic events within 90 days of clopidogrel withdrawal. The objective of this investigation was to explore the hypothesis that there is a transient “rebound” increase in platelet reactivity within three months of clopidogrel discontinuation.

### **Methods and Results**

In this prospective study, platelet function was assessed in patients taking aspirin and clopidogrel for at least 1 year following DES implantation. Platelet aggregation was measured using a modification of light transmission aggregometry in response to multiple concentrations of adenosine diphosphate (ADP), epinephrine, arachidonic acid, thrombin receptor activating peptide and, collagen. Clopidogrel was stopped and platelet function was reassessed 1 week, 1 month and 3 months later.

Thirty-two patients on dual antiplatelet therapy were recruited. Discontinuation of clopidogrel increased platelet aggregation to all agonists, except arachidonic acid. Platelet aggregation in response to ADP (2.5, 5, 10, 20  $\mu\text{M}$ ) and epinephrine (5, 20  $\mu\text{M}$ ) was significantly increased at 1 month compared to 3 months following clopidogrel withdrawal. Thus, a transient period of increased platelet reactivity to both ADP and epinephrine was observed 1 month after clopidogrel discontinuation.

## **Conclusions**

This study demonstrates a transient increase in platelet reactivity 1 month after clopidogrel withdrawal. This phenomenon may, in part, explain the known clustering of thrombotic events observed after clopidogrel discontinuation. This observation requires confirmation in larger populations.

## **Introduction**

The American College of Cardiology, American Heart Association and European Society of Cardiology, recommend that patients receive dual antiplatelet therapy for 1 year following myocardial infarction (MI) or drug-eluting stent (DES) revascularisation [1-3]. It is however unclear how patients should be treated after a year of aspirin and clopidogrel. The discontinuation of antiplatelet therapy in patients with established coronary artery disease (CAD) has become an important issue, as epidemiological studies have suggested an increased risk of adverse events when clopidogrel is discontinued after a year of therapy [4, 5]. *Ho et al* described a significant increase in the risk of recurrent MI and death within 90 days of clopidogrel cessation in 3,137 MI patients treated with percutaneous coronary intervention (PCI) or medical therapy [4]. The reasons for this clustering of ischaemic events after clopidogrel discontinuation are not clear, but could be related to a rebound hyperthrombotic period, possibly due to increased platelet reactivity [5].

Thrombotic complications following discontinuation of antiplatelet therapy have been recognised for almost a decade [6]. Indeed, aspirin withdrawal or non-adherence has been associated with a three-fold increase in ischaemic events in patients with CAD [7]. More recently, the premature discontinuation of clopidogrel has been associated with the development of stent thrombosis following DES implantation [8-11].

Recent clinical studies have challenged the association of clopidogrel withdrawal and adverse clinical events [12, 13]. Moreover, conflicting results have emerged from studies of platelet function following clopidogrel discontinuation [14-16]. *Lordkipanidze et al* observed rebound platelet reactivity following clopidogrel withdrawal in patients with CAD, treated with dual antiplatelet therapy for 1 year [14]. In contrast, *Sibbing et al* compared platelet aggregation in patients randomised to a strategy of tapered clopidogrel withdrawal to those who discontinued clopidogrel abruptly, and did not find any evidence of a rebound phenomenon in platelets [15].

Thus, it is not clear if clopidogrel withdrawal precipitates a period of increased platelet reactivity in patients taking long-term dual antiplatelet therapy. Furthermore, there remains a paucity of data characterising platelet function following discontinuation of chronic clopidogrel therapy [15]. While the increased risk of ischaemic events following clopidogrel withdrawal is observed for up to 90 days, no study to date has evaluated platelet reactivity beyond 1 month following clopidogrel discontinuation. The objective of the present investigation was to explore the hypothesis that there is a transient “rebound” increase in platelet reactivity within three months of stopping clopidogrel therapy.

## **Methods**

### **Patient population and study design.**

This study was approved by the Medical Research Ethics committee of Beaumont Hospital and conformed to the Declaration of Helsinki.



Patients with stable CAD were eligible for study inclusion if they fulfilled the following criteria; they had undergone PCI with DES > 1 year previously; had been taking 75mg of both aspirin and clopidogrel for a minimum of 12 months following PCI and, had no contraindications to clopidogrel discontinuation.

Patients were excluded if they were non-compliant with antiplatelet therapy; were taking antiplatelet drugs other than aspirin and clopidogrel (dipyridamole, ticlopidine), oral anticoagulants, steroidal or nonsteroidal anti-inflammatory drugs; had an acute cardiovascular event during the interval between PCI and study enrolment; a platelet count  $<125,000/\text{mm}^3$ ; haematocrit  $<25\%$ ; or active malignancy.

All subjects were recruited from the cardiology out-patient department in our institution and underwent screening with a detailed medical history, physical examination and laboratory analysis, including complete blood count, serum electrolytes, glucose, urea and creatinine. Compliance with antiplatelet therapy was assessed with a questionnaire prior to obtaining informed consent. Platelet function was assessed while patients were taking both aspirin and clopidogrel. In all patients, the thienopyridine was stopped and platelet function testing was repeated 1 week, 1 month and 3 months after clopidogrel discontinuation (Figure 1). Aspirin therapy was continued in all patients and alteration of other medical therapy was not permitted for the duration of the study. To assess the degree of variability in platelet reactivity over time, we also recruited 10 patients with known CAD on chronic aspirin therapy to act as a control group. These patients underwent platelet function testing at identical intervals to the patients who discontinued clopidogrel.

## Platelet Function Analysis

Platelet function testing measures the ability of platelets to aggregate or adhere in response to agonists [17]. A wide variety of *in vitro* platelet function assays are available, each with considerable disadvantages [17, 18]. Light transmission aggregometry (LTA) is considered to be the gold standard platelet function test but is time consuming, labor intensive and necessitates highly skilled laboratory staff. We developed a modification of LTA, which is rapid, inexpensive and is capable of assessing multiple platelet receptors and pathways simultaneously [19-23].

Early morning phlebotomy was performed on all subjects following an overnight fast. Uncuffed blood samples were drawn by a single phlebotomist from an antecubital vein using a 19-gauge butterfly needle and into 3.2% trisodium citrate, after discarding the first 5 ml of free flowing blood. Samples were centrifuged for 10 minutes at  $150 \times g$  and platelet-rich plasma (PRP) supernatant was collected. The PRP was not adjusted for platelet count.

Platelet function was measured in response to 5 platelet agonists: arachidonic acid (AA), adenosine diphosphate (ADP), epinephrine, thrombin receptor activating peptide (TRAP), and collagen. Similar to LTA, this assay measures light absorbance after the addition of soluble agonists to PRP. In brief, 180  $\mu\text{L}$  of platelet-rich plasma was added to the wells of a 96-well plate containing eight incremental concentrations of the agonists; arachidonic acid (500, 375, 188, 93.8, 46.9, 23.4, 11.8, 5.86)  $\mu\text{g mL}^{-1}$ ; ADP and TRAP (20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156)  $\mu\text{M}$ ; epinephrine (20, 5, 1.25, 0.313, 0.078, 0.0195, 0.00488, 0.00122)  $\mu\text{M}$ ; and collagen (190, 143, 71.3, 35.6, 17.8, 8.9, 4.45, 2.23)  $\mu\text{g mL}^{-1}$ .

Control wells containing saline buffer, platelet-rich and platelet-poor plasma were also prepared. Light transmission was measured using a 572-nm filter at 0, 3, 9, 15 and 18 minutes and the plate was constantly rotated at 1000 r.p.m. through a 1 mm orbit between measurements. Results were converted to percentage platelet aggregation based on the platelet-rich and platelet-poor plasma absorbance values, which represented 0% and 100% aggregation respectively. The maximal percentage aggregation measured for each agonist-concentration over the 18 minutes was calculated and plotted as dose-response curves. Experiments were performed with a Victor 3™ Multilabel plate reader (Perkin Elmer, Wellesley, MA, USA).

### **End Point Determination and Statistical Analysis**

The study hypothesis was that platelet reactivity would be increased at 1 month compared to 3 months following clopidogrel discontinuation. We calculated that we needed to include at least 31 patients to detect a 10% difference in ADP (5  $\mu$ M)-induced platelet aggregation, in order to give the study a 90% power with a one sided alpha value of 0.05.

Categorical variables were expressed as frequencies and percentages.

Continuous variables were analysed for a normal distribution with the Kolmogorov-Smirnov test (using p value > 0.2 as threshold). Normally distributed variables are presented as mean  $\pm$  standard deviation. Variables that did not follow a normal distribution are represented as median and interquartile range. The comparison of the dose-response curves ( $EC_{50}$ ) across the 4 study time points was performed using the extra sum-of-squares F-test. Comparisons of the maximal platelet aggregation between the 3 time points following

clopidogrel discontinuation were analysed using analysis of variance (ANOVA) with Bonferroni correction. The nominal level of significance was 5%. Statistical analyses were performed with SPSS (version 17, SPSS Inc., Chicago, Illinois).

## **Results**

Thirty-eight patients were screened for study inclusion between July 2008 and February 2009. Six patients were excluded, 5 due to non-compliance with antiplatelet therapy and 1 due to an abnormal screening complete blood count. Finally, 32 patients (78% male) were enrolled in the study, with a mean age of  $64 \pm 11$  years. The study population had a typical array of cardiovascular risk factors; 73% hypertension; 73% dyslipidaemia; 25% type 2 diabetes; 18% cigarette smokers; 41% obese (body mass index  $>30$  kg/m<sup>2</sup>); 28% chronic renal impairment (glomerular filtration rate  $< 60$  ml/min); and 63% had a prior MI. All patients were treated with aspirin and clopidogrel, with the majority taking HMG-CoA reductase inhibitors (94%), angiotensin converting enzyme-inhibitors (86%) and beta-receptor blockers (91%).

Platelet function was measured on 4 separate occasions and the dose-response curves for each agonist are presented in Figure 2. The pattern of recovery of platelet function following clopidogrel discontinuation was different for each agonist / signalling pathway. For each agonist, we compared the agonist concentration that gave a response halfway between baseline and maximum platelet aggregation (EC<sub>50</sub>) at each of the 4 study time points. The LogEC<sub>50</sub> values for ADP and TRAP-induced platelet aggregation were significantly

different ( $p < 0.001$ ), whereas there was no difference in the  $\text{LogEC}_{50}$  values for arachidonic acid, epinephrine or collagen ( $p = \text{NS}$ ). The comparisons of maximal platelet aggregation for each agonist/platelet-signalling pathway are presented individually below (Table 1 & Figure 3).

### **ADP-induced platelet aggregation**

Maximal platelet aggregation in response to 20  $\mu\text{M}$  ADP was  $43 \pm 16.5\%$  at study entry while patients were taking aspirin and clopidogrel. Discontinuation of clopidogrel resulted in increased ADP-mediated aggregation by 1 week ( $63.7 \pm 8.3\%$ ). The response to ADP increased further at 1 month ( $63.7 \pm 8.3\%$  vs  $71.8 \pm 6.1\%$ ,  $p < 0.001$ ). By contrast, there was a significant decrease in platelet aggregation in response to ADP at 3 months ( $71.8 \pm 6.1\%$  vs  $67.8 \pm 5.6\%$ ,  $p = 0.017$ ). This transient increase in ADP-induced platelet reactivity at 1 month was consistently observed in response to multiple concentrations of ADP (2.5, 5, 10, 20  $\mu\text{M}$ ).

### **Epinephrine-induced platelet aggregation**

Platelet aggregation in response to 20  $\mu\text{M}$  epinephrine was  $45.1 \pm 13.9\%$  while patients were on aspirin and clopidogrel. Following clopidogrel withdrawal, aggregation in response to epinephrine increased to  $51.5 \pm 15.9\%$  at 1 week, with a further rise to  $55.9 \pm 14.6\%$  at 1 month. Analogous to the decrease in platelet reactivity in response to ADP between 1 and 3 months, there was a significant reduction in epinephrine-induced platelet aggregation to  $49 \pm 16.5\%$  at 3 months ( $p = 0.039$ ). This observation was consistent for both 5  $\mu\text{M}$  and 20  $\mu\text{M}$  epinephrine concentrations.

### **Arachidonic Acid- induced platelet aggregation**

At study entry, the maximal platelet aggregation measured in response to 500  $\mu\text{g}/\text{mL}^{-1}$  arachidonic acid was  $2.2 \pm 5.1\%$ . Arachidonic acid-mediated platelet aggregation did not change throughout the study ( $p = 0.99$ ).

### **TRAP- induced platelet aggregation**

Maximal platelet aggregation in response to 20  $\mu\text{M}$  TRAP was  $66.6 \pm 12.6\%$  at study entry and increased significantly following clopidogrel withdrawal. At 1 week, aggregation in response to TRAP had increased to  $76.7 \pm 5.8\%$ , with a further increase to  $82 \pm 4.1\%$  by 1 month ( $p = 0.002$ ). However, there was no significant decrease in TRAP-induced platelet aggregation between 1 and 3 months ( $82 \pm 4.1\%$  vs  $80.4 \pm 6.2\%$ ,  $p = 0.816$ ).

### **Collagen- induced platelet aggregation**

At study entry, maximal platelet aggregation in response to 190  $\mu\text{g}/\text{mL}^{-1}$  collagen was  $72.3 \pm 7.3\%$ . Following clopidogrel discontinuation, collagen-mediated platelet aggregation increased to  $75.7 \pm 6.9\%$  at 1 week, with a further increase to  $78.4 \pm 10.4\%$  at 1 month. There was no difference in platelet aggregation in response to collagen between 1 and 3 months ( $78.4 \pm 10.4\%$  vs  $78.1 \pm 6.9\%$ ,  $p = 0.99$ ).

We observed considerable interindividual variability in platelet function following clopidogrel discontinuation. Platelet aggregation in response to ADP was significantly increased at 1 month compared to 3 months in 21 patients, thus two thirds of our patients demonstrated a transient increase in platelet reactivity

following clopidogrel withdrawal. The magnitude of this increase in platelet aggregation to ADP (5  $\mu$ M) ranged from 5.1 – 22.8% (Mean 11.5  $\pm$  5%). In contrast, platelet aggregation to ADP was similar at 1 and 3 months ( $\pm$  5%) in 11 patients. A multivariate analysis did not identify any measurable patient characteristic to be predictive of the increased response to ADP or epinephrine at 1 month.

## **Discussion**

The results of this study demonstrate that platelet aggregation in response to ADP and epinephrine was significantly increased at 1 month compared to 3 months after clopidogrel was discontinued. This result was consistent for multiple concentrations of ADP (2.5, 5, 10, 20  $\mu$ M) and epinephrine (5, 20  $\mu$ M) and occurred in two thirds of our patient population. Thus, our results demonstrate a temporal, transient increase in platelet reactivity 1 month following clopidogrel withdrawal.

Increased ADP-mediated platelet aggregation is characteristic of an impaired response to clopidogrel and defines high on-treatment platelet reactivity in patients taking dual antiplatelet therapy [24]. High platelet reactivity is associated with an increased risk of recurrent ischaemic events and stent thrombosis (ST) after PCI [25-29]. Recently, it has been suggested that the withdrawal of long-term clopidogrel therapy may induce a period of high platelet reactivity and therefore increase thrombotic risk [4, 5, 30, 31].

Numerous clinical studies have shown that the early interruption of clopidogrel therapy in recipients of DES results in a marked increase in the rates of stent

thrombosis (ST) and death [8, 10, 11]. In fact, discontinuation of dual-antiplatelet therapy is the most powerful predictor of ST during the first 6 months after stent implantation [32]. It is likely however, that ST in the setting of premature withdrawal of clopidogrel is caused by uninhibited platelet adhesion to both thrombogenic stents and disrupted vascular endothelium and is not due to rebound platelet hyperreactivity [33]. Several other factors that contribute to pathophysiology of ST have also been recognised, including patient [34], procedural [35] and, stent variables [36-38].

Recently, *Ho et al* described an excess of ischaemic events and increased mortality within 90 days of clopidogrel discontinuation in 3,137 patients who suffered an acute coronary syndrome [4]. Importantly, the rates of death and MI were higher in the 1,568 patients treated with medical therapy compared to those who underwent PCI. Thus, ischaemic events following clopidogrel withdrawal are not exclusively stent related. The clustering of events following clopidogrel withdrawal suggests a rebound hyperthrombotic period, possibly due to increased platelet reactivity. While the same authors have confirmed these findings in a recent follow-up analysis [5], conflicting data have also emerged [12, 13, 39]. A post-hoc analysis of the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance) trial, which compared the efficacy and safety of clopidogrel plus aspirin compared with aspirin alone in patients at high risk of cardiovascular events, did not observe a clinically detectable rebound effect [12]. Furthermore, a recent retrospective analysis of 2,254 patients who underwent coronary DES implantation only observed an increased risk of adverse events following



clopidogrel cessation if the thienopyridine was stopped within 6 months of stent implantation [39].

Studies of platelet function following clopidogrel discontinuation have also reported conflicting results [14-16]. *Lordkipanidze et al* compared platelet aggregation in 37 CAD patients scheduled to discontinue clopidogrel with 37 CAD patients taking only aspirin [14]. Platelet aggregation was measured repeatedly for 1 month using both VerifyNow® P2Y<sub>12</sub> and LTA in response to multiple concentrations of ADP (1 – 20 µM). There was no difference between the groups with the VerifyNow® P2Y<sub>12</sub> or in response to the higher ADP (10 – 20 µM) concentrations, however there was a significant increase in platelet aggregation in response to lower ADP concentrations (1 - 5 µM) in patients who stopped clopidogrel. In our study, we observed increased ADP-mediated platelet aggregation in response to both higher (20 – 10 µM) and lower ADP (2.5 – 5 µM) concentrations following clopidogrel discontinuation, though there was no difference at ADP concentrations < 2.5 µM. Similar to our study, the enhanced platelet reactivity in response to ADP was evident 1 month after clopidogrel discontinuation.

Contradictory results have been reported by *Sibbing et al*, who compared platelet aggregation in patients randomised to a strategy of tapered clopidogrel withdrawal (n = 35) to those who discontinued clopidogrel abruptly (n = 34) [15]. Platelet aggregation in response to ADP, TRAP and collagen was measured weekly up to 1 month following drug withdrawal using both LTA and multiple electrode aggregometry. No difference in maximal platelet aggregation to ADP was observed up to 1 month following clopidogrel discontinuation and the

authors concluded that there was no evidence for a rebound phenomenon in platelets. However, these investigators did not measure platelet aggregation beyond 1 month, despite clinical evidence suggesting an increase in adverse events for up to 90 days following clopidogrel cessation [4, 5]. Moreover, a trend towards decreased ADP-mediated platelet aggregation at 3 weeks following clopidogrel discontinuation is evident in this data. Based on our results, continued platelet function testing out to 90 days may have provided additional important information.

The Platelet Activity after Clopidogrel Termination (PACT) study, a prospective, randomised, double-blind, placebo-controlled crossover study, measured platelet reactivity in 15 healthy volunteers who received dual antiplatelet therapy for 14 days [16]. Platelet reactivity was measured repeatedly out to 45 days using whole blood flow cytometry and by LTA in response to both submaximal and maximal concentrations of ADP, TRAP and collagen. While the authors found no evidence for rebound platelet reactivity, these data may not accurately reflect the effect of long-term clopidogrel therapy on the recovery of platelet function following clopidogrel cessation in a cardiovascular population. We observed increased platelet reactivity at 1 month following clopidogrel discontinuation in patients with CAD taking long-term dual antiplatelet therapy (mean  $18 \pm 3$  months).

Two thirds of our patient population demonstrated a transient increase in platelet reactivity 1 month following clopidogrel discontinuation. The magnitude of the enhanced response to ADP ( $5 \mu\text{M}$ ) ranged from 5.1 – 22.8% (Mean  $11.5 \pm 5\%$ ). Given that an appropriate response to clopidogrel maybe

defined as a 10% reduction in ADP-mediated platelet aggregation [24], the increase in ADP reactivity that we observed 1 month following clopidogrel discontinuation is considerable. However, it is unlikely that a transient increase in platelet reactivity could solely account for the increased rate of ischaemic events described in the literature. Other possible precipitants for these events following clopidogrel withdrawal may include; delayed endothelial healing with DES; loss of protection from dual antiplatelet therapy; endothelial dysfunction and increased inflammation after clopidogrel withdrawal [40, 41]. Nevertheless, an increase in platelet reactivity is a potential risk factor for the development of acute ischaemic events [42].

The precise mechanism for the transiently enhanced platelet aggregation we observed is not known. Long-term clopidogrel therapy is associated with a reduction in inflammatory markers, in particular high sensitivity C-reactive protein and P-selectin [41, 43, 44], which are both been associated with ischaemic events following PCI [45, 46]. These anti-inflammatory effects appear to be unique to clopidogrel amongst antiplatelet agents, and may therefore be specifically linked to the P2Y<sub>12</sub> receptor [43, 44, 47-51]. The abrupt discontinuation of clopidogrel may therefore initiate a proinflammatory milieu, which results in increased ADP-mediated platelet aggregation.

A platelet rebound phenomenon would be expected to occur approximately 2 weeks following clopidogrel discontinuation, as circulating platelets would not be inhibited by the active metabolite of clopidogrel. Our observations and those of other investigators [14] of increased reactivity at 1 month following clopidogrel withdrawal are thus, difficult to explain. It is possible that the

inflammatory milieu induced by clopidogrel withdrawal peaks at 1 month or that chronic clopidogrel therapy affects the megakaryocyte, resulting in altered platelet function beyond the duration required for replacement of the platelet pool [52].

We observed a parallel increase in platelet aggregation in response to ADP and epinephrine 1 month after clopidogrel discontinuation. Catecholamines are known to potentiate the effects of other platelet agonists and in particular the platelet  $\alpha$ -2 adrenergic receptor contributes to high on-treatment platelet reactivity [53]. We have previously described a correlation between ADP- and epinephrine-mediated platelet aggregation in cardiovascular patients on dual antiplatelet therapy [19]. It is not clear why ADP and epinephrine-mediated platelet aggregation is linked as downstream signal transduction differs considerably between the Gi-coupled P2Y<sub>12</sub> receptor and the G<sub>z</sub>-coupled  $\alpha$ -2 adrenergic receptor. Further studies are required to investigate this interaction.

### **Study Limitations**

In this prospective study we did not measure platelet reactivity prior to or immediately after the initiation of clopidogrel. This may have provided a 'true baseline' platelet reactivity for each patient however, the majority of our cohort had stenting in the setting of acute MI, thus early platelet function testing would not have accurately reflected a true baseline measurement. As such, we elected to test each patient 3 months following clopidogrel cessation and believe that this reflects an accurate estimate of each patient's baseline platelet reactivity.

The precise mechanism of the unique enhanced response to ADP and epinephrine is unclear, but maybe due to other mechanisms of action of clopidogrel than the blockade of the P2Y<sub>12</sub> receptor [41]. We did not measure markers of inflammation such as CRP or P-selectin, however this has been extensively evaluated in the setting of clopidogrel discontinuation by other investigators [41, 43, 49, 50]. While most of our patients demonstrated increased platelet reactivity 1 month following clopidogrel cessation, no thrombotic events occurred during the study. Thus, we have not demonstrated a definitive association between increased platelet reactivity following clopidogrel discontinuation and recurrent ischaemic events. We measured platelet function at 3 predefined time points following clopidogrel discontinuation however, more frequent platelet function analysis may have yielded additional relevant information. The results of this study are observational and require confirmation in larger patient populations, tested with multiple assays of platelet function.

## **Conclusion**

This study demonstrates a transient increase in platelet reactivity 1 month after discontinuation of long-term clopidogrel therapy in a cardiovascular population. These findings may have clinical implications with respect to thrombotic events and merit further study in a larger population.

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## Figure Legends

### Fig 1

**Title** Study Protocol

**Legend** Schematic representation of the study protocol.

### Fig 2

**Title** Platelet function following clopidogrel discontinuation is agonist specific.

**Legend** Platelet aggregation to increasing concentrations of arachidonic acid, collagen, thrombin receptor activating peptide (TRAP), adenosine diphosphate, and epinephrine at study entry and 1 week, 1 month and 3 months following clopidogrel discontinuation. Each data point represents mean aggregation (%) to each concentration. The p – values are generated using the extra sum-of-squares F-test, and represent the comparison of the dose-response curves ( $EC_{50}$ ) across the 4 study time points. \*\*\*  $p < 0.001$ .

**Fig 3**

**Title** Platelet aggregation in response to ADP and epinephrine is uniquely increased one month after clopidogrel discontinuation.

**Legend** Platelet aggregation in response to selected concentrations of adenosine diphosphate, epinephrine, arachidonic acid, collagen, and TRAP at study entry and at 1 week, 1 month and 3 months following clopidogrel discontinuation. Each point indicates mean platelet aggregation (%) with standard error of the mean. The p – values are calculated with analysis of variance and the Bonferroni correction. TRAP, thrombin receptor activating peptide. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**Supplemental Fig 1**

**Title** Platelet aggregation in the control patients

**Legend** Platelet aggregation in response to selected concentrations of adenosine diphosphate, epinephrine, arachidonic acid, collagen, and TRAP at study entry and at 1 week, 1 month and 3 months later. Each point indicates mean platelet aggregation (%) with standard error of the mean.

**Table 1.****Platelet Function Profile Analyses**

Platelet Agonist	Study entry	1 Week	1 Month	1 Week vs 1 Month	1 Month	3 Months	1 Month vs 3Months
Arachidonic acid ( $\mu\text{g/mL}^{-1}$ )				p - value			p - value
500	2.2 $\pm$ 5.1	4.8 $\pm$ 6.2	6.2 $\pm$ 11.6	0.66	6.2 $\pm$ 11.6	3.4 $\pm$ 6	0.99
Adenosine diphosphate ( $\mu\text{M}$ )							
0.313	0.2 $\pm$ 1.9	0.6 $\pm$ 2.1	1.8 $\pm$ 4.9	0.55	1.8 $\pm$ 4.9	0.7 $\pm$ 2.4	0.59
0.625	2.1 $\pm$ 4.8	9.2 $\pm$ 9.9	13.9 $\pm$ 11.4	0.22	13.9 $\pm$ 11.4	9.1 $\pm$ 9.6	0.20
1.25	8.4 $\pm$ 9.8	26.3 $\pm$ 14.5	33 $\pm$ 11.9	0.18	33 $\pm$ 11.9	27.2 $\pm$ 15.8	0.30
2.5	18.2 $\pm$ 14.2	41.5 $\pm$ 12.8	47.3 $\pm$ 11.9	0.026	47.3 $\pm$ 11.9	42.1 $\pm$ 13.9	0.032
5	30.7 $\pm$ 16.2	51.6 $\pm$ 10.4	59.7 $\pm$ 9.6	<0.001	59.7 $\pm$ 9.6	53.7 $\pm$ 9.8	0.002
10	39.1 $\pm$ 16.7	59.5 $\pm$ 9.4	67.3 $\pm$ 7.6	<0.001	67.3 $\pm$ 7.6	62.2 $\pm$ 7.1	0.003
20	43 $\pm$ 16.5	63.7 $\pm$ 8.3	71.8 $\pm$ 6.1	<0.001	71.8 $\pm$ 6.1	67.8 $\pm$ 5.6	0.017
Epinephrine ( $\mu\text{M}$ )							
1.25	21.3 $\pm$ 11.3	21.2 $\pm$ 13.5	24.7 $\pm$ 13	0.45	24.7 $\pm$ 13	19.3 $\pm$ 15	0.125
5	30.7 $\pm$ 14.7	34.6 $\pm$ 15.4	39.6 $\pm$ 16.5	0.09	39.6 $\pm$ 16.5	32.9 $\pm$ 15.9	0.021
20	45.1 $\pm$ 13.9	51.5 $\pm$ 15.9	55.9 $\pm$ 14.6	0.14	55.9 $\pm$ 14.6	49 $\pm$ 16.5	0.039
TRAP ( $\mu\text{M}$ )							
5	53.1 $\pm$ 18.4	68.6 $\pm$ 8.4	72.1 $\pm$ 19.9	0.84	72.1 $\pm$ 19.9	73.2 $\pm$ 7.8	0.99
10	60.6 $\pm$ 12.3	73.5 $\pm$ 6.5	79.7 $\pm$ 4.2	<0.001	79.7 $\pm$ 4.2	77.3 $\pm$ 7.3	0.26
20	66.6 $\pm$ 12.6	76.7 $\pm$ 5.8	82 $\pm$ 4.1	0.002	82 $\pm$ 4.1	80.4 $\pm$ 6.2	0.82
Collagen ( $\mu\text{g/mL}^{-1}$ )							
71.3	12.5 $\pm$ 5.7	24.5 $\pm$ 20.7	23 $\pm$ 20.4	0.99	23 $\pm$ 20.4	21.9 $\pm$ 17.3	0.99
143	57.5 $\pm$ 13.3	65.4 $\pm$ 14.9	69.9 $\pm$ 12.1	0.57	69.9 $\pm$ 12.1	73.4 $\pm$ 10.1	0.69
190	72.3 $\pm$ 7.3	75.7 $\pm$ 6.9	78.4 $\pm$ 10.4	0.66	78.4 $\pm$ 10.4	78.1 $\pm$ 6.9	0.99

**Legend**

Data are expressed as mean  $\pm$  SD values of percentage of platelet aggregation. p - values represent differences in means in platelet aggregation calculated using repeated measures analysis of variance with the Bonferroni correction. There was no difference in response to the lower concentrations of each agonist at these intervals, thus we present data from the higher concentrations only. TRAP, thrombin receptor activating peptide.







