Dual antiplatelet therapy unmasks distinct platelet reactivity in patients with coronary artery disease.

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Dual antiplatelet therapy unmasks distinct platelet reactivity in patients with coronary artery disease

Running head: Platelet reactivity in coronary artery disease

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Summary. *Background:* Platelet induced thrombosis is a major risk factor for recurrent ischaemic events, although platelet function in patients with cardiovascular disease taking aspirin and clopidogrel is very poorly characterised. The aim of this study was to assess platelet reactivity in patients with cardiovascular disease taking aspirin and clopidogrel.

Methods: We developed a rapid assay to measure platelet aggregation in response to arachidonic acid, collagen, adenosine diphosphate (ADP), epinephrine and Thrombin Receptor Activating Peptide (TRAP) in 80 healthy volunteers. We then recruited 200 consecutive patients from outpatient clinics and the cardiac catheterisation laboratory and tested platelet function. Platelet aggregation induced by epinephrine is a marker of global platelet reactivity. We tested platelet function in 146 patients compliant with antiplatelet therapy. Platelet aggregation to epinephrine was divided into quartiles. The platelet response to the other agonists was analysed based on the response to epinephrine.

Results: Platelet reactivity increased significantly across the quartiles in response to epinephrine in normals and patients ($p<0.0001$). A significant increase in response across quartiles was seen with all agonists in normals ($p<0.001$). In contrast, a significant increase in response across quartiles was only seen with ADP in patients ($p<0.0001$). Hypertension, smoking and diabetes were significantly associated with increasing platelet reactivity to epinephrine ($p < 0.05$).

Conclusion: This study shows that platelet response differs between normals and patients on dual antiplatelet therapy. Dual antiplatelet therapy unmasks a distinct type of platelet reactivity in response to epinephrine and ADP but not other agonists, in patients with cardiovascular disease.

Keywords: coronary artery disease, dual antiplatelet therapy, epinephrine, platelet aggregation, platelet reactivity.

Introduction

Platelets play a critical role in patients with cardiovascular disease [1]. Antiplatelet drugs are effective, both alone and in combination, in the secondary prevention of cardiovascular disease [2, 3]. However, platelet function in patients on dual antiplatelet therapy with cardiovascular disease is not fully understood [4]. Several lines of evidence suggest that recurrent thrombotic events may be due to increased platelet reactivity in patients taking aspirin and clopidogrel. Moreover increased platelet reactivity may predict risk for future cardiovascular events [5-7]. There is a growing body of evidence suggesting that platelet reactivity is important in clinical practice [8-10]. Platelet function is not measured routinely in clinical practice because it is labour intensive, expensive and time consuming. Moreover the direct clinical applicability of standard assays such as light transmission aggregometry in patients with cardiovascular disease is questionable [4, 11].

Many assays of platelet function have been developed since light transmission aggregometry. Two of these, the PFA-100 ™ and the Verify Now ™, have advanced research in arterial thrombosis, especially in the area of monitoring antiplatelet therapy. The PFA-100 ™ has a major disadvantage, however, in that it is insensitive to monitoring the effect of clopidogrel and the Verify Now ™ device requires a separate cartridge for the assay of either aspirin or clopidogrel response, making it relatively expensive [12]. In addition, the role of these assays in the evaluation of platelet response to other agonists known to activate platelets is unclear.

Normal volunteers who have the greatest platelet reactivity to epinephrine have platelets that aggregate more to other agonists as well. This has been termed global platelet hyperreactivity. It has been suggested that a common mechanism may

exist to explain the phenomenon of global platelet reactivity [13]. More recently, the phenomenon of global hyperreactivity has been described in diabetic patients [6].

To understand platelet function in patients with cardiovascular disease taking dual antiplatelet therapy, we developed an assay that characterised the response to multiple agonists. We developed an assay that was rapid, inexpensive, and capable of assessing multiple platelet receptors and pathways simultaneously in patients on dual antiplatelet therapy. In contrast to normal volunteers and diabetics, we found that patients with cardiovascular disease on dual antiplatelet therapy have increased platelet reactivity in response to epinephrine and ADP alone. This response does not generalise to the agonists collagen, arachidonic acid and TRAP in patients compliant with their antiplatelet medication.

Methods

Patients

This study was approved by Medical Research Ethics Committees of both the Royal College of Surgeons and Beaumont Hospital and complied with the Declaration of Helsinki. Informed consent was obtained from all patients prior to phlebotomy.

Healthy volunteers

To establish the normal range of platelet response, we tested 80 healthy volunteers (male=40, female=40) who had not taken any medication known to affect platelet function in the previous 14 days. Blood was drawn before 10 AM and 2 hours after a light breakfast to minimise variation due to circadian rhythm. Blood was collected through a 19-gauge Butterfly® needle into a 30-ml syringe containing 3.2% sodium citrate. The first 3ml of blood was discarded. Blood was centrifuged for 10 min at 150*g*. Platelet-rich plasma (PRP) aspirated from the supernatant was placed in a reagent reservoir. Using a multi-channel pipette, the PRP was dispensed across wells in a 96-well plate (black isoplate ® with clear flat-bottomed wells, PerkinElmer) containing different concentrations of the agonists, arachidonic acid, collagen (type 1 soluble calf skin), Adenosine Diphosphate (ADP), epinephrine and Thrombin Receptor Activating Peptide (TRAP).

Patients on dual antiplatelet therapy

Patients with cardiovascular disease were recruited from the cardiology outpatient clinic and the cardiac catheterisation laboratory. All patients taking dual antiplatelet therapy who had received 4 or more doses of aspirin and clopidogrel or patients who received an equivalent loading dose of 300 mg of aspirin and clopidogrel in the previous 24 hours were screened for eligibility.

Patients taking GPIIb/IIIa inhibitors, warfarin, non-steroidal anti-inflammatory medication or any other medication known to affect platelet function were excluded. Patients with a serum creatinine > 150 mmol/l, or a platelet count of < 100,000 / mm^3, or who were pregnant, or had hepatic dysfunction (defined by hepatic enzymes more than twice the upper normal limit) were also excluded. After resting comfortably for at least 10 minutes, patients were phlebotomised and blood collected as described above. Demographic material was recorded on all patients.

Assessment of Drug Compliance

A standard questionnaire was completed by each patient to review compliance with antiplatelet therapy. Patients were excluded if they stated or if they suspected that they had not taken their medication on one or more occasion(s) in the previous 14 days. A significant response (≥20%) to arachidonic acid may indicate non compliance with antiplatelet therapy [14]. Therefore patients who demonstrated a platelet aggregation response of ≥20% to arachidonic acid were also excluded from this study. Finally, we compared the response to arachidonic acid between the inpatient and outpatient population as an index of compliance.

Novel Platelet Function Assay

To assess platelet function, we used a modification of light transmission aggregometry that we have previously described [15, 16]. In brief, 180 µl of PRP was added to each well of a 96-well plate containing different agonists. Light absorbance was measured at standard times. Light absorbance values were then normalised based on the PRP and PPP absorbance values which represented 0 and 100% aggregation. Using this, the percentage aggregation response for each concentration of each agonist was calculated and plotted as dose response curves (Fig. 1). To characterise maximal platelet aggregation in healthy volunteers and coronary artery disease patients taking

dual antiplatelet therapy we assayed increasing concentrations of 5 different agonists using the same novel platelet function test.

Platelet aggregation measured as a percentage of absorbance from baseline using a 572 nm filter was assayed at 0, 3, 9, 15 and 18 minutes. Between each of the standardised times, the plate was rotated at 1000 r.p.m. through a 0.1 mm orbit. The agonists used were arachidonic acid, collagen, ADP, TRAP and pharmacological concentrations of epinephrine. The final concentrations of the agonists arrayed were (500, 375, 188, 93.8, 46.9, 23.4, 11.8, 5.86) µg/ml for arachidonic acid; (190, 143, 71.3, 35.6, 17.8, 8.9, 4.45, 2.23) µg/ml for collagen; (20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156) µM for ADP and TRAP; and (20, 5, 1.25, 0.313, 0.078, 0.0195, 0.00488, 0.00122) µM for epinephrine. The agonist volumes used were 50 µl of arachidonic acid, 50 µl of collagen, 40 µl of ADP, 40 µl of epinephrine and 40 µl of TRAP. The 96-well plate was then read using a Victor 3™ Multilabel plate reader (Perkin Elmer, Wellesley, MA, USA). The time from blood draw until the end of the assay protocol was recorded.

Statistical analysis

Continuous variables were analysed for a normal distribution using the Kolmogorov-Smirnov test (using p value > 0.2 as threshold). Continuous variables following a normal distribution are expressed as a mean value +/- standard deviation. Comparisons between quartiles were analysed using one-way ANOVA for continuous variables and the Cochran-Armitage trend test for categorical variables. Comparisons between inpatient and outpatient response to arachidonic acid were analysed using Fisher's exact test. The nominal level of significance was 5% and multiple comparisons between quartiles were adjusted using the Bonferroni correction.

To assess assay reproducibility, we calculated the inter-assay (between assay precision) and the intra-assay (within assay precision) coefficients of variation. The between assay precision was analysed using 10 normals measured on 5 separate occasions at least one week apart. A value of <12% for the between assay precision is regarded as acceptable for assay validation [17]. The within assay precision was analysed from the same donor in response to the same agonist measured 10 times on the same day.

Results

To develop an assay of platelet function that was rapid, reproducible and which defined the maximal response to different agonists, we assayed increasing concentrations of different agonists at multiple time points. Concentrations of the agonists, arachidonic acid and collagen, ranging from 2.23-500 µg/ml and the agonists, ADP, epinephrine, and TRAP, ranging from 0.00122-20 µM were assayed. We assayed these concentrations in PRP from 80 healthy volunteers (40 males and 40 females). Since the time to maximal aggregation varies depending on the agonist used, aggregation was assessed at different time points until maximal aggregation occurred. Maximal aggregation was defined as no change in absorbance with incremental concentration of agonist at two consecutive time points. Maximal aggregation occurred at 18 minutes in response to 500 µg/ml of arachidonic acid, 190 µg/ml of collagen, 20 µM for each of ADP, epinephrine and TRAP in all cases. The average time from blood draw to completion of the assay was 41 ± 6 minutes.

To establish the reproducibility of the test, the between and within assay precision of the assay were calculated. The between assay precision of each agonist was; for arachidonic acid 500mg/ml - 12%, collagen 190mg/ml - 10%, ADP 20 µM – 8%, epinephrine – 11% and TRAP – 11%. The within assay precision was < 1% for all agonists, indicating excellent reproducibility.

Platelet reactivity was then tested in a cohort of patients taking antiplatelet agents. The assay detects the effect of aspirin alone and in response to aspirin and clopidogrel combined (supp Fig. 1S). A total of 200 consecutive patients with cardiovascular disease taking both aspirin and clopidogrel were screened from May 2007 to July 2007 and their platelet function assayed. Demographic information for the patients is shown in Table 1.

Platelet reactivity in patients on dual antiplatelet therapy

Since non-compliance with aspirin therapy may confound interpretation of platelet function testing, we attempted to control for this in a number of ways. We excluded patients who stated that they had missed one or more doses of their antiplatelet therapy in the previous 14 days (26/200, 13%). Secondly, since an aggregation response of ≥20% to arachidonic acid may indicate non-compliance, we excluded patients whose aggregation response to arachidonic acid was ≥20% (28/200, 14%). We then analysed the platelet data from the remaining 146 subjects. Since non-compliance is more likely in the outpatient population compared to the inpatient population [18], we compared the response to arachidonic acid between these two groups. The relative proportions of patients with a response to arachidonic acid of ≥20% between the inpatient and outpatient population were identical (supp Fig. 2S). Thus non-compliance seems unlikely to account for the results of our investigation.

Epinephrine has been shown to be a marker for global platelet reactivity in healthy volunteers [13]. Therefore we characterised platelet response to epinephrine in healthy volunteers and coronary artery disease patients on dual antiplatelet therapy. Overall the mean aggregation response to epinephrine was 80 ± 11% for the healthy volunteers. The mean aggregation response to epinephrine was 53 ± 16% in the patients; the response was highly variable in the patients and followed a normal bell shaped distribution. Platelet aggregation in response to submaximal concentrations of epinephrine (0.313μM) in healthy volunteers followed a bimodal distribution in agreement with previous work (Fig. 3S). In contrast there was no bimodal distribution in patients on dual antiplatelet therapy [13]. Platelet response variability was significantly higher in the patients compared to the healthy volunteers (p=0.0071) (supp. Fig. 4S). Platelet aggregation to 20 μM epinephrine was read at 18 minutes.

The maximal platelet aggregation response to epinephrine ranged from 16 - 80% in 146 patients and was then ranked from the lowest to the highest responder and divided into quartiles. The mean percentage platelet aggregation in response to epinephrine in each of the quartiles was 32 ± 7%, 47 ± 4%, 60 ± 4% and 72 ± 4% (Fig. 2). The mean response between each quartile was significantly different ($p<0.0001$). When we analysed platelet response to submaximal concentrations of epinephrine we find that in healthy volunteers the response to epinephrine predicted a greater response to all agonists. In contrast in the patients taking dual antiplatelet therapy the response to epinephrine was only seen in response to ADP and not to any of the other agonists (supp. Fig. 5S A & B). Smoking, hypertension and diabetes significantly influenced platelet reactivity ($p<0.05$) across the epinephrine quartiles.

We evaluated the influence of cardiovascular risk factors on platelet reactivity using trend testing. The relative number of subjects with a history of diabetes mellitus significantly increased as the level of platelet reactivity increased when we based the quartiles on the response to either epinephrine or TRAP ($p<0.05$) but did not influence platelet reactivity when we based the quartiles on the response to ADP or collagen. Similarly, hypertension significantly influenced platelet reactivity across the quartiles in response to either epinephrine or TRAP ($p<0.05$) but did not influence platelet reactivity in the response to either ADP or collagen. The relative number of subjects with a history of smoking significantly increased as the level of platelet reactivity increased based on the response to epinephrine only ($p<0.05$). The other risk factors did not influence platelet reactivity. In addition, trend testing found no link between cardiovascular medications and platelet reactivity (Table 1).

As described above the aggregation response to epinephrine was ranked from the lowest to highest responder and divided into quartiles. The corresponding

response to each agonist was then analysed for each individual within the original epinephrine quartiles.

The response to arachidonic acid was analysed for each individual within the original epinephrine quartiles. The mean platelet aggregation response for the entire cohort using arachidonic acid (500 µg / ml) was 8 ± 5% indicating that the TXA_2 pathway was adequately inhibited in this study population. The mean percentage platelet aggregation in response to arachidonic acid in each of the epinephrine quartiles was 7 ± 4%, 8 ± 5%, 8 ± 4% and 9 ± 5%. There was no significant difference between the quartiles in response to arachidonic acid (Fig. 3A). The response to collagen was analysed for each individual within the original epinephrine quartiles. The mean platelet aggregation response for the entire cohort using collagen (190 µg/ml) was 64 ± 15%. The mean percentage platelet aggregation in response to collagen in each of the epinephrine quartiles was 59 ± 17%, 67 ± 12%, 63 ± 16% and 67 ± 14%. There was no significant difference between these quartiles in response to platelet aggregation induced by collagen (Fig. 3B).

The response to TRAP was analysed for each individual within the original epinephrine quartiles. The mean platelet aggregation response for entire cohort using the agonist TRAP (20 µM) was 61 ± 12%. The mean percentage platelet aggregation in response to TRAP in each of the epinephrine quartiles was 57 ± 13%, 60 ± 10%, 62 ± 10% and 65 ± 13%. There was no significant difference between the quartiles in response to TRAP (Fig. 3C).

The response to ADP was analysed for each individual within the original epinephrine quartiles. The mean platelet aggregation response for the entire cohort using ADP (20 µM) was 40 ± 18%. The mean percentage platelet aggregation in response to ADP in each of the epinephrine quartiles was 30 ± 14%, 40 ± 19%, 39 ±

16% and 50 ± 15%. The second quartile in ADP response was significantly higher than the first and the fourth quartile was significantly higher than the third ($p<0.01$ and $p<0.005$, respectively). In summary, platelet reactivity increased significantly across the quartiles in response to epinephrine ($p<0.0001$). This increasing linear trend was also observed in response to ADP ($p<0.0001$) but was not detected with the other agonists suggesting that there is a distinct platelet reactivity in response to both epinephrine and ADP in patients with cardiovascular disease taking both aspirin and clopidogrel (Fig. 4).

Discussion

Platelets play a critical role in the pathogenesis of thrombosis in cardiovascular syndromes [19]. Several recent studies have identified variable platelet response in patients taking antiplatelet therapy [20-22]. However, platelet responsiveness in patients with coronary artery disease on dual antiplatelet therapy has not been well characterised. We developed an assay that could be preformed near the patient to assay platelet function in response to multiple agonists. Our assay is rapid with the average time from blood draw to results of platelet function being less than one hour. In detailed studies of normal healthy volunteers, Yee at al [13] have shown that platelet response to epinephrine predicts the response to other agonists, a term called "global platelet hyperreactivity". The results of the present investigation demonstrate in coronary artery disease patients taking both aspirin and clopidogrel, that epinephrine elicits a markedly heterogeneous response. This response was also seen with the agonist ADP. In contrast, there was no variation in response to the agonists, arachidonic acid, collagen or TRAP. Thus the response of platelets in patients with coronary artery disease taking dual antiplatelet therapy is different from normal healthy volunteers.

Compliance is a confounding variable in the assessment of patients on antiplatelet therapy. Indeed, it has been suggested that a lack of response to antiplatelet therapy may simply be explained by non-compliance [23]. To address the issue of compliance, all patients in this study were questioned in detail regarding their antiplatelet therapy. We excluded those individuals who had not taken their medication as prescribed in the previous 14 days. Arachidonic acid induced platelet aggregation of greater than or equal to 20% has been proposed as a definition of response to aspirin [14, 24-28]; however the definition of clopidogrel non-response

remains unclear [29, 30]. Since aspirin non-responsiveness may be due to non-compliance, we also excluded patients from our study whose platelets had a response to arachidonic acid of ≥20%. It has been suggested that an analysis of platelet response to antiplatelet therapy in inpatient and outpatient groups should be performed in interpreting this type of study as antiplatelet therapy is better controlled in an inpatient setting [18]. Therefore, we compared the response to arachidonic acid between the inpatient and outpatient populations. We found no significant difference in the platelet response to aspirin between inpatients and outpatients. Thus it seems unlikely that our results may be attributed to non-compliance with therapy.

In our investigation smoking, hypertension and diabetes significantly influenced platelet reactivity based on the response to epinephrine ($p<0.05$). This is in agreement with previous studies suggesting that these risk factors influence platelet reactivity [31-36]. Recent evidence from two studies examining platelet reactivity in normal volunteers and in patients with type II diabetes have demonstrated when a subject has increased platelet reactivity to one agonist that they have increased platelet reactivity to all agonists [6, 13]. These results are in marked contrast to the current study where patients on dual antiplatelet therapy had an increased response to only epinephrine and ADP. The reasons for this divergence are not readily apparent, but do not seem to be related to the drugs used in our population. It is interesting to speculate that the mechanisms of increased platelet reactivity in patients with coronary artery disease are different from both normal volunteers and type II diabetics. A unifying hypothesis that could explain the observed association between smoking, hypertension and platelet reactivity could be enhanced sympathetic activity.

There is a genetic component in coronary artery disease and in platelet reactivity in normal healthy volunteers [37, 38]. Recent work by Bray *et al* has

demonstrated that a component of the platelet response seen *in vitro* in patients with coronary artery disease is at least in part mediated by genetic factors [39]. Whether our results are due to common environmental factors shared in this cohort of patients or genetic factors is unclear. The increased response to the agonists ADP and epinephrine in contrast to other agonists suggests a common pathway possibly through adenylate cyclase that could be modified by either genetic (hypertension) or environmental factors (smoking) in patients with coronary artery disease. This pathway warrants further investigation as a marker of potential risk for future thrombotic events in patients with coronary artery disease.

In summary, we have developed an assay that is rapid, inexpensive, and capable of assessing multiple platelet receptors and pathways simultaneously in patients on dual antiplatelet therapy. In contrast to normal volunteers and diabetics, we found that patients with cardiovascular disease on dual antiplatelet therapy have increased platelet reactivity in response to epinephrine and ADP alone, which does not occur in response to the agonists, collagen, arachidonic acid and TRAP, in patients with coronary artery disease compliant with their antiplatelet medication. In this study we observed substantial interindividual variability in response to epinephrine, more so in the patients compared to the healthy volunteers. Whether more aggressive antiplatelet therapy is warranted in this group remains to be determined.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interests.

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Legends

Table 1 Patient Demographic data

Smoking, hypertension and diabetes significantly influenced platelet reactivity in response to epinephrine *($p < 0.05$). PPI, proton pump inhibitor, ACS, acute coronary syndrome, ACE, angiotensin converting enzyme, AT-II, angiotensin II.

Fig. 1. Dose response curves using 5 different agonists in 80 healthy volunteers. The curves represent aggregation responses to increasing concentrations of arachidonic acid, collagen, ADP, epinephrine and TRAP in 80 healthy volunteers. Each data point represents the mean aggregation (%) to each concentration plus or minus the standard error of the mean.

Fig. 2. Maximal platelet aggregation in response to epinephrine is variable in the presence of dual antiplatelet therapy (*** $p < 0.0001$)

Fig. 3. Increased platelet reactivity to epinephrine is not associated with increased platelet reactivity to arachidonic acid, collagen or TRAP

Figures 3(A), (B) & (C) demonstrates a non-significant difference between each epinephrine quartile for the agonists, arachidonic acid, collagen and TRAP, respectively. ns, non-significant

Fig. 4. Increased platelet reactivity to ADP is associated with increased platelet reactivity to epinephrine.

The increasing linear trend seen in response to epinephrine is also seen in response to ADP ($p < 0.0001$). * $p < 0.01$, ns, non-significant, ** $p < 0.005$

Table 1 Patient Demographic data

	Entire cohort (n=146)	**1st Quartile (n=37)**	**2nd Quartile (n=36)**	**3rd Quartile (n=36)**	**4th Quartile (n=37)**	**p Value**
Age(yrs)	**64±12**	64±15	65±10	65±11	63±12	0.88
Male, n (%)	**94(64)**	26(70)	23(64)	19(53)	26(70)	0.76
Risk Factors: n (%)						
Diabetes	**25(17)**	5(14)	2(6)	8(22)	10(27)	*
Smoking history	**107(73)**	21(57)	28(78)	26(72)	32(86)	*
Hypertension	**78(53)**	17(46)	20(56)	22(61)	26(70)	*
Dyslipidaemia	**96(66)**	27(73)	21(58)	22(61)	26(70)	0.88
Family History	**71(48)**	21(57)	12(33)	14(39)	24(65)	0.42
Past Vascular disease	**84(58)**	24(65)	16(44)	23(64)	21(57	0.88
ACS	**71(48)**	15(40)	17(47)	20(56)	19(51)	0.27
Medications: n (%)						
PPI	**99(68)**	23(62)	20(56)	29(80)	27(73)	0.09
β-Blocker	**125(86)**	31(84)	31(86)	31(86)	32(86)	0.75
Statin therapy	**137(94)**	33(89)	35(97)	33(92)	36(97)	0.28
ACE	**77(53)**	20(54)	20(55)	19(53)	18(49)	0.60
AT-II	**31(21)**	7(19)	5(14)	10(28)	9(24)	0.32

Smoking, hypertension and diabetes significantly influenced platelet reactivity in response to epinephrine * ($p < 0.05$). PPI, Proton Pump Inhibitor, ACS, Acute Coronary Syndrome, ACE, Angiotensin Converting Enzyme, AT-II, Angiotensin II.

Fig. 1

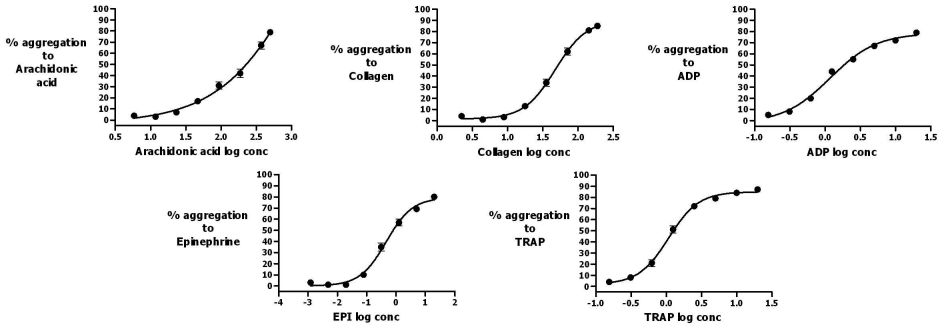


Fig. 1. Dose response curves using 5 different agonists in healthy volunteers. The curves represent aggregation responses to increasing concentrations of arachidonic acid, collagen, ADP, epinephrine and TRAP in 80 healthy volunteers. Each data point represents the mean aggregation (%) plus or minus the standard error of the mean.
212x84mm (300 x 300 DPI)

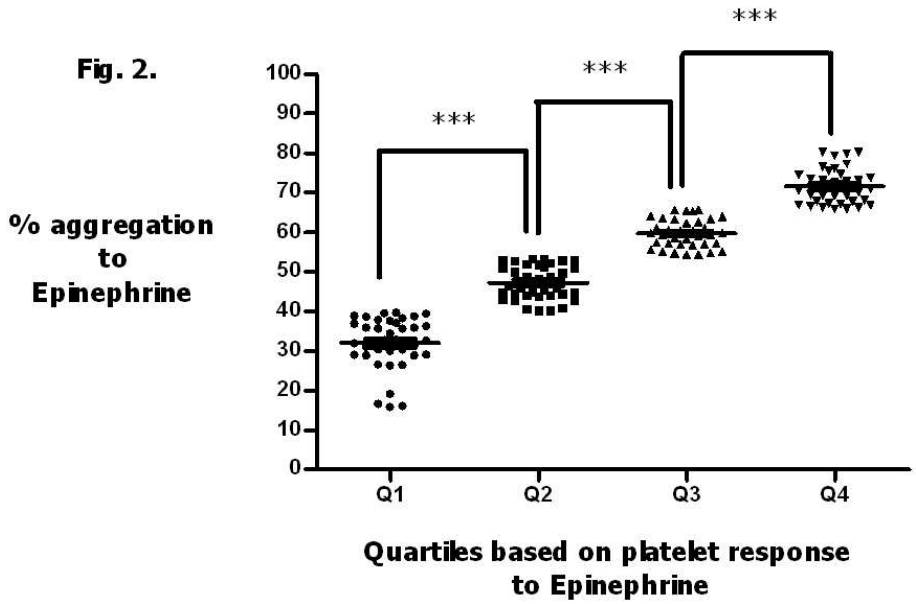


Fig. 2. Maximal platelet aggregation in response to epinephrine is variable in the presence of dual antiplatelet therapy (*** $p < 0.0001$)
81x53mm (300 x 300 DPI)

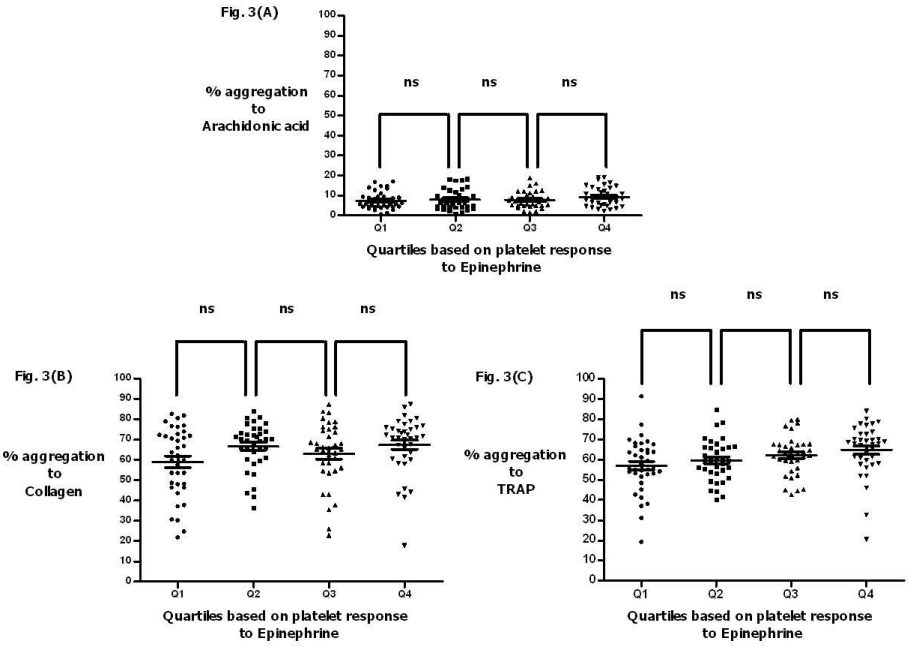


Fig. 3. Increased platelet reactivity to epinephrine is not associated with increased platelet reactivity to arachidonic acid, collagen or TRAP Figures 3(A), (B) & (C) demonstrates a non-significant difference between each epinephrine quartile for the agonists, arachidonic acid, collagen and TRAP, respectively. TRAP = Thrombin Related Activated Peptide, ns=non-significant
113x80mm (300 x 300 DPI)

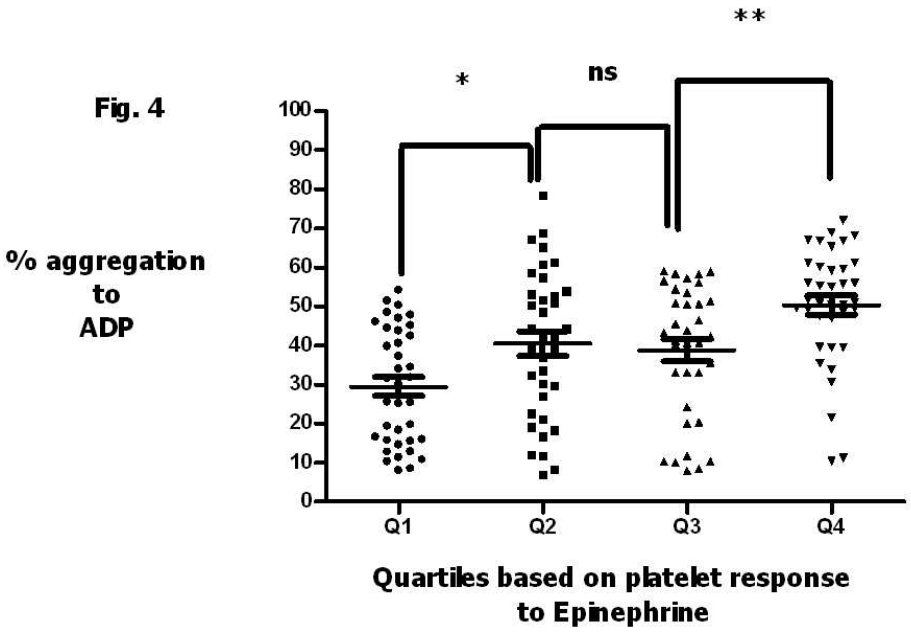


Fig. 4. Increased platelet reactivity to ADP is associated with increased platelet reactivity to epinephrine. The increasing linear trend seen in response to epinephrine is also seen in response to ADP ($p < 0.0001$). * $p < 0.01$, ns, non-significant, ** $p < 0.005$
81x55mm (300 x 300 DPI)

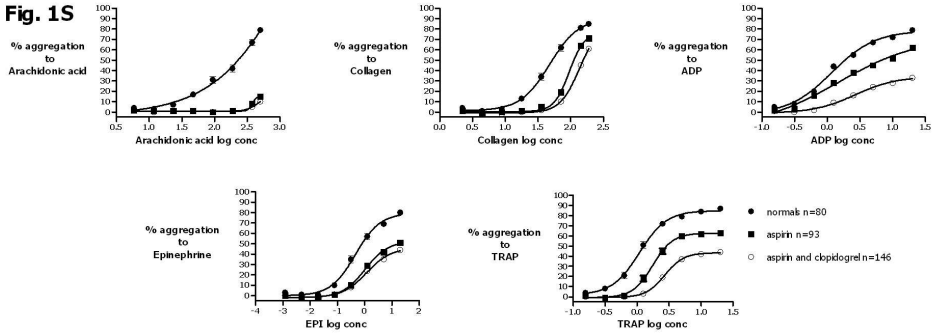


Fig. 1S. The 96 well platelet function assay detects the effect of both aspirin and clopidogrel across all agonists. This demonstrates that aspirin inhibits platelet aggregation to the agonists, arachidonic acid, collagen, ADP, epinephrine and TRAP. The addition of clopidogrel leads to further platelet inhibition. ● normals n=80, ■ on aspirin n=93, ○ on aspirin and clopidogrel n=146
166x65mm (300 x 300 DPI)

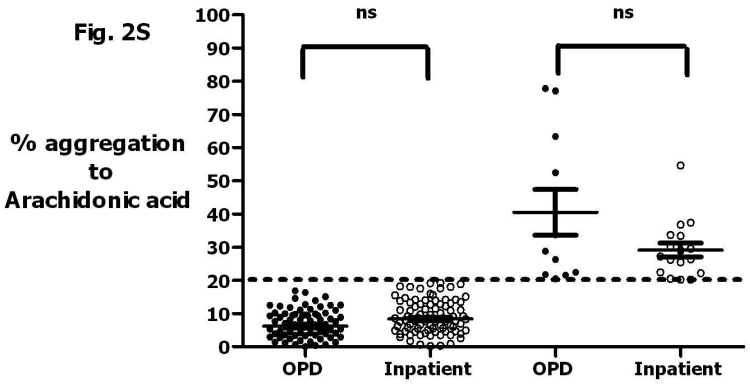


Fig. 2S. Platelet aggregation induced by arachidonic acid is the same in the inpatient and outpatient population Variation in response to antiplatelet therapy may be related to compliance. To address this we excluded those individuals with an aggregation response greater than 20%. To examine this further we determined whether the response to arachidonic acid varied in the outpatient versus the inpatient population and found that the relative proportion of patients was non-significantly different.
151x70mm (300 x 300 DPI)

Fig. 3S

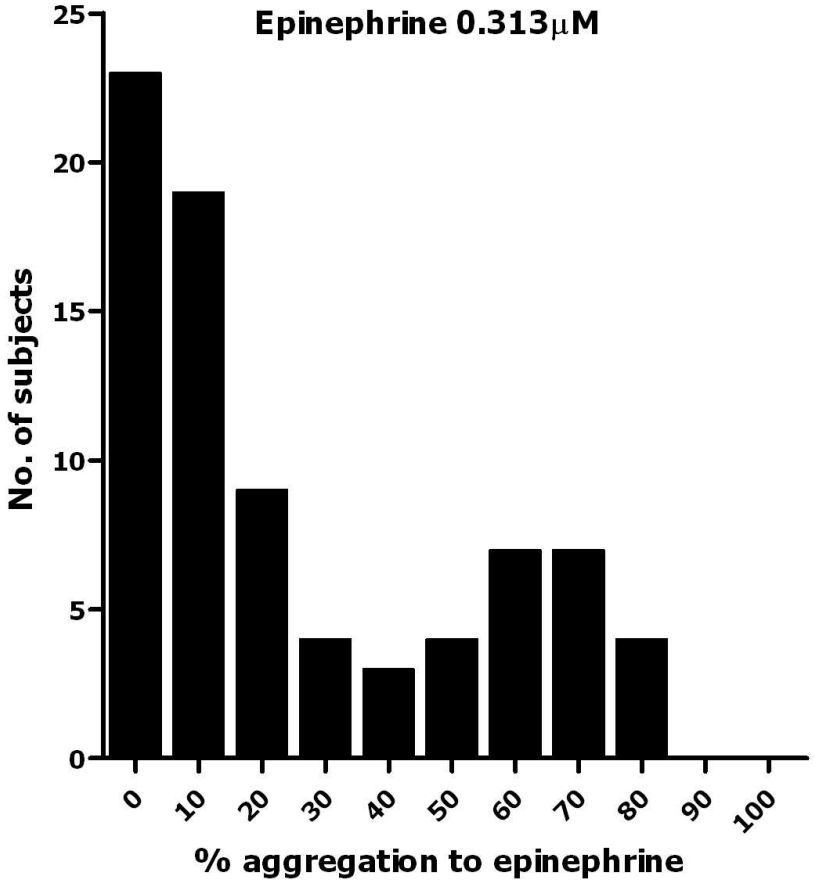


Fig. 3S. There is a bimodal distribution of aggregation in response to submaximal concentrations of epinephrine in healthy volunteers (n=80). This histogram depicts the number of subjects with a given level of aggregation to epinephrine at a concentration of 0.313μM.
108x103mm (300 x 300 DPI)

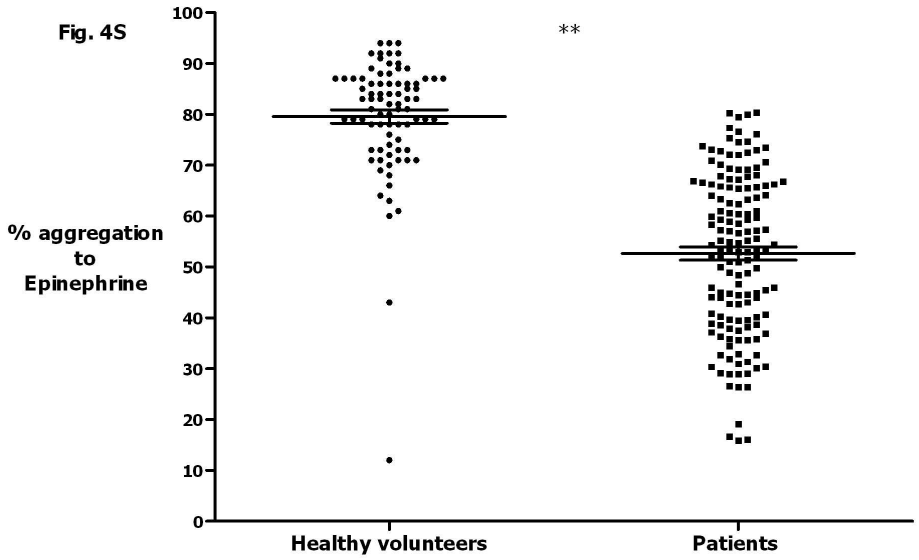
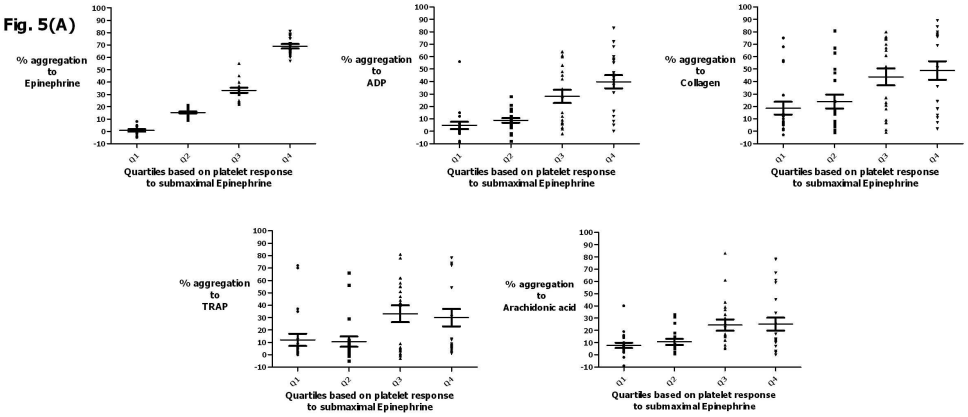


Fig. 4S. There is wider variability in platelet responses to epinephrine in patients taking dual antiplatelet therapy compared to healthy volunteers. The aggregation response to the maximum concentration of epinephrine was plotted for healthy volunteers (n=80) and patients taking dual antiplatelet therapy (n=146). The interindividual variability was significantly wider in patients compared to healthy volunteers. ** p=0.0071.
169x101mm (300 x 300 DPI)



207x96mm (300 x 300 DPI)

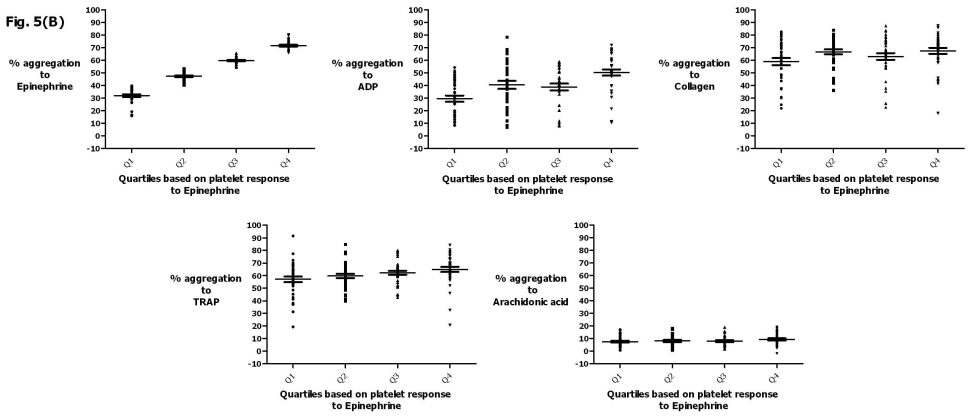


Fig. 5S (A) & (B). Healthy volunteers demonstrate global hyperreactivity in contrast to patients on dual antiplatelet therapy. In healthy volunteers shown in panel (A) the response to epinephrine predicted a greater response to all agonists whereas in the patients taking dual antiplatelet therapy seen in panel (B) the response to epinephrine was only seen in response to ADP and not to the other agonists.
255x116mm (300 x 300 DPI)