A Novel method of Thrombelastograph analysis aids assessment of antiplatelet therapy in percutaneous coronary intervention.

A thesis submitted to the University of London for the degree of Doctor of Medicine (Research).

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Alex Hobson

Barts and the London School of Medicine and Dentistry, Queen Mary, University of London.

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4 Declaration

No portion of this work has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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7 Acknowledgments

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8 The Author

I am currently a Clinical Fellow in Interventional Cardiology, completing my 6th year of Specialist Registrar training in Cardiology with a sub-specialty interest in coronary intervention. I am in the process of applying for Consultant posts. I embarked on this study to increase my knowledge of clinical research, as I believe it is particularly important to have an in depth understanding of the processes involved to allow me to make the most of the vast evidence base in percutaneous coronary intervention. Specifically I aimed to study the use of adjunctive pharmacology without which percutaneous intervention would not have become the powerful therapeutic tool which it is today.

9 Glossary

ACS	Acute Coronary Syndrome
PCI	Percutaneous coronary intervention
ST	Stent thrombosis
TEG	Thrombelastography
AA	Arachidonic acid
ADP	Adenosine diphosphate
MI	Myocardial infarction
VWF	Von Willebrand's Factor
STEMI	ST-segment elevation myocardial infarction
LTA	Light transmittance aggregometry
NSTEMI	Non ST-segment elevation myocardial infarction
DES	Drug eluting stent
cAMP	Cyclic adenosine monophosphate
EDTA	Ethylenediamine tetraacetic acid
VASP	Vasodilator-stimulated phosphoprotein phosphorylation
Act F	Activator F
MA	Maximum amplitude
AUC	Area under the response curve
COX-1	Cyclooxygenase – 1
Pmap	PlateletMapping

10 Abstract

Background

A near-patient test of responses to antiplatelet therapy would be of great value in percutaneous coronary intervention (PCI). However, at present, there are no widely accepted tests and varied definitions of "resistance".

Study aim

To establish whether (i) a novel modification of thrombelastography (short TEG) can detect individual responses to Aspirin and Clopidogrel in 15 minutes and if so (ii) establish normal ranges of response (iii) test responses in patients with previous stent thrombosis (ST) and (iv) compare results with the VerifyNow system.

Methods

(A) Thirty volunteers and 85 patients undergoing PCI were recruited. Blood tests were taken before and after loading doses of Aspirin and Clopidogrel and from stable patients on maintenance therapy with either Aspirin or Aspirin & Clopidogrel.
(B) Survivors of ST were identified from 3004 PCI patients and matched to patients without ST.
Analysis was performed using conventional TEG parameters, the novel short TEG Area under curve at 15 minutes (AUC15) and % clotting inhibition (%CIn) and VerifyNow.

Results

(i) Short TEG reliably detects effects of both Aspirin and Clopidogrel in 15 minutes. The short TEG %CIn correlates closely with response to treatment calculated by comparison with baseline ($R=0.93$, $p<0.001$ for Aspirin and $R=0.83$, $p<0.001$ for Clopidogrel).
(ii) Short TEG identifies important effects of gender on observed responses and evidence of cross-reactivity between Aspirin and Clopidogrel.

(ii) In patients with previous ST there were no differences in responses to Aspirin but greater reactivity whilst on Clopidogrel when assessed with short TEG and VerifyNow.
(iv) Short TEG correlated closely with results obtained with VerifyNow.

Conclusions

This modification has potential value in the rapid identification of patients responding poorly to Aspirin & Clopidogrel and as a bedside tool has potential to reduce risk of adverse clinical outcomes following PCI.

11 Introduction

As our understanding of the pathophysiology of cardiovascular events such as Myocardial Infarction (MI) and Acute Coronary Syndromes (ACS) evolves, the integral role of the platelet is increasingly recognised. Plaque rupture, platelet activation and aggregation and thrombus formation occur as a result of complex interactions between platelets, vascular endothelium, inflammatory cells and circulating proteins. These processes can result in vascular occlusion, ischaemia and infarction. Similarly during treatment, coronary vessel trauma and inflammation induced during the process of percutaneous coronary intervention (PCI) and stent implantation, as well as the poorly understood role of subsequent stent endothelialisation combine to make some patients susceptible to adverse thrombotic event, including stent thrombosis (ST).

Clinical studies have repeatedly shown that antiplatelet therapies improve outcomes in cardiovascular disease, including those patients undergoing PCI. As a result there has been increased awareness of the importance of antiplatelet therapies in both primary and secondary prevention of cardiovascular disease and in the treatment of acute events such as MI and ACS.

However, there is now well established evidence that responses, in terms of platelet function, are heterogeneous, with substantial variation in response to antiplatelet therapies. Despite this, conventional treatment with oral antiplatelet agents (such as Aspirin and Clopidogrel) involves administration of standard doses to all patients. Whilst it is still unclear to what extent variation in platelet function tests performed in isolation correlate with genuine effects on clotting tendency the current strategy of universally applied loading and maintenance doses of antiplatelet agents for all patients with coronary artery disease (CAD), including those undergoing PCI, may be improved. Some patients have a weak response and lack therapeutic protection, whereas others have an excessive response and are more susceptible to bleeding. Measuring patient responses to antiplatelet therapies may therefore be important in a) monitoring the therapeutic efficacy of antiplatelet therapies, b) guiding treatment modification, and c) improving outcomes by optimising antiplatelet therapy on an individual patient basis. There are however many unanswered questions, including:

(i) In whom should responses be monitored?

(ii) Which is the best test to use?

(iii) What cut-off values correspond to clinical outcome measures?

(iv) How should therapy be altered in those found to respond poorly?

(v) Would these approaches lead to improved outcomes?

A rapid and reliable method of assessing the contribution of platelets to clotting would be of considerable clinical value in answering these important questions and may help to improve outcomes for patients. It remains to be established however if identifying patients who appear to lack therapeutic protection and modifying their subsequent treatment would improve outcome.

11.1 The role of the platelet

Platelets were first described by Max Schultze (1825-1874) in 1865 and are now known to be vital components of normal haemostasis, adhering to injured vessel walls to restrict blood loss. However, they are also key participants in "pathological" thrombosis and the critical role of platelets in coronary artery thrombosis, stroke and peripheral vascular disease is now well understood.

Platelets are produced from bone marrow megakaryocytes at a rate of 10^{11} a day. Their normal lifespan in the circulation is 7-10 days explaining why the action of irreversible antiplatelet therapies (such as aspirin and clopidogrel) diminishes relatively rapidly with time. They are discoid in shape, measure 1.5-3 microns in diameter, are anuclear but retain the capacity to splice messenger RNA and synthesise some proteins. They also contain mitochondria and several types of granules, the contents of which are released upon activation.

Platelet activation is brought about by activators such as collagen (exposed when the vascular endothelial lining is damaged), thrombin (primarily through the protease-activated receptor-1 (PAR-1)), Adenosine diphosphate (ADP) (through P2Y1 and P2Y12 receptors) and other activators. Activation results in the release of granules which

contain coagulation factors, vasoconstrictors and other platelet activating factors leading to further platelet activation and platelet aggregation. These granules include dense bodies, (containing substances including ADP, adenosine triphosphate (ATP), serotonin, and calcium) and alpha granules (containing other compounds such as fibrinogen, factor V, vitronectin, thrombospondin and von Willebrand factor).
Platelet activation also results in conformational change to the platelet with the transport of negatively charged phospholipids to the platelet surface. These provide a catalytic surface further enhancing the cascade of platelet and coagulation system activation and increasing thrombin generation. Platelets also contract during aggregation (they have a high concentration of actin and myosin filaments) leading to clot retraction and strengthening.

11.1.1. Role of the platelet in plaque formation

Normal healthy vascular endothelium prevents platelet activation and adhesion through the production of inhibitory prostaglandins and nitric oxide. As a result the receptors on circulating platelets have a low affinity for platelet agonists, are unable to bind circulating soluble fibrinogen, and therefore platelet activation and clot formation does not occur. However, local disturbance in blood flow (e.g. at the site of a coronary artery bifurcation) can lead to minor injury of the vascular endothelium and this process is enhanced by known risk factors for cardiovascular disease such as hypertension. Endothelial damage exposes areas of the subendothelium which contain substances (such as collagen, von Willebrandt's factor and fibronectin) which promote platelet adhesion via platelet glycoprotein (or integrin) receptors even in their low affinity conformations. Adherent platelets undergo conformational change which includes the secretion of dense and alpha granules. These contain platelet factors, elastases and collagenases which act to increase vascular permeability and platelet derived growth factors which lead to vascular smooth muscle proliferation and migration into the intimal layer. Platelets therefore have an important role in initial plaque formation. Thus, a vicious circle can form where plaque formation leads to further disturbance in local blood flow and further injury to the

endothelium leading to further increases in platelet activity and subsequent plaque formation.

11.1.2. Role of the platelet in inflammation

The idea that platelets act as inflammatory agents is not new and was first proposed by Giulio Bizzozero in 1882. Inflammation is now known to be due to be a complex process characterized by interactions between leucocytes, endothelial cells and platelets. During adhesion to damaged endothelium activated platelets secrete pro-inflammatory cytokines, chemokines and other inflammatory mediators (such as interleukin-Iß and CD40L) which promote chemotaxis, lead to adhesion of leucocytes to the endothelium, and promote leukocyte activation and subsequent migration into subendothelial tissue (1,2). In addition platelets in atherosclerotic lesions secrete a variety of substances (such as platelet activating factor and macrophage inflammatory protein-1α) which promote further leukocyte chemotaxis and others (such as transforming growth factor ß, platelet derived growth factor and serotonin) which stimulate smooth muscle cell, fibroblast and collagen synthesis. Platelets (via their effect on chronic inflammation) therefore have a central role in plaque formation. In addition acute inflammation is now known to have a crucial role in thinning of the fibrous cap of atherosclerotic plaques and subsequent plaque rupture (3).

As well as the now well understood role of platelets in inflammation secondary to tissue injury it is also increasingly understood that platelets have important roles in both innate immunity and adaptive responses to microbial and antigen challenge (4).

11.1.3. Role of the platelet in thrombosis

Atherosclerotic plaques are rich in collagen, fibronectin, thrombospondin and tissue factor which when exposed promote platelet adhesion and activation via specific receptors. As highlighted above platelet adhesion and activation leads to morphological change with the formation of pseudopodia (pici) and granule secretion. The secretion of these granules, containing substances which are themselves platelet agonists, leads to additional platelet activation. Activated platelets promote the formation of thrombin

from prothrombin. Thrombin is the most potent activator of platelets and cleaves fibrinogen to form fibrin. This leads to further platelet activation, platelet aggregation and the formation of a platelet and fibrin plug. Plaque rupture initially leads to physiological platelet activation. In some instances a small clot is formed which then undergoes fibrinolysis. However activated platelets release substances (such as Plasminogen Activator Inhibitor-1) which inhibit fibrinolysis and at other times platelet activation and aggregation can become uncontrolled leading to intraluminal thrombus, ischaemia and infarction.

11.2 Antiplatelet therapy

An ideal antiplatelet agent would be safe, well tolerated, cheap, available in tablet form and have predictable, rapid, long lasting and ideally measurable and reversible effects. Importantly its use must improve patient outcomes.

In current clinical practice recurrent thrombotic events continue to occur despite the routine use of antiplatelet therapies. As a result there has been a trend towards increased intensity of antiplatelet therapy. However, all antiplatelet therapies increase the bleeding risk and with modern management of ACS / MI bleeding rates are increasing (5). In addition it is now known that bleeding is associated with an increased risk of recurrent ischaemic events and death even when the bleeding episode itself is not life-threatening (6). Data from the CRUSADE registry suggests that the commonest adverse event post non- ST segment elevation MI (NSTEMI) is now bleeding. In addition post hoc analysis of ACUITY has shown that bleeding post PCI conveys a greater mortality risk than peri-procedural MI (7).

There is a strong association between 30 day bleeding and 1 year mortality (8) and major haemorrhage has been shown to be an independent predicator of 1 year mortality in elective and urgent PCI (9). Even minor bleeding by the GUSTO criteria increases the risk of 30 day mortality by 60% (6).

In addition, in hospital bleeding in the ACUITY study was shown to increase the risk of subsequent ST. As well as the direct bleeding risk the risks of bleeding whilst on antiplatelet therapy often delay cardiac and non-cardiac surgery.

It is therefore no surprise that in some populations (e.g. CHARISMA (10) and ACUITY-PCI (11)) more intensive antiplatelet therapy has been associated with worse outcomes. The potential benefits of antiplatelet agents in terms of reducing ischaemic events therefore need to be carefully balanced with the increased risks of bleeding.

Current antiplatelet therapy includes Aspirin, the thienopyridines (principally Clopidogrel) and Glycoprotein IIb/IIIa inhibitors. These therapies are widely utilised. It is estimated that in the USA 29 billion Aspirin tablets are taken annually and sales of Clopidogrel totalled $3.77 billion in the 12 months to June 2006 (12).

11.2.1 Aspirin

Hippocrates records using an extract of willow bark to treat pain. The active compound (salicin) was isolated in the 1820's. Acetylsalicylic acid (Aspirin) was first produced in the 1850's and patented and marketed by Bayer in 1889. Aspirin is known to cause platelet inhibition by irreversible acetylation of cyclooxygenase-1 (COX-1) through the acetylation of serine residue 529, hence preventing conversion of Arachidonic Acid (AA) to prostaglandin –H and subsequent formation of the potent vasoconstrictor and platelet activator thromboxane A2. Aspirin has a rapid onset of action. Its half-life in the circulation is only 20 minutes but as its activity is irreversible the effects are relatively long lasting allowing dosing every 24 to 48 hours. In normal adults the antithrombotic effect of Aspirin is saturable at doses in the range of 75 – 100mg (13).

It is well established that long-term use of Aspirin in patients with vascular disease decreases morbidity and mortality from cardiovascular events by 25% and it is a cornerstone of secondary prevention treatment in the setting of coronary artery disease (14). In ACS Aspirin reduces the risk of progression to death and MI by 50% (15) and in ST segment elevation myocardial infarction (STEMI) Aspirin reduces the risk of death by 23% (16).

The role of Aspirin in primary prevention is still the subject of debate, although most trials support its use in high-risk patients (17); the potential benefit must however be

balanced with risk of bleeding. Current guidelines recommend a loading dose of >160mg if a rapid onset of action is required and 75-100mg daily thereafter (13).
Aspirin is also the cornerstone of treatment to prevent ST after PCI and stent insertion. Historically it was used as monotherapy or with anticoagulants with disappointing results. It is now utilised in combination with thienopyridines.

11.2.1.1 Current guidelines

Current UK guidelines recommend the use of Aspirin 75mg daily in all patients with established cardiovascular disease, for individuals over 50 with a 10 year cardiovascular disease risk of >20% and for diabetics receiving treatment for hypertension, over the age of 50 years or who have had diabetes for > 10 years. Aspirin 75mg is also recommended in patients with lone atrial fibrillation without other risk factors for thromboembolism.
For patients undergoing PCI American guidelines recommend 75-325mg before the procedure (300-325mg if not on chronic maintenance therapy at least 2 hours and preferably 24 hours before the procedure) and 325mg daily for at least 1 month after bare metal stents (BMS), 3 months for sirolimus eluting stents and 6 months for paclitaxel eluting stents. Chronic Aspirin therapy should be continued indefinitely after this point at a dose of 75-162mg daily (18).
European guidelines recommend that in patients not on chronic maintenance therapy an oral loading dose of 500mg Aspirin should be administered at least 3 hours prior to PCI, or at least 300mg given intravenously immediately prior to the procedure (19).
European guidelines state that there is no need for doses greater than 100mg daily for maintenance therapy post PCI and stent insertion.

11.2.1.2 Assessment of responses to Aspirin

Aspirin resistance is a genuine entity although difficult to define precisely and is reported in up to 59.5% of patients with stable coronary artery disease (20-23). Recent studies using methods which specifically analyse platelet aggregation in response to AA stimulation (COX-1 specific methods including modified thrombelastography (TEG)) suggest that the true incidence may be much lower (24,25); these studies have also

highlighted the importance of compliance. Attenuated responses to Aspirin on platelets have been reported in obese subjects (26), smokers (27) and in diabetics (28).
Some studies have shown that platelets from Aspirin-resistant patients appear to be more sensitive to the actions of ADP suggesting that the addition of alternative antiplatelet therapies that inhibit ADP-induced platelet aggregation to these patients would be therapeutically useful (29). It is possible that increased sensitivity to ADP and other platelet activators in some patients explains why assays that are not entirely specific to AA-induced activation give higher estimates on the incidence of Aspirin resistance. It is however possible that Aspirin has some effect on platelets via non COX-1 mediated pathways. For example Gurbel et al have shown that despite complete inhibition of COX-1 with low dose Aspirin (81mg), as assessed with AA-induced light transmissance aggregometry, there appeared to be dose-dependent increases in inhibition of collagen induced aggregation with higher doses of aspirin (30). Non COX-1 specific assays such as the VerifyNow Aspirin assay have also shown an apparent dose response with most non-responders to 81mg of Aspirin responding to 325mg (27).
However, the risk reduction observed with Aspirin therapy appears consistent for a wide range of doses (75mg to 1500mg daily) although doses lower than 75mg daily do appear to be associated with a significantly increased risk and higher doses are associated with higher rates of bleeding (15,31). In addition it has been suggested that enteric coated Aspirin, particularly at lower doses may delay the action of Aspirin (32).

11.2.2. Ticlopidine

Aspirin acts predominantly via one relatively weak pathway of platelet activation and despite its use thrombotic events still occur. Alternative or additional antiplatelet therapy may therefore provide additional benefit.
Ticlopidine is a thienopyridine which irreversibly inhibits the platelet P2Y12 receptor. It is a prodrug which is transformed into its active metabolite by metabolism by hepatic cytochrome P-450.
Ticlopidine is effective in the secondary prevention of MI and stroke (33) and has been shown to be effective in preventing stent occlusion when used in combination with Aspirin. In the FANTASTIC study which compared Aspirin and Ticlopidine with Aspirin

and Warfarin after implantation of BMS there was a dramatic reduction in cardiac events (mainly due to reductions in acute and subacute ST to less than 1%) in the Ticlopidine arm. Haemorrhagic complications were also dramatically reduced in those on Ticlopidine compared to Warfarin (34).

The use of Ticlopidine has now largely been superseded by Clopidogrel (section 11.2.3) due to side effects (principally neutropaenia) and tolerability (35). Neutropaenia occurs in 2.4% and can be fatal, although it usually resolves within 2 to 3 weeks of drug cessation. A full blood count is recommended every 2 weeks for the first 3 months of therapy. Rashes, diarrhoea, nausea, vomiting and abdominal pain all limit its tolerability.

11.2.3 Clopidogrel

Clopidogrel is a thienopyridine that, through irreversible inhibition of platelet P2Y12 receptors inhibits ADP-induced platelet aggregation and inhibits the conformational change of platelets so that fibrinogen can no longer bind to the glycoprotein (GP) IIb/IIIa receptor. It has largely superseded Ticlopidine because of better tolerability and safety and several studies showing that it is at least as effective in preventing stent thrombosis (36-40) and has a faster onset of action (41). Clopidogrel sales worldwide are now worth more than any other drug other than Atorvastatin (12). It is a pro-drug that is metabolized by hepatic CYP3A4 to its active metabolite.

There is now a sound evidence base for the use of clopidogrel and it is now widely used in patients with, or at high risk of, cardiovascular disease. The CAPRIE trial suggested that Clopidogrel 75mg daily was marginally more effective than aspirin in prevention of vascular events in a high-risk population (42). Data from CURE demonstrate that the addition of Clopidogrel to Aspirin in patients with ACS conveys prognostic benefit by reducing further cardiovascular events (43). More recently CHARISMA has yielded a benefit with the addition of Clopidogrel to Aspirin in all patients with symptomatic atherosclerosis although there was a suggestion of harm in those with asymptomatic disease or multiple cardiovascular risk factors alone (44). Furthermore CLARITY and COMMIT provide evidence for its short-term use in STEMI (45,46).

11.2.3.1 Use of Clopidogrel in PCI patients

In the context of PCI and stent insertion a combination of Aspirin and thienopyridine is markedly superior to Aspirin and an oral anticoagulant (34,36,47,48).

As it takes a few days for Clopidogrel to have its full effect, a loading dose is usually administered in the context of PCI followed by 75mg daily. Data from CREDO and PCI-CURE support the use of a 300mg loading dose prior to PCI and long-term (9-12 month) maintenance therapy after stent insertion (49,50). In the CREDO trial outcomes were improved only when the 300mg loading dose was given at least 6 hours prior to intervention (49). Further, a *post hoc* analysis of this trial suggests that 28 day outcomes were only significantly improved when the loading dose was administered at least 15 hours prior to angioplasty (51). Several studies have suggested that a 600mg loading dose provides additional benefit. ISAR-REACT and ISAR-SWEET trials strongly suggested a beneficial clinical effect of the 600mg loading dose, but were not randomised trials comparing 600mg to 300mg doses (52,53). The ARMYDA-2 trial randomised 255 patients to either 300 or 600mg loading doses of clopidogrel 4 to 8 hours prior to PCI. There was a significant decrease in cardiovascular events in the 600mg group with no increase in the haemorrhagic risk (54). A further randomised trial of ACS patients receiving loading doses of Clopidogrel at least 12 hours prior to PCI also found a significant decrease in cardiovascular events at 1 month in those receiving 600mg (55). These results are contradicted by the ARMYDA – 5 study comparing pre-treatment clopidogrel with treatment at the time of PCI (56), and a large study of nearly 2500 patients which failed to show improved outcomes with doses of >300mg (57). However the sample size in ARMYDA-5 was small and the larger study was retrospective and compared the total dose of Clopidogrel administered in a 24 hour peri-procedural period (10 hours prior to and 14 hours after PCI). This study compared two groups of over 1000 patients: One group receiving 300mg and a second receiving a mean of 458mg. The majority in the "high dose" group only received 375mg and all those in the "low dose" group who had percutaneous intervention would have gone on to receive maintenance Clopidogrel but by chance did not receive this during the studied 24 hour period.

There is therefore evidence of considerable benefit from Clopidogrel in large studies on diverse groups of patients, although there remains controversy about optimal doses.

11.2.3.2 Current guidelines

American guidelines suggest that a 300mg loading dose of Clopidogrel should be administered at least 6 hours before PCI. They recommend that doses of greater than 300mg are reasonable to achieve higher levels of antiplatelet activity more rapidly. These guidelines recommend that dual antiplatelet therapy with Aspirin and Clopidogrel 75mg daily is continued for at least 1 month and up to 1 year after insertion of a BMS, for at least 3 months after sirolimus eluting stent insertion and 6 months after paclitaxel eluting stents. They recommend that dual antiplatelet therapy is continued for 12 months in patients receiving drug eluting stents (DES) who are not at high risk of bleeding (18). European guidelines recommend a loading dose of 300mg of Clopidogrel in patients with stable angina at least 6 hours prior to PCI and the same loading dose as soon as possible in ACS patients. They recommend dual antiplatelet therapy with Aspirin and Clopidogrel 75mg daily for at least 3-4 weeks after insertion of a BMS in stable angina, 9-12 months after insertion of any stent in ACS and 6-12 months after insertion of DES in stable angina (19).

11.2.3.3 Assessment of antiplatelet effect of Clopidogrel

There is considerable inter-individual variability in platelet inhibition in response to Clopidogrel (58-61). Gurbel et al showed an almost normal distribution of response to a 300mg loading dose of Clopidogrel followed by 75mg daily in patients undergoing PCI and stent insertion using ADP-induced aggregation (62). These findings have been replicated in stable patients undergoing planned PCI (63), and in a combination of over 500 patients and healthy volunteers using ADP-induced light transmittance aggregometry (LTA) (60). Patients with a raised body mass index, metabolic syndrome (64), type two diabetes mellitus (65) and patients with ACS (66) have an increased sensitivity to ADP (leading to increased platelet adhesion and aggregation to ADP and less observed response to Clopidogrel). Genetic variability in CYP3A4 may also lead to differences between individuals (67).

There may also be an effect of initial loading dose on observed responses. Six hundred mg loading doses have shown increased speed of onset and less variability in response compared to 300mg doses (68,69) and an associated improvement in outcome (the ARMYDA-2 study was not powered for outcome measures but did show lower levels of peri-PCI biomarkers (54)). ALBION and ISAR-CHOICE have included doses up to 900mg. ALBION suggested an increased effect with 900mg doses (70). However, in ISAR-CHOICE there was no difference in effect between the 600 and 900mg loading doses and also no difference in levels of the active metabolite of Clopidogrel suggesting that absorption or metabolism may be a limiting factor (71). Using 45 healthy volunteers Price et al also showed no difference in the magnitude of response or the time to maximal response using VerifyNow P2Y12 assays between 600mg and 900mg loading doses but again confirmed the superiority of both doses compared to 300mg in terms of platelet inhibition (72). Two sequential 600mg loading doses given 24 hours apart have also been shown to increase platelet inhibition compared to a single dose (73).

The dose of maintenance therapy also has a role in observed responses. The standard and licensed maintenance dose of Clopidogrel is 75mg daily. However studies have shown that a 600mg loading dose of Clopidogrel administered to patients on maintenance therapy with 75mg daily leads to additional inhibition of platelet aggregation (74) and it has also been shown that maintenance therapy with 150mg Clopidogrel leads to greater inhibition of platelet aggregation than a 75mg daily dose when assessed with optical aggregation and VerifyNow (75,76).

There is however, ongoing debate as to the optimal method of assessment and whether existing methods of assessment are capable of predicting benefit or risk. There are significant differences in baseline platelet aggregation, and a higher level of baseline aggregation correlates with total mortality at long-term (13 years) follow up (77). It is therefore questionable whether response to treatment (ie percentage change from baseline) is an appropriate measure. There is some evidence that post-treatment platelet reactivity (which would take account of higher baseline activity) is a more valid method of assessment. There is also evidence however that Clopidogrel has a beneficial effect,

even when no differences in platelet reactivity can be measured using existing methods of assessment. For example in the COMMIT study a beneficial effect of Clopidogrel was obtained after only a single 75mg dose, when the vast majority of patients would be classified as non-responders by any currently utilised criteria (46). In addition Clopidogrel is likely to have significant effects that, whilst they may be mediated by platelets, are not measurable. For example Han et al randomised 600 patients to either 150 or 75mg of Clopidogrel for the first month after PCI. All patients then received 75mg Clopidogrel for 1 year (78). They showed no significant differences in outcome at 1 month but a significant difference in MI, revascularisation and major adverse cardiac events at 18 months. This could be explained by a disease-modifying or anti-inflammatory effect of Clopidogrel which cannot be measured using current techniques.

It is also possible that a rebound effect may occur after cessation of Clopidogrel. Ho et al demonstrated a clustering of adverse events in the 90 days after cessation of Clopidogrel (79).

The optimal duration for Clopidogrel therapy is still unknown. There is some evidence that 12 months of therapy is superior to 6 months (80) and data from the GHOST registry suggests that >12 months duration of dual antiplatelet therapy may be beneficial (81).

11.2.3.4 Therapeutic manipulation in poor responders

The ACC/AHA/SCAI 2005 guidelines recommend increasing the dose of Clopidogrel maintenance therapy in patients at high risk from ST in whom Clopidogrel response is suboptimal. However, there have been few studies into therapeutic manipulation, particularly looking at outcome measures, and the level of evidence is only IIb/C.
The OPTIMUS study looked at 64 Diabetic patients (76). The maintenance dose of Clopidogrel was randomised to either 75mg or to 150mg daily in the 40 initial poor responders. The level of response (as measured by LTA) was improved with the 150mg dose. However there was still wide variability in the observed response and only 40% of the initial “non-responders” became responders at the higher dose.

Two trials are in progress which will help ascertain whether higher doses of Clopidogrel may improve outcome; GRAVITAS and OASIS-7. In addition ISAR-SAFE will provide data on the optimal duration of Clopidogrel therapy post PCI.

11.2.4 Glycoprotein IIb/IIIa inhibitors

Platelet activation leads to conformational change in glycoprotein IIb/IIIa inhibitors. This increases their affinity for binding fibrinogen. The final and obligatory pathway of platelet aggregation involves fibrinogen binding glycoprotein IIb/IIIa receptors on different platelets. Glycoprotein inhibitors inhibit this binding of fibrinogen to the platelet glycoprotein IIb/IIIa receptors and therefore inhibit the final common pathway of platelet aggregation.

11.2.4.1 Abciximab

Abciximab is a monoclonal antibody which binds to this receptor preventing the binding of fibrinogen. It has a short plasma half life but strong affinity for the receptor. Some receptors remain occupied for weeks, however overall platelet aggregation returns to normal within 24 to 48 hours of drug cessation. Abciximab also binds vitronectin receptors on endothelial cells (82) and MAC-1 receptors on leucocytes (83), although the clinical significance of this is unknown. Trials have shown benefit in high risk patients undergoing PCI (84,85) and in primary PCI (86-88). A metanalysis has confirmed that the benefit is sustained with a decrease in death and MI up to 3 years of follow up (89). There is also a clinical benefit from Abciximab in NSTEMI where ISAR-REACT 2 showed improvements in MI and target vessel revascularization which were maintained up to 12 months (90). There is however evidence that in the context of adequate pre-loading with Clopidogrel it provides no incremental benefit in elective PCI (90) and no benefit in ACS patients in whom PCI is not planned (92).
It is licensed as an adjunct to antithrombotic and oral antiplatelet agents in complex PCI. The National Institute for Clinical Excellence (NICE) has recommended considering its use in high-risk ACS and NSTEMI when early PCI is indicated but delayed.

11.2.4.2 Eptifibatide and Tirofiban

Eptifibatide is derived from a protein within the venom of the south-eastern pygmy rattlesnake. It binds reversibly to platelets and therefore unlike Abciximab has a short half-life. Tirofiban is a synthetic version of a compound found in the venom of the saw-scaled viper and again has a rapid onset and short duration of action with coagulation returning to normal within four to eight hours of its cessation. Both of these agents are licensed for use in preventing early MI in patients presenting with unstable angina and NSTEMI. Abciximab is the preferred agent for high-risk PCI.

11.2.5. Other available antiplatelet agents

11.2.5.1 Dipyridamole

Dipyridamole exerts an antiplatelet effect through increasing platelet cyclic adenosine monophosphate (cAMP) levels. This is probably achieved through blocking adenosine uptake into red blood cells. Adenosine modulates platelet function via its action on the platelet A2A receptor (which increases cAMP levels). By blocking adenosine uptake Dipyridamole therefore increases the amount of adenosine having this inhibitory effect on platelet function. It is licensed as an adjunct to anticoagulants for patients with prosthetic heart valves and is also used together with Aspirin in the secondary prevention of ischaemic stroke and transient ischaemic attacks. The ESPS-2 study demonstrated that modified release Dipyridamole plus Aspirin is more effective than either alone in the secondary prevention of ischaemic stroke (93).

11.2.5.2 Cilostazol

Cilostazol inhibits c AMP phosphodiesterase III in platelets and also inhibits the production of platelet derived growth factor from endothelial cells (94). It inhibits platelet aggregation and also acts as an arterial vasodilator. Its main use (and license in the UK) is in patients with intermittent claudication (as a vasodilator). There is some evidence however that it may be as effective as Ticlopidine and Clopidogrel after coronary stent implantation (95,96). Concerns have been raised however in a study randomizing the use of bare metal and paclitaxel-eluting stents in which Cilostazol was

used (not randomised) in some patients. In this study 5 out of 37 patients receiving Cilostazol experienced ST compared to 1 of 138 receiving thienopyridines (97).
It has also been studied in combination with aspirin and clopidogrel as triple therapy. It leads to greater inhibition of ADP-induced platelet aggregation than Aspirin and Clopidogrel alone (98). As well as the observed increased antiplatelet effect, the addition of Cilostazol to standard dual antiplatelet therapy in this one study was shown to decrease the rate of ST after coronary artery stent insertion. This study was however non-randomised, included only BMS and had a relatively small sample size.

11.2.5.3 Prasugrel

Prasugrel is a thienopyridine with important differences in its metabolism to Clopidogrel. Both are pro-drugs, however there are faster, more consistent and higher levels of active metabolite after the administration of Prasugrel than after Clopidogrel. As a result there is a more rapid, pronounced and reliable inhibition of platelet activation and aggregation after Prasugrel administration (99). Post hoc analysis of JUMBO-TIMI 26 has revealed that Prasugrel may be associated with decreased thrombotic events when compared to Clopidogrel (100). The TRITON-TIMI 38 trial, a larger phase III trial comparing Clopidogrel (although only a 300mg loading dose and administered in 75% of cases at the time of, rather than before, PCI) with Prasugrel 60mg loading and maintenance therapy for up to 15 months in 13,000 ACS patients demonstrated a significant improvement in net clinical benefit with Prasugrel (all-cause death, MI, stroke and major bleed) with a hazard ratio of 0.87 (101). There was however an increase in bleeding complications, particularly in patients with previous stroke or transient ischaemic attacks in whom the bleeding risk outweighed the benefits and in the elderly and those with a low body weight in whom the benefits were balanced by the risks. Overall treatment of 1000 patients with Prasugrel as compared with Clopidogrel would prevent 23 MI's at the expense of 6 non bypass surgery related TIMI major haemorrhage. Of note the major driver of event rate was peri-procedural MI and early ST. Prasugrel has a more rapid onset of action than Clopidogrel and therefore the use of a relatively low loading dose administered at the time of PCI rather than in advance of the procedure would undoubtedly have favoured Prasugrel. Some of the potential weaknesses of this study

have been answered with PRINCIPLE-TIMI 44. This study has evaluated the antiplatelet effects of Prasugrel 60mg versus Clopidogrel 600mg. This showed higher levels of platelet inhibition with Prasugrel after 30 minutes than Clopidogrel at 6 hours. Therefore Prasugrel may have beneficial effects even when a 600mg loading dose of Clopidogrel is administered in advance of the procedure.
A further trial, TRILOGY ACS is planned which will use a reduced dose of Prasugrel in those sub-groups identified as at high risk of bleeding in TRITON-TIMI 38.

11.2.6 Emerging antiplatelet therapies

There are several antiplatelet therapies currently in phase III clinical trials.

1. **Cangrelor** is a directly acting stabilized ATP analogue which is administered intravenously. It provides rapid and pronounced platelet inhibition via the P2Y12 receptor and may challenge the place of glycoprotein inhibitors in PCI. It has the benefit of rapid recovery of platelet function within 60 minutes of the infusion being discontinued as it has a short plasma half-life of only 3-5 minutes (102).
There remain concerns as to how to best integrate the use of Cangrelor into clinical practice, particularly when to administer loading doses of oral antiplatelet therapies, as Cangrelor inhibits the ability of Clopidogrel and Prasugrel to exert their therapeutic effect (103).

2. **Ticagrelor** (AZD 6140) is an oral directly acting and reversible antagonist of the P2Y12 receptor. It does not need to be metabolized although it is metabolized to an active metabolite which contributes to its effect. The DISPERSE and DISPERSE 2 trials were phase IIa, randomised and blinded studies of Ticagrelor against Clopidogrel and showed faster and more consistent platelet inhibition with Ticagrelor (at doses of 100 and 200mg twice daily) than Clopidogrel 75mg daily (104). These studies highlighted possible side effects of dyspnoea and pauses which may be due to effects of adenosine (105,106). The results of the PLATO trial, a phase III trial comparing outcomes with the use of Ticagrelor and Clopidogrel in over 18,000 ACS patients was presented at the

European Society of Cardiology Congress in 2009. Ticagrelor (a 180mg loading dose followed by 90mg twice daily was used) achieved a statistically significant primary efficacy endpoint (time to first occurrence of death from vascular cause, MI or stroke) over Clopidogrel (a 300mg loading dose with provision for a further 300mg dose at the time of PCI, followed by 75ng daily) with comparable safety data.
There are other potential antiplatelet therapies which are in earlier stages of development:

1. **SCH 530348** is an oral platelet protease-activated receptor-1 (PAR-1) antagonist which has completed phase 2 trials, showing good tolerability and no increase in major bleeding, even when used in addition to Aspirin and Clopidogrel (107).
2. **PR-15; DZ-6976** are agents that inhibit the activity of platelet anti-collagen receptors:
3. **DG-041** is an EP3 receptor antagonists which potentiates the protective effects of Prostaglandin E2.
4. **BX 667** is an orally active reversible P2Y12 inhibitor which has shown a wider therapeutic index than Clopidogrel in preliminary animal studies (108).

11.2.7 Other medication and dietary factors affecting platelet function.

Many other medications have been shown to affect platelet function. Non-steroidal anti-inflammatory drugs other than Aspirin have been shown to increase the bleeding time and to predispose to bleeding after invasive procedures. Even Paracetamol has been shown to have some affect on both in vitro and in vivo platelet function (109). Beta-lactam antibiotics at sustained high doses have also been shown to impair platelet function. Gold, Quinidine, Quinine, Cimetidine, Digoxin, Furosemide, nitrates, Propranolol, calcium channel antagonists, phenothiazines and tricyclic antidepressants have also been associated with an antiplatelet effect but this is unlikely to be clinically relevant in healthy individuals (110). Contrast media (including at the concentrations obtained during diagnostic angiography) also impair platelet function (111,112). Herbal remedies and dietary components such as ethanol, garlic, red pepper, caffeine and fish oils have also been shown to impair in vitro platelet function (113-117).

11.2.8 Systemic disease affecting platelet function

Renal failure and liver failure can cause platelet dysfunction. Uraemia can lead to severe bleeding due to abnormalities of platelet adhesion, aggregation and secretion. Multiple myeloma, Waldenstrom's macroglobulinaemia and various autoimmune disorders are associated with the production of antiplatelet antibodies. Myeloproliferative disorders, leukaemia and myelodysplasia can lead to the production of abnormal and dysfunctional platelets. In addition reticulated (young) platelets have been shown to be harder to inhibit with aspirin (118).

There are also inherited disorders of platelet function such as Glanzmann's thrombasthenia, an autosomal recessive disorder associated with mucocutaneous bleeding due to abnormal glycoprotein IIb/IIIa receptors.

As highlighted in section 11.1.2 there are important interactions between leucocytes and platelets. Leucocytes have an important role in modulating platelet function via CD 39 receptors which convert ATP to ADP and also remove ADP from the circulation. For this reason ATP does not cause platelet activation in platelet rich plasma but does in whole blood (secondary to conversion to ADP via leucocytes). However the overall effect of increased leucocyte numbers is to inhibit platelet function via the removal of ADP.

11.3 Methods of assessing responses to antiplatelet therapy

Historical methods of measuring platelet activation and function are time consuming and cannot be performed at the bedside. Recently, several assays have been developed which show some potential as point of care tests of the effects of antiplatelet medication. These include the PFA-100 (Dade Behring), the Accumetrics VerifyNow system, Plateletworks (Helena Laboratories), the Cone and Plate(let) analyser (DiaMed) and the modified Thrombelastography (TEG) PlateletMapping system (Haemoscope Corporation. Niles, Illinois, USA). Commonly utilised methods of assessment are summarised in Table I overleaf.

Assay	Pros	Cons	Assess responses to Aspirin (A) or Clopidogrel (C)	Methodology
Platelet Aggregometry	Highly specific for platelet aggregation in response to specific platelet agonists.	High cost, poor reproducibility, high sample volume, sample preparation, time and skill required. Aggregation is only one aspect of platelet function	A + C	Measures *ex vivo* glycoprotein IIb/IIIa dependent platelet aggregation. Can be performed on platelet rich plasma by turbidometry or on whole blood by electrical impedance. Response to aspirin is assessed using stimulation with Arachidonic Acid and response to clopidogrel using stimulation with ADP.
VASP Platelet Reactivity Index	Specific biomarker for platelet P2Y12 receptor activation	Costly, complex assay	C	VASP is a protein that is phosphorylated in the presence of P2Y12 stimulation. It is therefore a biomarker of P2Y12 stimulation and can be measured using flow-cytometric based techniques.
Biomarkers of Arachidonic Acid Metabolism	Largely dependent on platelet COX-1	Levels are not platelet specific. Little data to support use	A	Aspirin suppresses production of thromboxane B2, a stable metabolite of thromboxane A2 which can be measured in serum and 11-dehydro-thromboxane B2, measurable in the urine.
PFA-100	Ease of use. Whole blood assay	Non-specific as highly dependent on Von Willebrand factor	A	A whole blood assay measuring time for occlusion of an aperture in a membrane under high stress shear conditions. A cartridge containing a membrane coated with collagen and epinephrine has been used to study the effects of Aspirin.
VerifyNow	Ease of use, automated, rapid, whole blood assay	Further data required on optimal cut-off values	A + C	Rapid, automated whole blood assay measuring agglutination of fibrinogen-coated beads in response to specific agonists for Aspirin, thienopyridines and Glycoprotein IIb/IIIa inhibitors.
Plateletworks	Whole blood assay. Ease of use	Requires cell counter. Little evidence for use	A + C	Calculates platelet aggregation by comparing platelet count in EDTA collection tubes with citrate tubes with collagen and ADP stimulation. Uses standard cell counter.
Thromb-elastograph (TEG) Pmap	Whole blood assay. Information on plasmatic as well as cellular aspects of coagulation	Sample preparation.	A + C	Blood is placed in an oscillating cup within which a torsion wire is suspended. As blood clots, fibrin strands link the cup and torsion wire. The resulting torque generates an electrical signal which is plotted to produce a TEG trace. Specific platelet activators allow the effects of antiplatelet therapies to be detected.

Table I Common assays of response to antiplatelet therapy

11.3.1 The Bleeding time

The first test of platelet function, developed in the early 1900's, was the bleeding time. It utilises a standardized *in vivo* wound and measures the time to cessation of bleeding. This is largely dependent on platelets but is not specific to platelet function, also depending, for example, on the concentration of Von Willebrandt's factor. It is also insensitive, has high inter-operator variability and can lead to scar formation (119). It is therefore no longer recommended for assessing responses to antiplatelet therapy.

11.3.2 Platelet aggregometry

Optical aggregation is the historical gold standard test of platelet reactivity. The Global Platelet Function Working Group recommend that response to Aspirin is assessed using light transmittance aggregation using stimulation with 1mmol AA with residual aggregation of >10% signifying lack of benefit. They also recommend its use to measure responses to Clopidogrel using 10mmol ADP with an aggregation of >50% used to indicate lack of benefit. Its first uses were reported independently by Born G and O'Brien JR in 1962. It can be performed on platelet rich plasma by turbidometry (120,121) or on whole blood by electrical impedance (122). It measures platelet glycoprotein IIb/IIIa dependent aggregation. However as outlined previously this is only one aspect of platelet function and does not directly measure platelet adhesion, the platelets effect on thrombin generation, the release of granules or the initiation of inflammation.

Responses to antiplatelet therapies using platelet aggregometry have been shown to correlate with outcomes (20,123,124). There are however disadvantages including high cost, poor reproducibility, a high required sample volume, the need for sample preparation, the length of the assay time and the level of skill required. As a result it is performed only in specialised situations and is not suitable as a rapid point of care test (125). In addition it measures the effects of antiplatelet therapy on isolated platelets rather than in the context of blood clotting as a whole entity. Whilst this has some advantages (i.e. it is not affected by concurrent anticoagulant medication) it does not

necessarily provide the most clinically relevant information on prediction of the patients' overall risk of arterial thrombosis.

Multiple electrode platelet aggregometry has more recently been reported as a method which can be performed in whole blood in only 10 minutes and has been shown to correlate with standard light transmissance aggregometry (126).

11.3.3 PFA – 100

The PFA-100 is a whole blood assay that measures the time for occlusion of an aperture in a membrane under high stress shear conditions. A cartridge containing a membrane coated with collagen and epinephrine has been used to study the effects of Aspirin. There is evidence of a higher incidence of clinical events in patients found not to respond to aspirin by PFA-100 (127). The PFA-100 is, however, not a clear indicator of the effects of Clopidogrel (128). In addition the results, in common with the bleeding time, are highly dependent on von Willebrandt's factor, which is increased by PCI (129). It is therefore unable to differentiate between increased platelet reactivity due to PCI and a reduced response to Aspirin and is more a marker of generalized high platelet reactivity. However, whilst non-specific high residual platelet reactivity assessed using the PFA-100 may well predict patients at increased risk of thrombotic complications. Indeed, it has been shown to have a higher sensitivity and specificity than AA-induced platelet aggregometry in predicting myocardial necrosis in patients post stenting in the context of NSTEMI (130).

11.3.4 Plateletworks

The plateletworks system uses collection tubes with ethylenediamine tetraacetic acid (EDTA) and collagen and ADP to stimulate platelet aggregation. Platelet aggregation is then examined by measuring platelet count in a standard cell counter. This system is not well studied but there is some evidence for its use in detecting responses to thienopyridines and glycoprotein IIb/IIIa inhibitors (131,132).

11.3.5 Biomarkers of thromboxane metabolism

Aspirin suppresses production of thromboxane B_2, a stable metabolite of thromboxane A_2, which can be measured in serum. 11-dehydro-thromboxane B_2 levels (a stable metabolite found in the urine) can also be assessed. In patients on Aspirin urinary 11-dehydro thromboxane B2 levels have been correlated with clinical outcome (15). However levels are not platelet specific and sensitivity and reproducibility are unknown.

11.3.6 Vasodilator-stimulated phosphoprotein phosphorylation (VASP)

VASP is a protein that is phosphorylated and de-phosphorylated in the presence of P2Y12 stimulation. It is therefore a biomarker of P2Y12 stimulation that can be measured in the serum using flow-cytometric based techniques. It has been shown to correlate to a degree with ADP-induced optical aggregation ($R=0.559$; $p=0.02$) in the assessment of responses to Clopidogrel (133). Two groups have shown significant differences in VASP phosphorylation between patients with previous ST and PCI controls (134,135). Its use in assessment of response to Clopidogrel is supported by the Global Platelet Function Working Group who suggest a Platelet Reactivity Index of >76 equates to lack of therapeutic effect or "resistance".

11.3.7 P-Selectin expression

P-Selectin is a leucocyte adhesion receptor stored in platelets and endothelial cells which is transmitted to the platelet surface upon platelet activation. ADP induced P-Selectin expression can be measured using flow cytometric techniques, as can platelet-leucocyte aggregates, providing an assessment of response to clopidogrel.

11.3.8 Platelet adhesion

The preliminary step in platelet activation is often platelet adhesion. Platelet adhesion (for example to type 1 collagen microfibrils) can be measured in biological milieu, however whilst clinically relevant it is extremely difficult to do in practice.

11.3.9 VerifyNow

The VerifyNow system was formerly known as the Ultegra rapid platelet function analyzer. It is a rapid, automated whole blood assay that measures agglutination of fibrinogen-coated beads in response to specific agonists for Aspirin, the P2Y12 receptor (for thienopyridines) and Glycoprotein IIb/IIIa inhibitors. It therefore works on the principle of turbidimetric aggregometry and measures platelet to platelet aggregation in a glycoprotein IIb/IIIa dependent manner. The fibrinogen coated beads are added to augment this signal. Its use has been approved by the US Food and Drug Administration. Its advantages are that it can be used at the point of care, is easy to use, does not require sample preparation, only requires a 2ml blood sample and that results are rapidly available. Whilst the assay only takes 2 minutes for P2Y12 receptor inhibitors and 5 minutes for Aspirin as citrated samples are used it is recommended by the manufacturers that assays are not run for a minimum of 20 minutes for the P2Y12 receptor assay and 30 minutes for the aspirin assay. Results obtained with the VerifyNow system have been shown to correlate closely with optical aggregation (136-138). In the setting of PCI both Aspirin and Clopidogrel resistance as measured by VerifyNow have been correlated with an increased incidence of peri-procedural myocardial infarction (139,140). Increased platelet reactivity whilst on Clopidogrel assessed with the VerifyNow P2Y12 assay has also been shown to correlate with adverse clinical outcomes after discharge following PCI (140). Response to glycoprotein inhibitors assessed with VerifyNow has also been shown to be an independent predictor of major adverse cardiac events (142).

11.3.9.1 Aspirin assay

This uses AA as an agonist. Malinnin et al studied its use in determining the effects of a 325mg Aspirin loading dose. They found close correlation with ephedrine-induced optical aggregation (143). In the setting of PCI aspirin resistance as measured by VerifyNow Aspirin assay has been correlated with an increased incidence of peri-procedural myocardial infarction (140). Chen et al investigated 151 patients attending for routine PCI. All patients received Clopidogrel pre-loading, a 300mg loading dose of Aspirin > 12 hours prior to the procedure and 75mg of Aspirin on the morning of PCI. 19.2% of patients met the manufacturer's recommended criteria for Aspirin resistance (Aspirin reaction units (ARU) of >550 after treatment). Elevations in CK-MB and Troponin I were more common in Aspirin resistant patients on multivariate analysis (Odds ratio 2.9, p=0.015).

Dichiara et al (144) investigated responses to ADP and collagen stimulation in stable patients with high platelet reactivity to AA (ARU >550). These patients exhibited increased reactivity to ADP and collagen stimulation suggesting that VerifyNow may however identify a generalized high platelet reactivity phenotype, rather than specific response to Aspirin.

11.3.9.2 P2Y12 receptor assay

The P2Y12 receptor assay uses a combination of ADP and prostaglandin E1 to stimulate platelet activation via the P2Y12 receptor. ADP stimulates platelets via both the P2Y12 and P2Y1 receptors. Prostaglandin E1 is utilised to suppress the ADP-induced P2Y1-mediated increase in intracellular calcium levels. Malinin et al performed a validation study on the P2Y12 receptor assay using the specific P2Y12 inhibitor 2-methylthio-AMP. They found strong agreement between the results using the VerifyNow P2Y12 assay and those of ADP and prostaglandin E1 induced optical aggregation. Readings were not influenced by age, platelet count, haematocrit, fibrinogen, cholesterol or triglyceride levels. They concluded that this assay was a sensitive device suitable for monitoring platelet P2Y12 receptor inhibitors (137). In the VERITAS study they studied responses to Clopidogrel using this assay in 147 patients, with a history of vascular disease or

multiple risk factors, before and after Clopidogrel treatment. Whilst they showed a mean reduction in Platelet Response Units (PRU) of 64 +/- 25% (baseline mean PRU 306; post 450mg loading dose 128; post 75mg maintenance therapy x7/7 102) there was some overlap with baseline samples due to high inter-individual variability (145). In the setting of PCI Clopidogrel resistance as measured by VerifyNow has been correlated with an increased incidence of peri-procedural myocardial infarction (139).
Von Beckerath et al (75) used the VerifyNow P2Y12 assay in parallel with ADP-induced optical aggregation to assess responses to maintenance doses of Clopidogrel. They found using both assays that there was significantly greater inhibition with maintenance doses of 150mg daily (mean PRU 60+/-72), than with 75mg daily (mean PRU 117+/- 64).

11.3.9.3 GP IIb/IIIa receptor assay

Steinhubl et al studied 485 patients undergoing PCI in whom the use of a glycoprotein inhibitor was planned (142). They measured inhibition after the administration of a glycoprotein inhibitor (84% received Abciximab which was administered as a bolus and followed by an infusion). Using the VerifyNow Glycoprotein assay (at the time called the Ultegra rapid platelet function assay) they found significant variability in response. Poor response (<70% platelet inhibition 8 hours after the start of therapy) was associated with a significantly higher major adverse clinical event rate (25% vs. 8.1%, p=0.009).

It is noteworthy that the utility of the Aspirin and Clopidogrel assays is limited in some emergency patients as their use is not recommended within two weeks of Abciximab therapy.

11.3.10 Thrombelastograph platelet mapping system

Investigation into the role of a modified version of the Thrombelastograph (TEG) Platelet Mapping system in the assessment of individual responses to antiplatelet agents is the focus of this thesis and TEG will therefore be covered in depth in section 11.5.
In standard TEG blood is placed in an oscillating cup. Suspended within the cup by a torsion wire is a stationary pin. When blood is in its liquid form cup oscillation has no

impact on the pin. As blood clots, fibrin strands link the pin and the cup and changes in the viscoelasticity of the blood are therefore transmitted to the pin. The resulting torque generates an electrical signal whose magnitude can be plotted as a function of time to produce a TEG trace (146,147). The TEG trace can be analysed to provide several parameters defining the speed and strength of clot formation. Unmodified TEG provides a non-specific assessment of global haemostasis; the effects of some abnormalities are obscured by other more dominant components of the coagulation system (such as thrombin). The use of specific platelet activators and activators of fibrin formation allow the effects of antiplatelet therapies to be detected. With these modifications TEG correlates closely with optical aggregation in the assessment of the effects of antiplatelet agents (148). In the context of PCI Mobley et al found a good correlation between the two techniques when used to detect the effects of Clopidogrel (149). A close correlation between modified TEG and optical aggregation has also been found when used in the detection of Aspirin resistance (25).

11.4 Evidence linking responses to antiplatelet agents with outcome

Table II overleaf summarises the currently available evidence linking results of assays of response to antiplatelet therapy with adverse clinical outcomes.

11.4.1 Peripheral Vascular Disease

Mueller et al studied 100 patients on Aspirin therapy after peripheral limb percutaneous balloon angioplasty (123). Responses to Aspirin assessed with whole blood aggregometry predicted male patients who were at an elevated risk of vessel re-occlusion. Ziegler et al found similar results using the PFA-100 in a smaller cohort (52 patients on aspirin) undergoing peripheral limb angioplasty (150).

Assay	Correlation of results with adverse clinical events?	Reference
Platelet Aggregometry	The degree of ADP-induced aggregation post PCI is higher in those with subsequent ischaemic events.	Gurbel P 2005
	Those with poorest response to Clopidogrel, assessed with ADP-induced aggregation after a 600mg loading dose of Clopidogrel and prior to PCI, have higher levels of adverse events within 30 days of PCI.	Hochholzer W 2006
	Low response to Clopidogrel by ADP-induced aggregation significantly increased the occurrence of cardiovascular events and death within three months of PCI.	Geisler T 2006
	In a cohort of 60 consecutive patients undergoing primary PCI for STEMI 7 of 8 recurrent ischaemic episodes in the 6 month follow up period occurred in patients in the lowest quartile of platelet inhibition.	Matetzky S 2004
	Poor response to Aspirin and Clopidogrel by light transmission aggregometry has been found in patients with previous ST.	Wenawesar P 2005
	Poor response to Clopidogrel by light transmission aggregometry has been found in patients with previous ST.	Gurbel P 2005
VASP Platelet Reactivity Index	High ADP-induced aggregation whilst on Clopidogrel is predictive of stent thrombosis.	Buonamici P 2007
	Independent predictor of stent thrombosis in patients on Clopidogrel.	Blindt R 2007
	Significant differences in VASP phosphorylation between patients with previous stent thrombosis and PCI controls.	Frere C 2007 Gurbel P 2005
Biomarkers of Arachidonic Acid Metabolism	In patients on Aspirin high levels of urinary 11-dehydro thromboxane B2 are associated with a higher risk of cardiovascular death.	Eikelboom J 2002
PFA-100	Has been shown to have greater sensitivity and specificity than AA-induced platelet aggregometry in predicting myocardial necrosis post PCI in the context of NSTEMI.	Marcucci R 2007
VerifyNow	Poor response to Aspirin has been correlated with an increased incidence of peri-procedural myocardial infarction post PCI.	Chen W 2004
	High platelet reactivity whilst on Clopidogrel associated with increased events, including stent thrombosis.	Price M 2008 Hobson A 2008
	Increased risk of death, ACS and stroke (adjusted hazard ratio 2.71) in Aspirin non-responders with stable coronary artery disease.	Cheng X 2005
Thrombelast-ography (TEG)	High platelet reactivity by TEG PlateletMapping demonstrated in patients with previous stent thrombosis.	Hobson A 2008
	On combining two measures from standard TEG demonstrated an odds ratio of 38 for ischaemic events following PCI.	Gurbel P 2005

Table II Assays of response to antiplatelet therapy and outcome

11.4.2 Cerebrovascular disease

Grotemeyer et al studied 180 patients admitted with stroke and showed that a poor response to Aspirin (high residual platelet reactivity) before hospital discharge predicted adverse outcome (recurrent stroke, myocardial infarction and vascular death) during 24 month follow up (151). Major endpoints were seen in 4.4% of Aspirin responders and 40% of non-responders (one third of patients were classified as non-responders).
Grundmann et al used the PFA-100 in 35 patients with known cerebrovascular disease (152). Patients without an event for >24 months all responded well to Aspirin, patients with a recent event had a 34% rate of non-response. They concluded that Aspirin non-response may contribute to failure of secondary prevention. However, the sample size was small, PFA-100 is non-specific and increased platelet reactivity secondary to the recent event could have influenced the results.
Englyst et al investigated 45 patients with ischaemic stroke and found higher rates of Aspirin resistance (using TEG PlateletMapping) than in controls (153). Aspirin resistance was also found to be associated with stroke severity and more common in lacunar than embolic strokes.

11.4.3 Coronary artery disease

Coronary artery disease kills more than 110,000 people in England every year. It is also a major cause of morbidity. In England more than 1.4 million people suffer with angina, 275,000 have heart attacks each year and it is the commonest reason for hospitalisation. Coronary artery disease is caused by atherosclerosis and plaque deposition. Symptoms occur due to myocardial ischaemia and infarction, but in many cases it remains asymptomatic. Angina occurs when stable atherosclerosis causes inadequate myocardial blood supply with increased myocardial workload (and therefore increased myocardial oxygen demand). The spectrum of diseases that include ACS and MI are caused, in the majority of cases, by a common underlying pathophysiology; atherosclerotic plaque rupture or erosion. The likelihood of plaque rupture and erosion is determined by the strength of the plaque's fibrous cap (increasingly understood to be related to an acute

inflammatory process) and the stresses to which it is exposed (related to wall stress and blood flow across the intimal surface of the plaque (154). Plaque rupture leads to a rapidly escalating vascular inflammatory response involving the adhesion, activation and aggregation of platelets, thrombus formation, the release of vasoactive mediators and microembolisation. Local factors as well as factors influencing systemic hypercoagulability affect the degree of subsequent thrombosis and hence the clinical presentation.

Improved understanding of the pathophysiological processes outlined above explains the current focus of medical therapies in the form of anti-platelet agents (Aspirin and Clopidogrel), thrombolytics, anti-coagulants and anti-inflammatory agents (such as high dose statins). As outlined above the use of Aspirin and Clopidogrel improve outcomes in cardiovascular disease. However responses to these medications vary with some patients respond poorly.

Eikelboom et al assessed Aspirin resistance in patients enrolled in the HOPE (Heart Outcomes Prevention Evaluation) study using urinary 11-dehydro thromboxane B2 levels (a marker of in vivo thromboxane generation) (15). There was a 1.8 times higher risk of the composite endpoint of MI, CVA or death from vascular disease, and a 3.5 times higher risk of cardiovascular death, in the quartile with the highest levels (representing least effect of Aspirin) compared with the lowest quartile.

Gum et al studied responses to 325mg Aspirin with optical aggregation in 326 stable cardiovascular patients. During the follow up period of 679+/- 185 days Aspirin resistance was associated with an increased risk of the composite endpoint of death, MI or stroke (adjusted hazard ratio 4.14) (155).

Cheng et al assessed aspirin resistance using the VerifyNow Aspirin assay in 422 patients with stable coronary heart disease. They found an increased risk of death, ACS and stroke (adjusted hazard ratio 2.71) during follow up in Aspirin non-responders (ARU >550) (156).

Mobley et al have demonstrated (using optical aggregation, Ichor plateletworks assay and TEG) that 30% of patients undergoing coronary angiography were resistant to Clopidogrel (131).

11.4.3.1 ACS

Clopidogrel resistance has been associated with increased risk of recurrent thrombotic events in patients with acute MI. In a cohort of 60 consecutive patients undergoing primary PCI for STEMI Matetsky et al found that 7 of 8 recurrent ischaemic episodes in the 6 month follow up period occurred in patients in the lowest quartile of platelet inhibition (assessed by ADP-induced platelet aggregation) (157).

11.4.3.2 PCI

Coronary angioplasty was first introduced by Andreas Gruentzig in 1977 as a nonsurgical method for coronary arterial revascularization. Since its introduction the technology associated with PCI has developed rapidly through several phases. Simple balloon angioplasty was prevalent during the period 1977 to 1987. Adjuncts to balloon angioplasty, including atherectomy and laser assisted angioplasty followed between 1988 and 1992, after which the use of intracoronary stents has increased progressively (158). Acute vessel closure occurred commonly following balloon angioplasty, with a rate of 2-7%. However, with the routine use of stents and the use of adjunctive pharmacotherapy this figure has fallen to 1-2% (159).

Procedural complications are lower with PCI than with coronary artery bypass surgery, as are the cost of the procedure and the length of hospital stay. Patients receiving PCI return to work sooner than those undergoing surgery and are able to exercise more one month post procedure. As a result PCI is now the most common procedure used in the invasive treatment of coronary artery disease with more than one million procedures performed in the United States each year and an estimated two million performed annually worldwide (ACC/AHA/SCAI, 2005). In the UK, the number of patients treated by PCI exceeded the number of those treated with surgical revascularisation for the first time in 1997 and the ratio of PCI to coronary artery bypass grafting (CABG) had increased to 2.5:1 by 2004 (158).

Balloon angioplasty and coronary stenting cause trauma to the endothelium of the blood vessel wall exposing subendothelial contents such as collagen and vWF to the blood. This inevitably leads to platelet activation, aggregation and activation and there is therefore a risk of subsequent thrombotic occlusion. Initial stent trials used Aspirin monotherapy or Aspirin and vitamin K antagonists to try and reduce the risk of ST. However, rates of acute ST were 15-20% for Aspirin monotherapy (160) and with Aspirin and Warfarin dual therapy rates remained high (3.5% at the time of hospital discharge) and there were very high bleeding rates. The introduction of dual antiplatelet therapy with Aspirin and a thienopyridine (initially Ticlopidine) reduced the risk of ST to less than 1% with BMS and also reduced bleeding complications.

However, the incidence of restenosis (re-narrowing) within early stents remained in the region of 15-20%. As a result DES were developed which release pharmacological agents to inhibit the response to injury responsible for restenosis (through vascular smooth muscle cell migration and proliferation). With the use of DES restenosis and target-vessel revascularisation is reduced to under 10% (161).

There are concerns however that the thrombotic risk may be greater with DES and premature discontinuation of antiplatelet therapy after PCI and DES insertion appears to be particularly hazardous and is associated with subsequent mortality (162).

Gurbel et al have shown in patients post PCI that the degree of ADP-induced platelet aggregation (by LTA) was significantly more pronounced in those with subsequent ischaemic events (68). Hochholzer et al showed higher levels of adverse events within 30 days of PCI in those with poorest response to Clopidogrel assessed with ADP-induced aggregation after a 600mg loading dose of Clopidogrel and prior to PCI (163). Geisler et al also showed that a low response to Clopidogrel (by ADP-induced aggregation) significantly increased the occurrence of cardiovascular events and death within three months of PCI (164). Chen et al utilised the VerifyNow Aspirin assay to assess Aspirin resistance (165). They found Aspirin resistance to be associated with a higher incidence of myonecrosis (2.9 times higher incidence of rise in CK-MB and troponin I) post PCI. A high VerifyNow PRU (Platelet Response Units – measuring platelet reactivity whilst on Clopidogrel) has also been associated with an increased risk of post-discharge adverse events post PCI. Patients above an arbitrary cut-off value of >235 had significantly

greater adverse events post hospital discharge (141). These studies suggest that poor response to antiplatelet therapy is associated with adverse outcome after PCI. Several studies now suggest that as well as premature discontinuation of antiplatelet therapy, poor response to therapy may render individuals at risk of ST.

11.4.3.3 Stent Thrombosis

PCI and stent insertion is now the commonest method of coronary revascularisation in the UK. The initial, relatively common, limitation of restenosis and the subsequent need for repeat revascularisation in patients treated with BMS has been dramatically reduced as a result of widespread deployment of DES. Key to this strategy has been the use of Clopidogrel and Aspirin to reduce the rate of ST (36). ST is an important, potentially life-threatening complication of coronary stent placement. There is current concern regarding the frequency of ST in PCI patients receiving DES, in whom, despite current optimal therapy, the frequency in large 'real world' series remains around 1% (166,167). This concern has been further stimulated by meta-analysis of data derived from randomised studies suggesting an excess in late and very late ST in patients receiving DES compared with BMS (168). Specifically, there have been reports of late thrombosis occurring after DES implantation particularly on reduction or cessation of antiplatelet therapy (169-172).

The agents released from DES are bound in the majority of cases by a polymer to the stent struts. These agents activate signal transduction pathways and inhibit cell proliferation. As a result, although primarily aimed at preventing vascular smooth muscle cell proliferation and migration, they also impair re-endothelialisation which may lead to delayed arterial healing and incomplete stent coverage. This has been confirmed both at autopsy and by angioscopy in vivo (173,174). The drugs have also been shown to induce tissue factor expression, impair endothelial function and activate platelets which may result in a prothrombogenic environment (175-177). In addition hypersensitivity reactions to the polymer have been reported, which may lead to increased inflammation and platelet activation (178).

There are therefore theoretical reasons why longer periods of intense antiplatelet therapy may be required to prevent ST in patients with DES. Indeed, whilst the pathophysiology of ST is likely to be multifactorial (179), there are some data (highlighted below) suggesting that one aetiological mechanism may be individual relative hypo-responsiveness to anti-platelet therapy (63,135,180-184).

Case Reports

Ruef and Kranzhofer have reported a case of two vessel ST occurring in the context of Aspirin resistance (183).

Von Beckerath et al reported a case of ST which had occurred whilst on Clopidogrel and demonstrated a failure to metabolise Clopidogrel into its active metabolite in this patient (71).

Schafer et al reported a case of Late ST in a diabetic patient who appeared resistant to Clopidogrel whilst on dual antiplatelet therapy by PFA-100, ADP-induced optical aggregation and VASP phosphorylation (185).

Retrospective Studies

Wenaweser et al studied 23 patients with ST, 50 matched controls and 9 healthy volunteers. They utilised AA- and ADP-induced optical aggregation to detect responses to Aspirin monotherapy, and dual therapy with Aspirin 100mg and Clopidogrel 75mg daily. Resistance to both Aspirin and Clopidogrel was more common in the ST group than in the patients or healthy volunteers (182).

In the CREST study Gurbel et al studied 20 patients with previous ST and compared their responses to Clopidogrel with 100 age-matched patients without ST using ADP- and AA-induced optical aggregation, total and activated glycoprotein IIb/IIIa after stimulation with ADP and VASP phosphorylation. ST patients had higher levels of platelet reactivity to ADP, higher levels of glycoprotein IIb/IIIa receptor expression when exposed to ADP and a higher ratio of VASP to phosphorylated VASP. They found that 60% of patients with ST had high platelet reactivity (135).

Barragan et al utilised VASP phosphorylation to assess the effects of Clopidogrel. They found a highly statistically significant difference in responses to Clopidogrel between 16 patients who presented with ST and 30 PCI patients without ST (180).
Ajzenberg et al studied 10 patients with previous ST (analysis was performed only 4.5+/-3.4 days after ST), 22 stented patients without ST and 17 healthy volunteers. They assessed AA- and ADP-induced optical aggregation and shear-induced platelet aggregation. There was no significant difference in optical aggregation between the ST group and stented controls. However, shear induced platelet aggregation was significantly higher in ST cases than in controls (184).
Morel et al identified poor response to Clopidogrel assessed with VASP phosphorylation, but not ADP-induced aggregation, in patients with previous ST compared to matched controls (186).

Prospective studies

Buonamici et al prospectively studied 804 patients receiving DES. Response to Clopidogrel was assessed after a clopidogrel 600mg loading dose. All were treated with Aspirin 325mg and Clopidogrel 75mg maintenance therapy until 6 month follow up. They found that 13% were non-responders to Clopidogrel (defined using ADP-induced optical aggregation). ST occurred in 8.3% of non-responders and 2.3% of responders ($p<0.001$). They found that non-response to Clopidogrel was the strongest predictor of ST with a hazard ratio of 3.08 (181).
Muller et al used optical aggregation to assess ADP-induced platelet aggregation on platelet-rich plasma to assess responses to Clopidogrel in 105 patients with coronary artery disease undergoing elective PCI. They found that 5 to 11% of patients were non-responders to Clopidogrel (defined as less than 10% inhibition compared to baseline values 4 hours after administration of a 600mg Clopidogrel loading dose. Subacute ST occurred in 2 patients both of whom were non-responders. They also analysed 3 further patients who had already suffered ST. All three patients had high levels of platelet aggregation but percentage inhibition could not be calculated as they had no baseline samples for comparison (63).

In a cohort of 99 patients identified as being at high risk of ST Blindt et al demonstrated that ADP-induced aggregation and VASP phosphorylation were significantly higher 72-96 hours post stenting in those with angiographically confirmed ST. VASP phosphorylation was an independent predictor of ST (187).

11.5 Thrombelastography

The Thrombelastograph® Haemostasis System (TEG, Haemoscope Corp, IL, USA) provides an overall assessment of haemostatic function (188,189). It provides a graphic representation of clot formation and lysis. First developed in 1948, it was used initially as a research tool (190). In the last 20 years development and modernisation of TEG has facilitated its utility in the clinical management of bleeding and haemostasis where it is used to guide clotting factor replacement, platelet transfusion and in fibrinolysis treatment (191). Recent modifications have further added to its potential applications.

11.5.1 Principles of TEG: The test and how it works

Kaolin activated blood at 37^{o}C is placed in a cylindrical cuvette (cup) that oscillates by 4 degrees 45' at a frequency of 0.1 Hertz. Suspended within the cup by a torsion wire is a stationary pin. As the cup oscillates there is a 1mm gap between it and the pin. The wire acts as a torque transducer (146,192) (Figure 1).

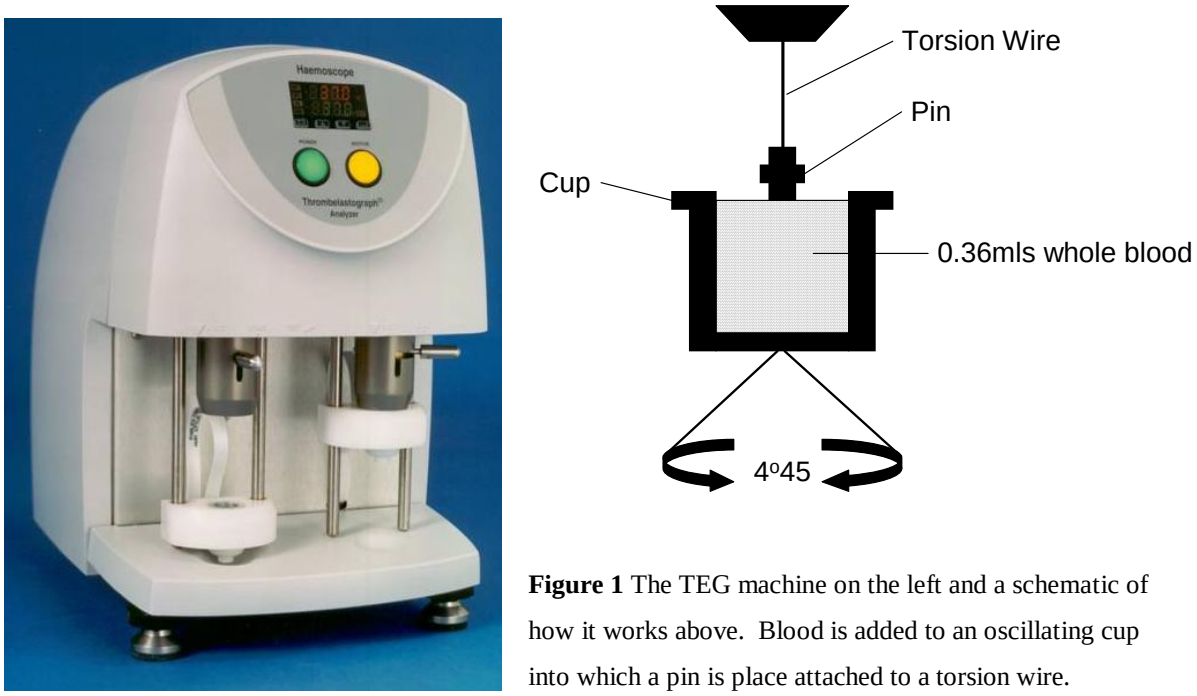

Figure 1 The TEG machine on the left and a schematic of how it works above. Blood is added to an oscillating cup into which a pin is place attached to a torsion wire.

When whole blood is in its liquid form cup oscillation has no impact on the pin. As blood clots, fibrin strands link the pin and the cup and changes in the viscoelasticity of the blood are therefore transmitted to the pin. The resulting torque generates an electrical signal whose magnitude can be plotted as a function of time to produce a TEG trace (146,147) (Figure 2).

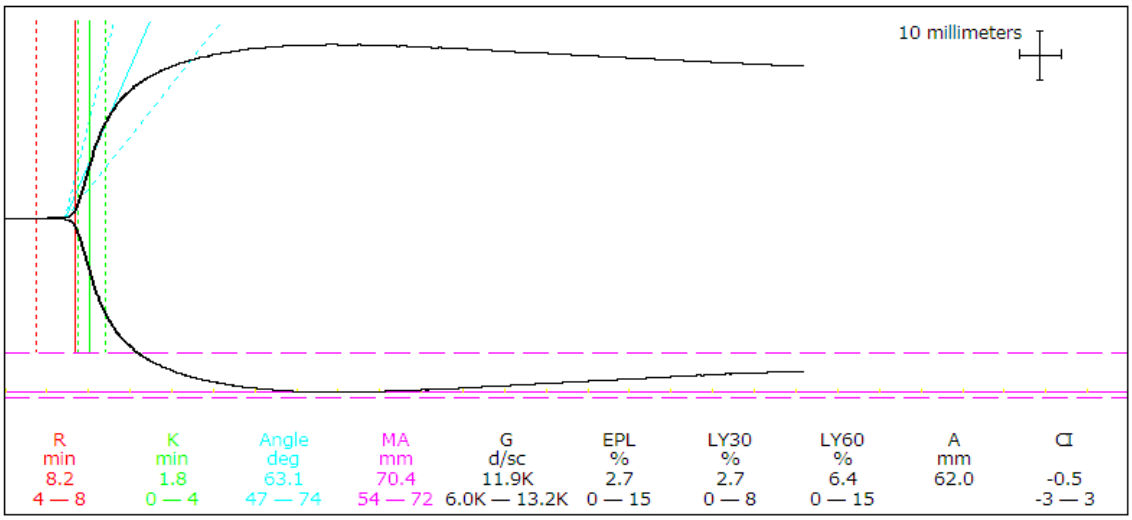

Figure 2 A TEG trace.

Thus, as blood clots there is a progressive increase in the signal amplitude to a maximum. The standard TEG trace can be analysed to provide several parameters defining the speed and strength of clot formation (Table III). Normal haemostasis involves the controlled activation of clot formation, spontaneously balanced by mechanisms of clot lysis; therefore a truly global analysis of haemostatic function requires assessment of both the fibrinolytic and coagulation systems. TEG measurements incorporate both of these components by using the parameter of viscoelasticity of clotting blood. This assessment is dependent on a) cellular and plasma components b) the activity and concentration of coagulation elements as well as c) procoagulant and d) fibrinolytic activity. The TEG trace can therefore provide continuous real time information on the viscoelastic properties of the evolving clot from the time of initial fibrin formation, through platelet aggregation, fibrin cross linkage and clot strengthening to clot lysis (193). Analysis can determine (a) the speed of clot generation (b) its strength and (c) its stability (194). Table III below summarises commonly assessed TEG variables.

Parameter	**Description and rationale for assessment**
R time	Reflects the time to initial fibrin formation. Relates to plasma clotting factor and inhibitor activity
K time	The time taken for the blood to achieve a fixed level of viscoelasticity. Assesses the rapidity of fibrin cross linking
α angle	The angle formed by the gradient of the initial trace. Represents the speed of clot formation
MA (maximum amplitude)	This indicates the strength of the clot and reflects the activity of fibrin and platelets

Table III Commonly assessed TEG Parameters.

Clotting is a dynamic process. Conventional tests such as activated partial thromboplastin time and platelet count and function assess isolated components of the haemostatic system and are unable to predict the role of these components in the context of haemostasis as a whole. The advantage of TEG is that it incorporates the interaction of all of the components of coagulation including platelets, fibrin, clotting factors, and thrombin as well as providing information about the quality of the clot (195). TEG has been shown to be superior to either activated clotting time (ACT) or conventional tests at diagnosing postoperative coagulopathies (196) and can help predict post operative blood loss (197).

11.5.2 Recent Modifications

Unmodified TEG provides a non-specific assessment of global haemostasis; the effects of some abnormalities are obscured by other more dominant components of the coagulation system (such as thrombin). Recent modifications to TEG allow more precise identification of abnormalities and have improved its ease of use and reproducibility (see Table IV overleaf).

Reagent used	Rationale for use
Citrate	Enables prolonged storage of samples before analysis
Heparin	Inhibits thrombin allowing the contribution of fibrin and platelets to be assessed
Heparinase	Reverses the effect of heparin, e.g. in patients on cardiopulmonary bypass
Activators (e.g. Celite, Kaolin, Tissue Factor)	Speed up result acquisition
Glycoprotein IIb/IIIa inhibitors	Inhibit platelet function allowing the contribution of fibrinogen to be assessed
Antifibrinolytic drugs (e.g. Tranexamic acid)	Reverse fibrinolysis
Activator F^{TM} (Reptilase and Factor XIIIa)	Activates fibrin formation without affecting platelets
AA	Activates platelets via the production of thromboxane A2. This pathway is affected by aspirin
ADP	Activates platelets via P2Y1 and P2Y12 receptors. Thienopyridines inhibit the P2Y12 ADP receptor.

Table IV TEG Reagents

Modifications include using sample activators to speed up result acquisition, and citrated samples to allow a longer delay before testing (198-200). Alternatively blood can be taken into heparinised tubes, again allowing a longer delay before testing and also eliminating the effect of thrombin, allowing assessment of the contribution of platelets and fibrin to the clot. The addition of a platelet glycoprotein IIb/IIIa inhibitor in vitro inhibits platelet aggregation and allows the relative contribution of fibrinogen to haemostasis to be assessed. The TEG trace produced in this context correlates with the plasma fibrinogen concentration (201). Other modifications (including the use of specific platelet activators and activators of fibrin formation) allow the effects of antiplatelet

therapies to be detected. Whilst these are summarised in Table IV they will be covered in detail later. Together these modifications allow analysis of the functional importance of different components of the haemostatic system. This may make specific diagnosis and targeting of therapy possible. Furthermore; potential therapies can be tested on patient's blood ex-vivo to predict the clinical response before administration (198).

11.5.3 Limitations of TEG

Haemostasis is associated with a wide range of normal values due to extensive variability in components of the haemostatic system including platelet count and function, glycoprotein IIb/IIIa receptor number and fibrinogen concentration. Ideally therefore, each patient should have baseline TEG measurements before initiating a treatment or procedure so that there is an internal, individualised reference for change. Difficulties with validation and standardisation probably accounts for why TEG has not been universally accepted by haematologists (200). To some extent these issues have been overcome by the use of computer software to analyse the TEG trace which allows for standardisation of results. Further standardisation has been achieved by use of disposable cups and pins, individual temperature control and use of activators such as kaolin to standardise the initiation of the clotting process. One fundamental challenge relating to the potential clinical applicability of TEG is whether it is only useful in the assessment of a change in clotting behaviour, or whether "snapshot" values will be useful. Despite these modifications however the use of standard TEG remains very limited as whilst problems with validation and standardisation have been largely overcome the technique remains cumbersome and time-consuming.

11.5.4 Current Clinical Applications

One of the main roles of TEG in clinical practice is in hepatobiliary surgery where it is used to monitor haemostasis and guide therapy (202). It has been shown to be more effective than conventional tests at assessing the risk of bleeding in this complex area (203,204). TEG has been used in liver transplantation since 1980 where it has been shown to reduce transfusion requirements (200). In obstetrics TEG can be used to

differentiate between the normal hypercoagulable state in pregnancy and the coagulopathic hypercoagulable state associated with pre-eclampsia. TEG has also been applied to obstetric patients to identify those at risk of potentially dangerous bleeding from an epidural (205).

In cardiac surgery it is well established that cardio-pulmonary bypass disturbs the haemostatic system in a number of ways including (i) haemodilution of procoagulants, fibrinogen and platelets (206) (ii) a reduction in levels of coagulation factors (iii) the use and reversal of heparin and (iv) preoperative administration of platelets (207). It has been demonstrated that routine use of TEG during cardiac surgery reduces transfusion requirements and, in addition, when transfusion was required, the TEG group were able to employ more specific therapy by identifying the cause of the coagulopathy (194). TEG can also be useful in the intraoperative period; for example, the use of heparinase in perioperative TEG studies is able to neutralize the effects of heparin administered during cardio-pulmonary bypass. Further, hypothermia used during cardiac surgery can affect coagulation in ways not detected by standard coagulation tests. In contrast, temperature adapted TEG can detect abnormalities in the hypothermic patient enabling effective treatment of coagulopathy (188).

As well as its use in the management of haemostasis TEG has more recently been investigated as a marker of risk for thrombotic events. In a study of 240 non-cardiac post-operative patients there was a significantly higher incidence of thrombotic events, including myocardial infarction, in those with maximum amplitude (MA) of >68mm on TEG (208). Gurbel et al have also shown that increased MA on TEG (both pre and post clopidogrel loading at the time of procedure) provides a predictive tool for ischaemic events following PCI. On combining two measures from a standard TEG trace; MA and a short R time (see Figure 2) they demonstrated an odds ratio for ischaemic events in the six months following PCI of 38 (209). However, because of the limitations outlined above, this novel marker of risk following PCI has not entered routine clinical use.

11.5.5 TEG "PlateletMapping™"

In standard TEG the maximum amplitude (MA) is largely dependent on thrombin. Thrombin is a powerful platelet activator and overwhelms the effect of other less potent platelet activators such as AA and ADP. In the presence of thrombin it is possible to detect some effect from potent antiplatelet agents such as Glycoprotein IIb/IIIa inhibitors (210), but the effect of other antiplatelet agents remains obscured (211). However, by taking blood into a tube that contains heparin, thrombin is inhibited. The subsequent addition of Activator F^{TM} (Act F) generates a fibrin network in which platelets can interact independent of thrombin. Without alternative sources of platelet activation there is minimal platelet activation and therefore minimal response on the TEG curve (low MA). However, other platelet activators (AA or ADP) can be added and (in the absence of inhibition of their specific pathways of action (eg with Aspirin or Clopidogrel respectively)) this increases the MA. Maximal platelet activation generates a curve similar to unmodified TEG in the presence of thrombin. The effect of antiplatelet medication can therefore be established by comparing the unmodified TEG curve (representing maximal platelet activation) and the modified TEG curve with either AA- or ADP-stimulation.

Aspirin achieves platelet inhibition by permanent inactivation of COX-I, an enzyme in platelet AA metabolism. The effect of Aspirin can therefore be calculated by comparing the unmodified curve in the presence of thrombin (maximal platelet activation), the heparinised sample with Act F alone (no platelet activation) and the modified TEG curve with AA-stimulation (residual platelet activation due to AA in the presence of Aspirin). The effect of Clopidogrel, a direct ADP inhibitor and the glycoprotein IIb/IIIa antagonist, Abciximab, on platelets can be assessed in a similar fashion, utilising ADP-induced platelet aggregation. These modifications are summarised in Table IV. This system is marketed by Haemoscope as the "Platelet Mapping Kit" (TEG Pmap). With these modifications TEG Pmap correlates closely with optical aggregation in the assessment of the effects of antiplatelet agents (148). In the context of PCI Mobley et al found a good correlation between the two techniques when used to detect the effects of Clopidogrel (149). A close correlation between modified TEG and optical aggregation has also been found when used in the detection of Aspirin resistance (25).

11.5.5.1 Pros and Cons of TEG Pmap

TEG Pmap has potential advantages as a method of analysing responses to antiplatelet therapy. TEG has the potential to be used in a point-of-care manner (it is already widely used in cardiothoracic surgical units in the UK). Unlike other point-of-care assays (such as the PFA-100) it has the ability to detect responses to both Aspirin and Clopidogrel. It also has the ability to determine the summative effect of multiple medications (unlike the VerifyNow assay which cannot be utilised for 14 days after administration of Abciximab and the PFA-100 which is unable to detect the effects of Clopidogrel). It is a whole blood assay that also provides information on clot formation and clot lysis.

11.5.5.2 Methods of TEG Pmap Analysis

The effects of antiplatelet agents can either be calculated by comparing a sample on antiplatelet therapy with a baseline sample (212) or by utilising the "percentage platelet inhibition" (25,148). The percentage platelet inhibition in response to AA- or ADP-stimulation is calculated by comparing the clot with fibrin alone with maximal platelet activation due to thrombin and platelet activation due to AA or ADP. The percentage platelet inhibition due to Aspirin can therefore be calculated using the formula:

$$\text{\% platelet inhibition} = 100 - \frac{\text{MA AA channel} - \text{MA Fibrin channel}}{\text{MA Thrombin channel} - \text{MA fibrin channel}} \times 100$$

Whilst this has attractions in making the results of TEG Pmap easily interpretable there are potential drawbacks. First, the percentage platelet inhibition is calculated using the MA. However, in the setting of antiplatelet agents, there can be a considerable delay (often over 1 hour) before the MA is obtained (eg. Figure 3).

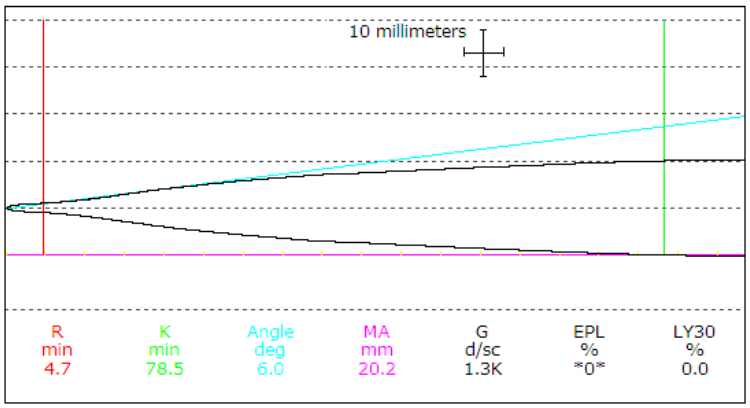

Figure 3 In this example of an AA-stimulated trace following the administration of Aspirin, the MA was obtained after 74 minutes.

Results are therefore slow to obtain and contain information on solely the clot strength and not the speed of clot formation. In addition the percentage platelet inhibition subtracts the response seen in the fibrin channel in an attempt to describe the effect of platelets on whole blood coagulation. However, fibrin is itself involved in platelet aggregation via its action on the integrin alpha (IIb) beta (3) receptors and is integral to thrombus formation (213). It is therefore possible that this means of calculation negates one of the potential benefits of TEG (namely that it has the ability to detect clinically relevant changes in overall blood clotting (including the effects of fibrin) rather than isolated platelet function). It may however mean that results are more likely to correlate closely with methods of assessing isolated platelet function such as turbimetric optical aggregation.

Ideally TEG analysis could be performed more rapidly and allow an assessment of the effects of antiplatelet agents on whole blood coagulation incorporating the effects of fibrin and thrombin and without the need for a baseline reference sample. Methods of TEG analysis and the development of our novel method are discussed in detail in section 13.2.

11.6 The rationale for the studies

A rapid and reliable test of response to antiplatelet therapy suitable for use in the context of PCI would be clinically useful. TEG has been shown to detect responses to antiplatelet therapy and has potential advantages in that it is a whole blood assay, provides information on overall clotting tendency as well as responses to both Aspirin and Clopidogrel and is potentially suitable for use at the point of care. However current methods of assessment are cumbersome and time-consuming. We therefore set out to develop and then investigate a novel method of analysis with a view to making TEG Pmap suitable for use in the context of PCI.

11.7 Hypothesis and aims

Our hypothesis was that with a novel method of analysis TEG Pmap could be a useful tool in the assessment of responses to antiplatelet therapy in the context of PCI. The aims of this study were therefore to 1) develop a novel method of TEG Pmap analysis making it more suitable for routine clinical use and (2) then investigate the use of TEG Pmap as a clinical tool in assessment of responses to antiplatelet therapy in the context of PCI. We also aimed to compare and contrast TEG Pmap with the VerifyNow assays of response to Aspirin and Clopidogrel.

11.7.1 Detailed Study aims

The detailed aims of this study were therefore to:

A) Establish a novel approach to TEG Pmap analysis "short TEG" with a view to:

i) speeding up result acquisition

ii) incorporating clot kinetics

iii) abolishing the need to use 4 channels as part of the "PlateletMap."

iv) making the technique faster, easier and therefore more suitable for use in routine clinical practice.

B) Provide a framework for the potential use of this method of analysis by:

i) assessing baseline variability in short TEG responses.

ii) assessing the ability of short TEG to detect differences due to antiplatelet therapy with both Aspirin and Clopidogrel.

iii) assessing the reproducibility of short TEG in determining responses to antiplatelet therapy.

iv) determining "normal" ranges of response to antiplatelet therapy using short TEG.

v) identifying the specificity of the assay for responses to Clopidogrel and to Aspirin.

vi) comparing this novel method of assessment with currently accepted methods of TEG analysis.

vii) identifying if there are important differences due to baseline demographics in short TEG responses.

viii) identifying if levels of response differ in high risk patients.

ix) establishing whether dose adjustment may improve response both on a population basis and specifically in individuals identified as initial poor responders.

C) The VerifyNow system is currently the most widely used assay for assessing responses to Aspirin and Clopidogrel despite relatively little evidence to support its use. We have utilised the VerifyNow system in parallel to TEG throughout this study so that we can compare and contrast the results obtained with those obtained with TEG.

12 Methods

12.1 Study participants

12.1.1 Volunteers

i) Data was retrospectively analysed from preliminary studies carried out by Dr R Swallow and Dr R Agarwala.

ii) 30 additional volunteers on no medication were recruited.

12.1.2 Patients

In total 110 patients were recruited at the Wessex Cardiac Centre. These comprised:

i) 70 patients on maintenance therapy with Aspirin receiving loading doses of Clopidogrel prior to invasive coronary investigation.

ii) 20 patients with a history of definite ST whilst on dual antiplatelet therapy for at least 14 days.

iii) A further 20 closely matched patients on maintenance therapy with Aspirin and Clopidogrel for at least 14 days.

12.1.3 Exclusion criteria

Volunteers: Individuals were excluded if they were taking had taken antiplatelet therapy or non-steroidal anti-inflammatory medication in the last 14 days, if they had a history of bronchial asthma, peptic ulceration, bleeding tendency or previous sensitivity to anti-platelet agents, if they were pregnant or if surgery was planned within 2 weeks.

Patients: Individuals were excluded if: they had a history of bronchial asthma, peptic ulceration, bleeding tendency or previous sensitivity to anti-platelet agents; if they were pregnant; had surgery planned within 14 days; were found to have significant derangement of full blood count (Haemoglobin <100, Platelets <100, Haematocrit < 0.30); or had antiplatelet or anticoagulant therapy other than Aspirin or Clopidogrel administered within 14 days or if the use of a glycoprotein IIb/IIIa inhibitor was planned during PCI.

12.2 Ethical approval

All participants gave written informed consent prior to commencing the study as part of trials receiving ethical approval from the Southampton & South West Hampshire Research Ethics committee B or the Isle of Wight, Portsmouth & South East Hampshire Research Ethics committee. Copies of participant information sheets and consent forms are shown in the Appendices.

12.3 Research and Development department approval

All trials were registered with Southampton University Hospitals Trust Research and Development department and obtained Research and Development departmental approval.

12.4 Study Methods

12.4.1 Venesection

I performed all venesection in the patients studied. I am grateful for the help of Zeshan Qureshi (Intercalated BSc student) who performed venesection in 20% of the volunteer samples analysed under my supervision.

Venesection was performed from the antecubital fossa using a tourniquet. Blood was taken using an 18 gauge needle. Using a three way tap (Figure 4) the first 2 mls of blood was drawn into a 5ml syringe and later discarded. Then 10mls of blood was withdrawn into a 10 ml syringe and from there immediately injected into a 6ml vacutainer containing 102iu of lithium heparin and mixed by gentle inversion 5 times. Samples were analyzed within one hour. For VerifyNow analysis blood from the same sample was immediately injected into either 1 or 2 (depending on whether response to one or two drugs was being assessed) 2 ml 3.2% sodium citrate vacutainers.

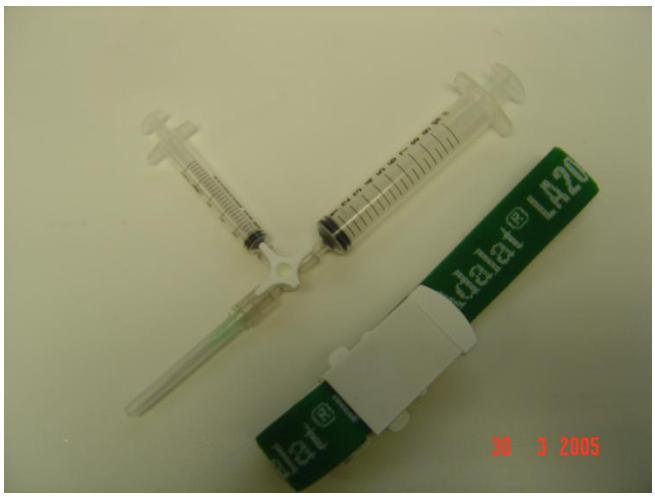

Figure 4 Venesection set-up

12.4.2 TEG PlateletMapping Methods

Samples were analysed using a computerised TEG Analyser (Haemoscope Corp, IL). Electronic quality controls were performed on a daily basis for all channels and both Level I and Level II wet quality controls were performed on a monthly basis. All reagents were allowed to reach room temperature before being reconstituted and were then utilised within 120 minutes.
The four channels used were (1) kaolin (the "thrombin channel"); (2) act F alone (the "fibrin channel"); (3) act F + AA (the "AA channel") and (4) act F + ADP (the "ADP channel"). For the thrombin channel 1ml of blood from the lithium heparin vacutainer was mixed with 1% kaolin solution (Haemoscope Corp, IL), 500µl of this was then added to a tube containing 4.0 iu of heparinase I and then 360µl of this mixed sample was placed in a cup containing 2.0 iu of lyophilized heparinase I for analysis. For the other three channels 10µl Activator F, a mixture of reptilase and factor XIII, (Haemoscope Corp, IL) was placed in each cup. 100µl (1mM) of AA was placed in the AA channel cup and 100µl of ADP (2µM) was placed in the ADP channel cup. 360µl of heparinised blood was then added to the Activator F, AA and ADP channel cups and mixed with the reagents. Samples were run until the MA had been reached or until 60 minutes had elapsed.

12.4.3 Presentation of TEG results

MA results in millimetres are derived directly from the TEG software and presented to 1 decimal place. Calculated AUC results (millimetre-minutes) are rounded to the nearest

whole number. Results are presented as the mean +/- 95% confidence interval of the mean.

12.4.4 VerifyNow Methods

Electronic quality control was carried out on a daily basis. Level 1 and Level 2 Quality Controls were carried out on every new batch of both Aspirin and P2Y12 assays (every 25 assays). Venesection was performed as specified as above. Vacutainers were mixed gently by inversion 5 times immediately after filling. Analysis was performed between 20 and 60 minutes of venesection for P2Y12 assays and 30 and 60 minutes for Aspirin assays. As recommended by the manufacturer non-responsiveness to Aspirin was specified as ARU >550. Response to Clopidogrel was assessed by comparison with a baseline sample with <30% inhibition considered to represent non-response.

12.4.5 Presentation of VerifyNow results

Results were recorded as presented on the machine. Group results are presented as the mean +/- confidence interval of the mean. Comparison was also performed between the numbers of non-responders as classified above.

12.4.5 Statistical Methods

Statistical advice was obtained from Dr Ghasem Yadegarfar PhD, Medical Statistician at the Research and Development support unit, Southampton University Hospitals.
TEG and VerifyNow results met criteria for normal distribution. Correlation between different methods of analysis was performed using Pearson Correlation. Comparisons between groups were performed using two-group two-tailed t-tests and between timepoints using one-group two-tailed t-tests. Comparison between numbers of non-responders was performed using Fisher's exact tests. $\chi 2$ (Chi squared) tests were used for categorical data. At all times a p value of <0.05 was considered to represent significance.

13 RESULTS

13.1 Reproducibility of TEG

13.1.1 Introduction

A previous study on healthy blood donors who denied exposure to antiplatelet therapy has shown analytical variation of about 5% in TEG Pmap variables (214). I performed several sets of experiments to confirm the reliability and reproducibility of TEG Pmap in (i) individuals naïve to antiplatelet therapy and (ii) in the assessment of response to antiplatelet therapy at the outset of this study.

13.1.2 Methods

For assessment of intra-individual variability venesection was performed on 20 different occasions, each at least two weeks apart and at least two weeks from the administration of any antiplatelet medication or non-steroidal anti-inflammatory medication.
Venesection was performed as specified in the general methods section. Analysis was performed by TEG Pmap as specified in the general methods section.
For assessment of inter-individual variability I retrospectively analysed the first baseline sample recorded for each volunteer investigated by our group (a total of 56 individuals). Venesection was again performed at least two weeks from the administration of any antiplatelet or non-steroidal anti-inflammatory medication.

13.1.3 Assessment of intra-individual baseline variability.

In one volunteer 20 baseline samples were taken for TEG Pmap at least 2 weeks apart and at least 2 weeks from the administration of any antiplatelet therapy. Results of the four channels of the TEG Pmap are shown in Table V and Figure 5 overleaf.

	Mean +/- 95% CI	Coefficient of variation (%)
Fibrin	6.5+/-1.3	45.8
Thrombin	60.1+/-1.8	6.2
ADP	63.5+/-1.6	7.7
AA	61.1+/-2.1	5.8

Table V Intra-individual baseline variability in TEG Pmap.

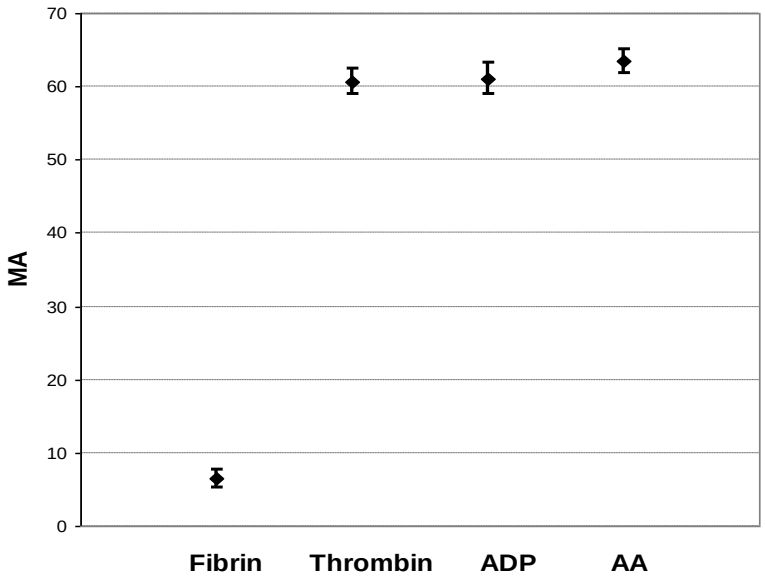

Figure 5 Intra-individual baseline variability in TEG Pmap

The coefficient of variability is in the order of 5 to 8% for the Thrombin, ADP and AA channels. The higher coefficient of variability for the Fibrin channel is largely due to the

lower recorded MA in this channel. As can be seen from Figure 5 above, the confidence intervals remain tight.

13.1.4 Assessment of inter-individual baseline variability.

I assessed the first baseline sample obtained from a total of 56 volunteers. All volunteers reported that they had not taken antiplatelet or other relevant medication for at least 14 days. Results are shown in Table VI and in Figure 6 below and overleaf. There is greater observed variability, particularly in the fibrin channel.

	Mean +/- 95% CI	**Coefficient of variation (%)**
Fibrin	13.9+/-3.1	86.3
Thrombin	64.5+/-2.0	11.9
ADP	59.8+/-2.5	15.9
AA	61.7+/-3.1	19.2

Table VI Inter-individual baseline variability in TEG Pmap.
(Samples taken from 56 volunteers on no antiplatelet medication, presented as the mean +/- 95% confidence interval of the mean and as the % coefficient of variation.)

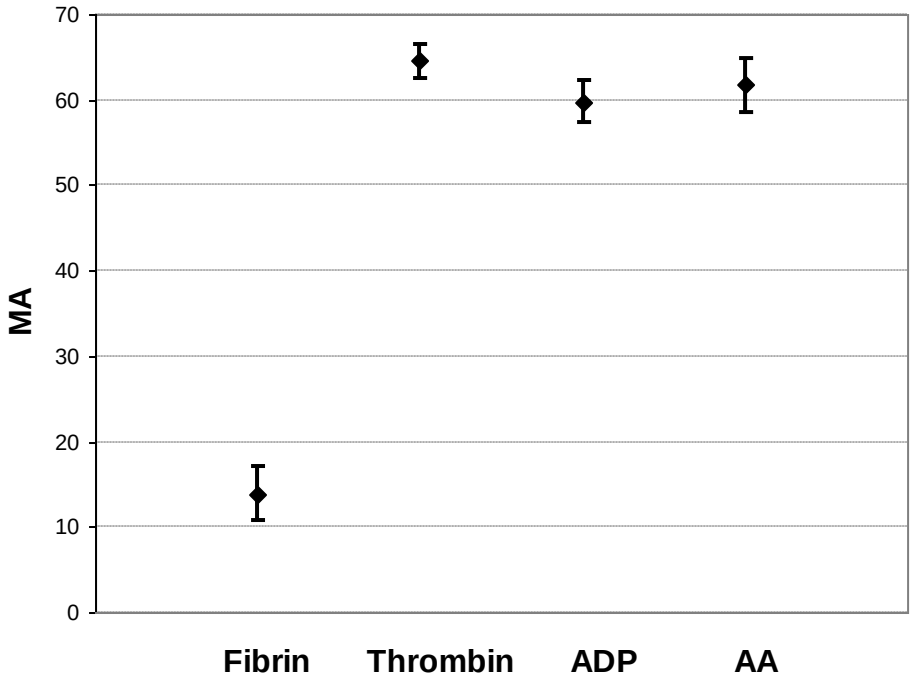

Figure 6 Inter-individual baseline variability in TEG Pmap
(Samples taken from 56 volunteers on no antiplatelet medication, presented as the mean +/- 95% confidence interval of the mean.)

13.1.5 Assessment of Intra-individual variability in response to therapy

One volunteer received 10 doses of Aspirin 300mg (at least 2 weeks from any other antiplatelet therapy). Blood tests were taken immediately before and 6 hours after drug administration. There was a reliable and reproducible decrease in the MA of the AA channel from 62.8 +/- 1.8 to 9.2 +/- 2.3 (see Figure 7 overleaf).

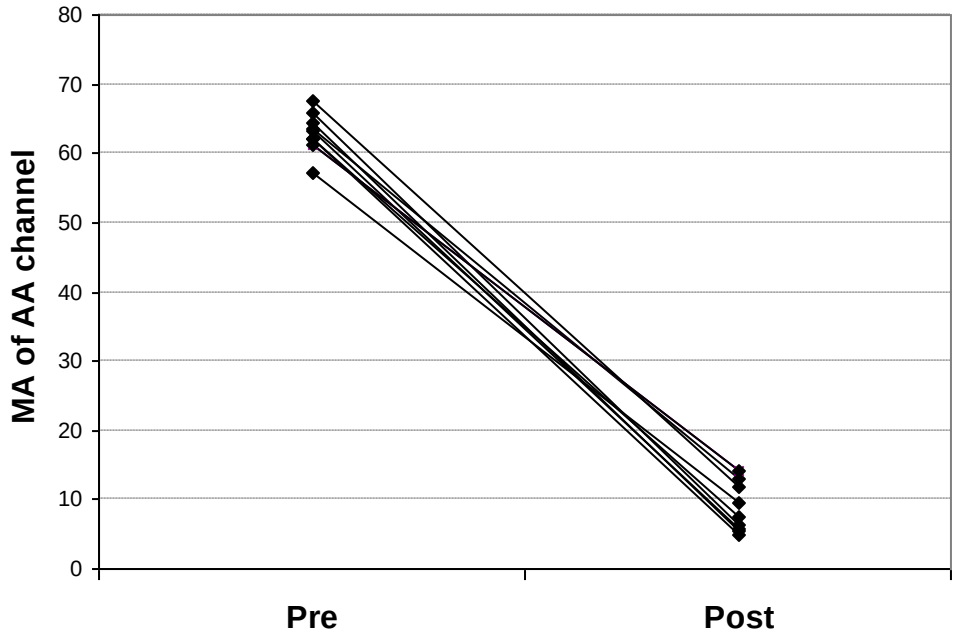

Figure 7 Intra-individual response to Aspirin therapy. (The MA of the AA channel immediately prior to and 6 hours after administration of a 300mg dose of Aspirin to one volunteer on 10 separate occasions.)

13.1.6 Summary

These experiments confirm that TEG Pmap has little intra- and inter-individual variability and reliably reflects response to antiplatelet therapy. There does however appear to be more variability in the Fibrin channel, even accounting for the lower values for MA obtained in this channel.

13.2 Developing a novel method of TEG analysis.

13.2.1 Introduction

Following the administration of Aspirin and Clopidogrel there is significant inhibition of AA- and ADP-induced clotting respectively. Whilst this can lead to visually obvious changes to the TEG trace it is unclear how best to calculate, and present, the effects of therapy from the results obtained.

For example Figure 8 below demonstrates the effects of Aspirin on AA-induced clotting in 35 volunteers administered a 300mg dose of Aspirin. There are obvious and significant reductions in the MA of the AA channel (all $p<0.0001$) compared to the baseline sample at all subsequent timepoints, but no significant changes in other TEG channels.

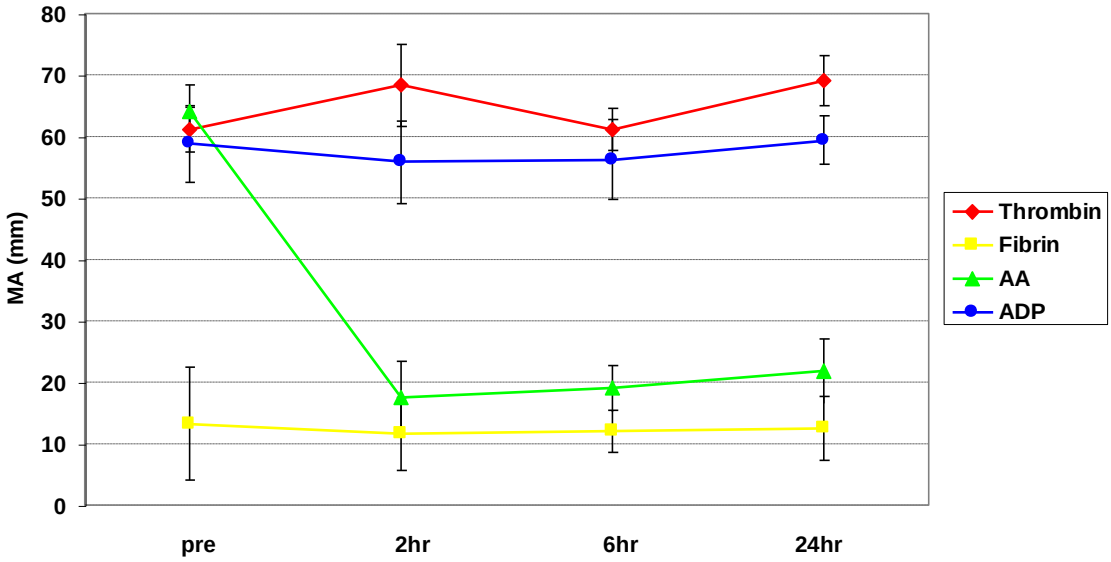

Figure 8 TEG Pmap. Effects of Aspirin on MA.

Mean MA (+/- 95% confidence interval) of all 4 TEG channels before and 2, 6 and 24 hours after the administration of a 300mg dose of Aspirin to 35 volunteers.

The individual results are shown in Figure 9, demonstrating good separation of baseline samples and those after Aspirin. These results intuitively suggest that TEG is capable of detecting responses to Aspirin. However, how best should results be standardized and presented to give the clinician reliable and intuitive information on response to treatment?

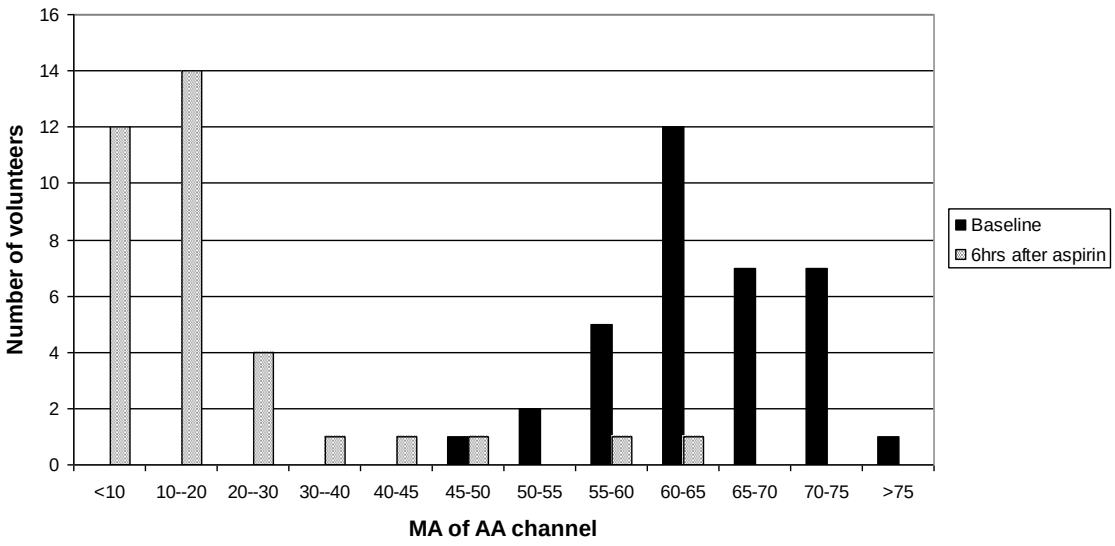

Figure 9 MA of the AA channel at baseline and 6 hours after the administration of 300mg Aspirin in 35 volunteers.

13.2.2 Existing Methods of TEG Pmap Analysis

By conventional analysis the effects of antiplatelet agents can either be calculated by comparing the MA of a sample whilst on antiplatelet therapy with a baseline sample (212) or by utilising the "percentage platelet inhibition" calculated using the MA (25,148).

13.2.2.1 Comparison with a baseline sample

This group has previously calculated the percentage change from baseline of each parameter (212). They subtracted the MA values of the Fibrin channel from ADP and AA values to calculate the non-fibrin (and thus predominantly platelet) contribution to the clot. The percentage change from baseline in the AA- and ADP-activated traces following the administration of antiplatelet agents was calculated to assess individual subject's responses, thus providing an indication of relative response to a certain dose of a drug.

For example inhibition due to Aspirin at time x =

$$\frac{(\text{MA AA baseline} - \text{MA fibrin baseline}) - (\text{MA AA time x} - \text{MA fibrin time x})}{(\text{MA AA baseline} - \text{MA fibrin baseline})} \times 100$$

13.2.2.1.1 Examples of comparison with a baseline sample

To demonstrate the sensitivity of TEG and the profound effect of Aspirin on the TEG response we used TEG Pmap AA assays in a volunteer who had taken no recent medication (215). On two occasions (more than 14 days apart) a baseline venous blood sample was drawn (having discarded the first 2mls) after which a 300mg Aspirin tablet (M&A Pharmachem Ltd.) was licked by the volunteer. In Test 1 an Aspirin was licked once (dose 7.5mg derived from pre- and post lick weight); in Test 2 an Aspirin was licked three times (dose 23.4mg). The baseline sample was immediately analysed by TEG as were further blood samples at two and four hours after licking the Aspirin.
A substantial attenuation in AA-stimulated blood clotting is seen after three licks of Aspirin (Figure 10).

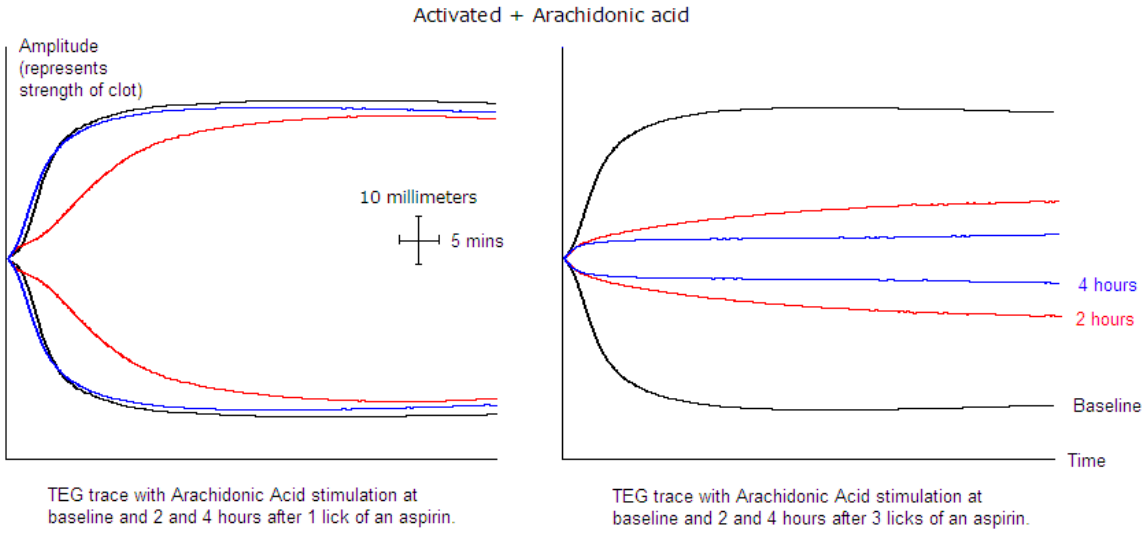

Figure 10 TEG traces showing the response to 1 and 3 licks of Aspirin. Following one lick (left) there is little discernible difference at baseline (black line) and at two (red line) and four (blue line) hours after the lick. By contrast, after three licks (right, dose 23.4mg) there is a substantial time-dependent inhibition of *ex vivo* clotting.

Comparison with the baseline sample using the technique suggested by this group previously is possible in this instance as the individual received a one-off dose of Aspirin. Percentage inhibition compared to baseline was 74% 2 hours and 96% 4 hours after the three lick dose but 1% at 2 hours and -10% 4 hours after one lick of Aspirin.

13.2.2.1.2 Problems with analysis dependent on comparison with baseline

The method used by this group previously requires a baseline sample for comparison. Whilst this is useful in individuals who are being exposed to the medication for the first time (as in the example above) it is of no use in those on maintenance therapy. Also by comparing the before and after results the magnitude of the trace can vary significantly without any bearing on the results. For example an individual with a large pre-treatment clot and a moderate response to the antiplatelet medication may have a greater post treatment clot than an individual with a small pre-treatment clot and only limited

response to the medication. Therefore despite a result indicating a greater effect of the medication the patient may still have a greater tendency to thrombosis.

13.2.2.2 The Percentage Platelet inhibition

The percentage platelet inhibition in response to AA- or ADP-stimulation is calculated by comparing the clot with fibrin alone with maximal platelet activation due to thrombin and platelet activation due to AA or ADP. The first published use of this technique was by Craft et al (148) and it has also been used by P Gurbel and his group at the Sinai Centre for Thrombosis Research (25).

For example the percentage platelet inhibition due to Aspirin can be calculated using the formula:

$$\text{\% platelet inhibition} = 100 - \left(\frac{\text{MA AA channel} - \text{MA Fibrin channel}}{\text{MA Thrombin} - \text{MA fibrin}}\right) \times 100$$

Whilst this has attractions in making the results of TEG Pmap easily interpretable there are potential drawbacks (see Section 13.2.2).

13.2.2.2.1 Example of using the Percentage Platelet inhibition

Using the example above the "percentage platelet inhibition" can also be calculated: percentage inhibition due to Aspirin was 74% 2 hours and 92% 4 hours after the three lick dose but 0% at 2 hours and 2% 4 hours after one lick of Aspirin.

13.2.2.2.2 Problems with analysis using the Percentage Platelet inhibition

The "percentage platelet inhibition" is calculated using the MA. This result is computed by the TEG software. However, in the setting of antiplatelet agents, there can be a considerable delay (often over 1 hour) before the MA is obtained (For example in Figure 10 the MA is reached after 45 minutes in the AA trace at the 2 hour timepoint).

Results can therefore be slow to obtain and in addition results contain information on solely the clot strength and not the speed of clot formation.

In addition the percentage platelet inhibition subtracts the response seen in the fibrin channel in an attempt to describe the effect of platelets on whole blood coagulation. However, fibrin is itself involved in platelet aggregation via its action on the integrin alpha (IIb) beta (3) receptors and is integral to thrombus formation (213). It is therefore possible that this means of calculation negates one of the potential benefits of TEG (namely that it has the ability to detect clinically relevant changes in overall blood clotting (including the effects of fibrin) rather than isolated platelet function). It may however mean that results are more likely to correlate closely with methods of assessing isolated platelet function such as turbimetric optical aggregation.

13.2.3 Developing a novel method

Ideally TEG analysis could (i) be performed more rapidly, (ii) incorporate information on both the speed and strength of clot formation, (iii) allow an assessment of the effects of antiplatelet agents on whole blood coagulation, incorporating the effects of both fibrin and thrombin, (iv) be performed without the need for a baseline reference sample and (v) be easy to understand and (vi) transferable between timepoints and patients. Further, ideally it would provide information not solely on the response to antiplatelet medication but also on the important measure of overall tendency to thrombosis whilst on medication.

13.2.3.1 The Area under the Curve

Utilising the area under the response curve (AUC) (Figure 11) has potential advantages.

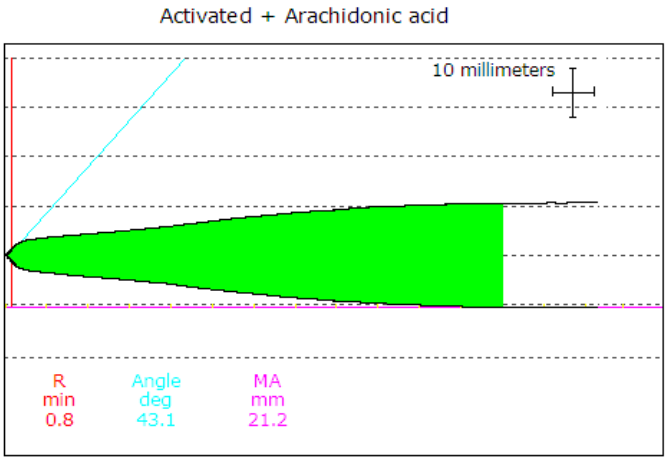

Figure 11 The area under the response curve is highlighted in green.

The AUC is dependent on both the rate of rise and maximum amplitude of the graph and therefore incorporates information on both clot kinetics (the speed of clot formation) and the strength of clot formation.

Figure 12 below shows 2 traces with AA-stimulation following administration of Aspirin which have identical MA (representing clot strength) but in which the speed of clot formation is very different.

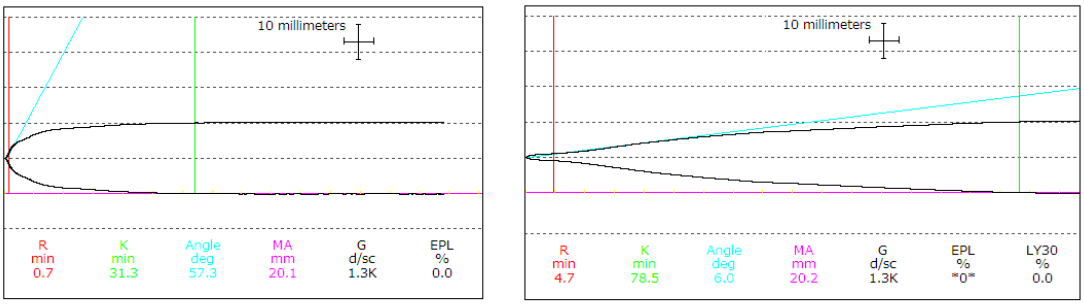

Figure 12 Whilst the MA is nearly identical the speed of clot formation is much reduced in the trace on the right compared to the left.

This is reflected in differences in the measurement of the area under the curve (Figure 13 below).

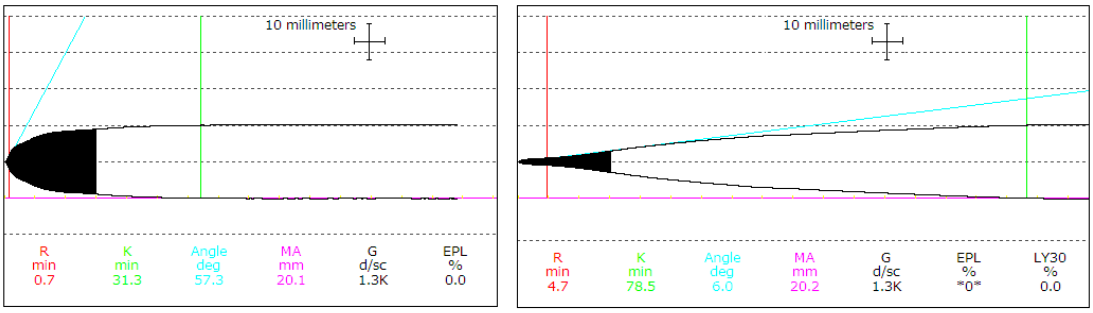

Figure 13 The area under the curve at identical timepoints (AUC) has now been highlighted in these traces showing a dramatic difference in the AUC due to the difference in the speed of clot formation despite almost identical MA (final clot strength).

The AUC can also be measured at a fixed timepoint, rather than the MA which is reached at different timepoints depending on the individual, the channel and any therapies administered (eg after 22 minutes in the left hand curve in figure 13 above and after 74 minutes in the curve on the right). Finally the AUC can potentially be measured at an earlier timepoint than the MA resulting in more rapid result acquisition and a greater assay throughput. The pros and cons of the MA and the AUC are compared in Table VII overleaf.

	Maximum Amplitude	**Area under the curve**
Existing evidence base for use?	Yes	No
Measurement reflects:	Strength of clot formed	Composite measure incorporating rate of clot formation and clot strength
Measurable at reproducible timepoint?	No	Yes
Measurable at early timepoint	No	Yes
Automated results?	Yes	No

Table VII Comparison between the Maximum Amplitude and the Area under the response Curve.

The AUC has previously been used at 60 minutes (AUC60) by this group (211). They demonstrated that the AUC60 could detect differences in response to both aspirin and clopidogrel. However at 60 minutes the AUC is largely dependent on the MA and the effect of the rate of rise of the trace is largely obscured. The use of an earlier timepoint would weight the effect of rate of rise of the trace and the amplitude of the trace more equally (incorporating more information on clot kinetics) as well as providing more rapid result acquisition. We therefore decided to assess the use of the Area under the response curve at 15 minutes (AUC15).

13.2.3.1.1 Calculating the area under the curve.

I helped develop a purpose specific software programme in conjunction with Dr Graham Petley in the Department of Medical Physics and Bioengineering (Areafinder 2.1) to calculate the area under the TEG response curve at any timepoint. From the original TEG trace we develop an enclosed shape from which the area can be calculated. We measure the actual Maximum Amplitude of the trace obtained (this does not necessarily

correspond to the MA calculated by the computer software (eg if the MA has not been reached or is inaccurate) and the time run by the trace to scale the image in both dimensions. The process is demonstrated in Figure 14 below.

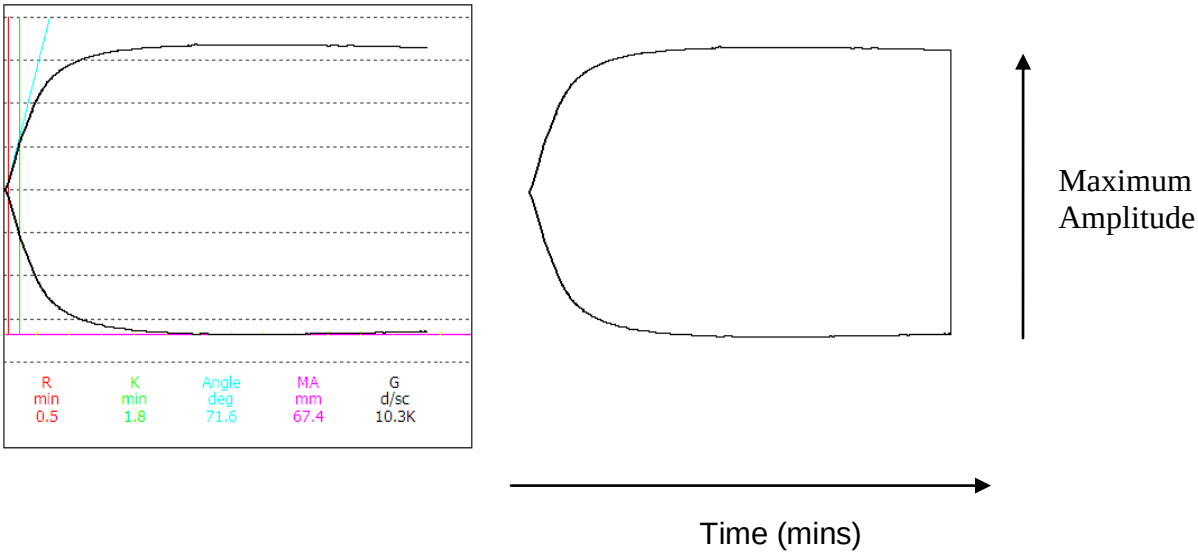

Figure 14 Calculating the AUC: The original TEG trace is shown on the left. From this an image is generated of an enclosed object from which the area can be calculated at any timepoint using AreaFinder 2:1. The maximum amplitude of the trace and the time elapsed are used to provide the scale in both dimensions.

13.2.3.2 The "percentage clotting inhibition"

Whilst the AUC may provide a more rapid and reliable method of assessing individual TEG traces the optimal method of calculating responses to antiplatelet therapy is unknown. Comparison with a baseline sample is seldom practical and the "percentage platelet inhibition" also has potential drawbacks as outlined above. With the group, I developed the percentage clotting inhibition (%CIn) to describe as closely as possible the absolute effect of an anti-platelet medication on the overall *ex-vivo* clotting response of an individual as a snapshot result.

This novel parameter the %CIn due to Aspirin and Clopidogrel is calculated by comparing AA- or ADP-induced clotting responses with response to thrombin, which

represents an invariable internal control. This therefore includes the effect of both platelets and fibrin on blood clotting to allow a more complete assessment of the effects of anti-platelet agents on whole blood coagulation. Furthermore, the new method employs the more rapidly acquired AUC15 rather than the MA to provide a more complete picture of the coagulation process, incorporating both the strength and speed of clot formation.

Thus, for example, the Percentage clotting inhibition (%CIn) due to Clopidogrel would be calculated using the formula:

%CIn= 100- ((AUC15 of ADP channel / AUC15 of Thrombin channel) x 100)

13.2.4 Summary

Using the AUC and the %CIn could theoretically provide rapid and clinically useful results. I went on to compare the use of the AUC and the %CIn against established methods of analysis.

13.3 Comparison between novel and established methods of TEG analysis.

13.3.1 Introduction

In order to demonstrate the potential utility of the novel method of TEG analysis using the AUC and the %CIn I first wanted to establish that it was possible to detect differences due to antiplatelet therapy using these techniques (section 13.3.3). Once this was established I compared the results obtained with conventional analysis using the MA and the %PIn with those of the novel methods of analysis in larger groups of volunteers and patients. The aim was to establish that using the AUC and the %CIn provided more rapid results, without the need for the Fibrin channel, but gave comparable results to those obtained with the slower and more cumbersome traditional method of analysis.

13.3.2 Methods

The preliminary work to establish the ability to detect differences due to antiplatelet therapy with the AUC was performed in our first 10 volunteers administered a 300mg dose of Aspirin. Venesection was performed at baseline (at least two weeks from the administration of any antiplatelet or non-steroidal anti-inflammatory medication) and two and 24 hours after witnessed administration of a 300mg dose of Aspirin.

The comparison between (i) the AUC and the MA and (ii) the %CIn and the %PIn was performed in 30 volunteers and 10 patients administered Aspirin, Clopidogrel and dual antiplatelet therapy as specified in Table VIII overleaf. I analysed a total of 560 individual TEG traces for both the AUC15 and the MA and calculated the %PIn and %CIn for both Aspirin and Clopidogrel in 160 cases. Statistical analysis was performed using Pearson correlation.

Group Size	Volunteer / patient	Anti-platelet therapy administered	Time of sample collection
10	Volunteer	Aspirin 300mg loading dose	Pre and 2,6 and 24 hours after Aspirin
10	Volunteer	Aspirin 75mg daily for 7 days	Pre and post 7 days of Aspirin
10	Volunteer	Clopidogrel 600mg loading dose	Pre and 2,6 and 24 hours post Clopidogrel
10	Patient	Aspirin 75mg daily for >28days Clopidogrel 600mg loading dose	Pre and 2,6 and 24 hours post Clopidogrel

Table VIII TEG: Comparison between novel and established methods of analysis: Groups studied.

13.3.3 Detection of differences using the AUC15.

First I performed a preliminary investigation into using the area under the curve (AUC) to detect differences in response to antiplatelet therapy. I assessed responses in 10 healthy volunteers who had blood tests before and 2 and 24 hours after administration of Aspirin. The MA was recorded and the AUC calculated at both 60 minutes (AUC60) and 15 minutes (AUC15). Analysis showed significant decreases in the MA, the AUC60 and the AUC15 after Aspirin administration, confirming the ability of the AUC at both 60 and 15 minutes to detect differences due to Aspirin. (Figure 15 overleaf)

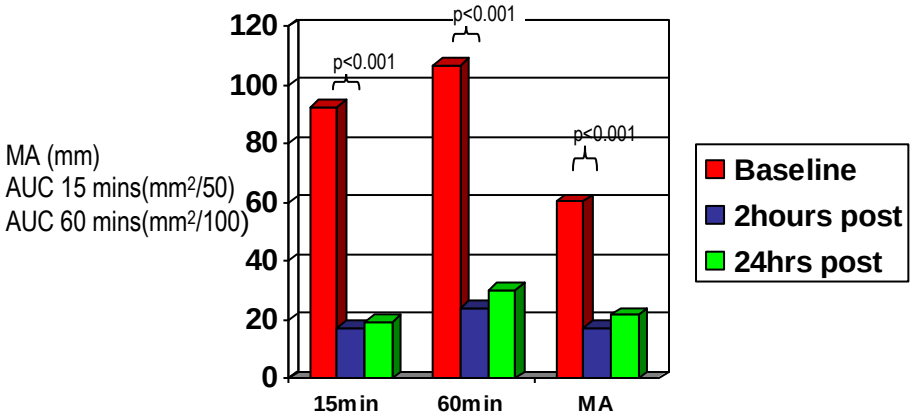

Figure 15 TEG: Comparison between the MA, AUC15 and AUC60 (after administration of 300mg Aspirin to 10 healthy volunteers).

Having confirmed that the AUC could demonstrate differences in response to antiplatelet therapy after only 15 minutes I went on to perform a correlation between the AUC15 and the MA in a greater number of subjects exposed to Aspirin, Clopidogrel or dual antiplatelet therapy.

13.3.4 Correlation between the AUC15 and the MA

A comparison was performed between the MA and the AUC15 results in 30 healthy volunteers and 10 patients receiving antiplatelet therapy using Pearson correlation. Blood samples were analysed before and at multiple timepoints after administration of Aspirin, Clopidogrel or dual antiplatelet therapy. A total of 560 traces were analysed and incorporated into the correlation. There was a very close overall correlation (R=0.964, p=<0.01), see Figure 16).

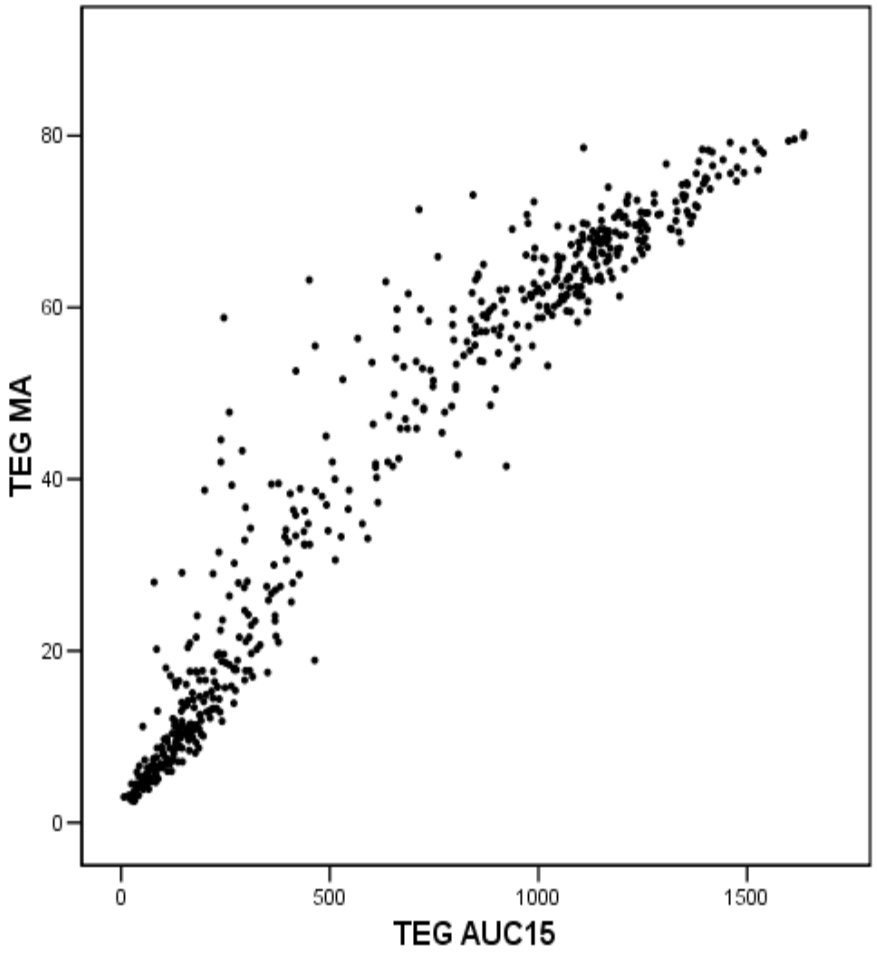

Figure 16 TEG: A scatterplot showing the correlation between the MA and AUC15 (in thirty healthy volunteers and 10 patients undergoing elective percutaneous intervention (R=0.964, n=560, p<0.01)).

There were also close correlations for each individual channel (Thrombin: R=0.824, n=160, p<0.001; Fibrin: R=0.789, n=160, p<0.001; APD: R=0.963, n=160, p<0.001; AA: R=0.951, n=160, p<0.001) and when patients (R=0.916 ,n=160, p<0.001) and volunteers (R= 0.901, n=400, p<0.001) were considered individually.

13.3.5 Correlation between the %PIn and %CIn

I also performed a correlation between the calculated %PIn using the MA and the %CIn using the AUC15. There was a close correlation between the two methods of analysis ($R=0.903$, $n=320$, $p<0.01$). (Figure 17)

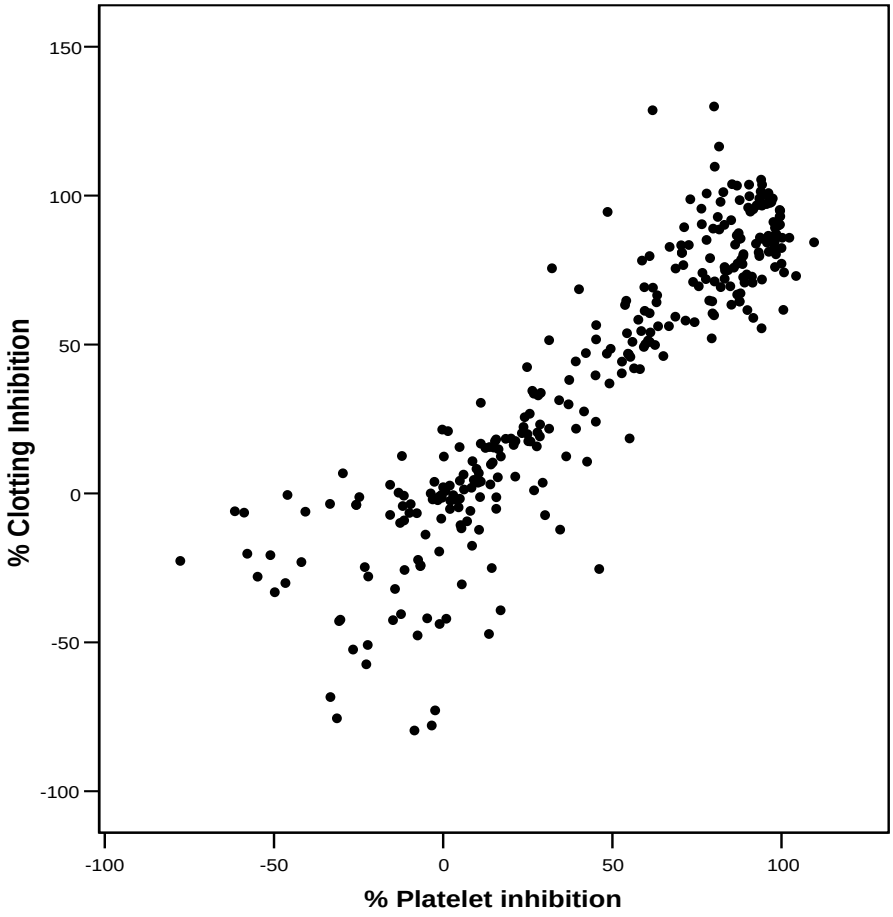

Figure 17 TEG: A scatterplot showing the correlation between the %PIn and %CIn (n=160).

13.3.6 Summary

These results confirm that the AUC15 can detect differences in response to antiplatelet therapy and that these results correlate strongly with the MA. The %CIn calculated using the AUC15 also correlates well with the %PIn, but can be calculated without the need for a fibrin channel and in only 15 minutes. With the favourable results of these analyses I

set out to investigate the potential of TEG, using the AUC15 and %CIn “short TEG” as a clinical tool to assess responses to antiplatelet therapy and identify risk.

13.4 Normal Ranges

13.4.1 Introduction

Despite the increasing body of evidence demonstrating that responses of individual patients to antiplatelet therapies are heterogeneous, and that the level of response is linked to outcome, assessment of response to antiplatelet therapy is still rare in clinical practice. Some recent guidelines recommend monitoring response to antiplatelet therapy in high risk individuals and modifying therapies in those who appear to respond poorly (18). However there is as yet no generally accepted method of assessment. Responses to antiplatelet drugs remain difficult to assess clinically, with no widely available and generally accepted point-of-care assays, and no standardised definitions of adequate response or "resistance". In addition many assays require a baseline sample to calculate the response to treatment. Other assays assess platelet reactivity whilst on antiplatelet therapy.

An ideal test for monitoring response to antiplatelet therapies would be (a) rapid, (b) available at the point-of-care, (c) capable of assessing responses to a wide variety of antiplatelet therapies and (d) provide reliable, reproducible and easily comparable results without the need for a baseline sample. Conventional definitions of "resistance" to antiplatelet drugs, including Aspirin and Clopidogrel, are determined using laboratory assays of isolated platelet function. The limitations of such methods for the clinician include that (a) they are time consuming, (b) expensive, (c) require special training to perform and (d) often yield higher levels of resistance than clinical treatment failure would suggest. The availability of rapid point-of-care tests of platelet responsiveness providing clinically relevant and easily interpretable results from a single sample would provide clinicians with powerful tools to enable the detection of patients at increased risk due to relative hyporesponsiveness to initial standard therapy and potentially guide prophylactic treatment modification in these patients. To establish such point-of-care assays and in order to determine abnormal responses, it is however necessary to first determine what constitutes a normal response and to compare results obtained whilst on

antiplatelet therapy with those obtained by comparison with baseline samples. This is particularly important as (i) baseline platelet reactivity varies (ii) responses to antiplatelet therapy change over time and (iii) there is cross-reactivity between different antiplatelet agents which can complicate analysis.

Using the AUC and the %CIn short TEG could provide a rapid assessment of an individual's clotting response whilst on Aspirin and / or Clopidogrel without requiring a pre-treatment baseline value. This is potentially highly clinically relevant in the field of PCI, particularly in the context of drug eluting stents. However, in order to determine what constitutes an abnormal response it is of course first necessary to establish what are "normal responses" to antiplatelet therapy.

In this study we have assessed (a) responses to loading doses of Aspirin and Clopidogrel and (b) time-dependent short TEG measurements of platelet reactivity whilst on maintenance therapy with both aspirin and dual antiplatelet therapy. Our aim was to establish reference ranges for responses to Aspirin and Clopidogrel, in order to facilitate the use of short TEG as a "snapshot" to assess responses of individual patients to these antiplatelet agents.

If validated the use of short TEG could allow tailored therapy for patients due to undergo PCI in order to minimise risk of subsequent ischaemic events.

13.4.2 Methods

40 volunteers on no medication, 65 patients on maintenance therapy with Aspirin 75mg daily and 20 patients on maintenance therapy with dual antiplatelet therapy of Aspirin and Clopidogrel following percutaneous intervention were recruited according to specific protocols. Individuals were excluded if they were taking or had recently taken non-steroidal anti-inflammatory medication, anticoagulants or antiplatelet therapy other than that specified in the study. Volunteers were excluded if they had a history of bronchial

asthma, peptic ulceration, bleeding tendency or previous sensitivity to anti-platelet agents. Patients were excluded if they had had a coronary event within 28 days or suffered thrombotic or haemorrhagic complications following PCI.

In all subjects venesection and sample analysis was performed as previously described.
Platelet reactivity after treatment was measured using the short TEG AUC15 of the AA or ADP channels (for Aspirin and Clopidogrel respectively)
Response to treatment was established by comparing the short TEG AUC15 results after administration with those obtained at baseline.
The Percentage Clotting Inhibition (%CIn) was calculated as described previously.

Loading dose results are presented as the mean +/- 95 % confidence intervals of the mean at each timepoint. Results following the Clopidogrel loading dose are presented graphically as the median responses and 5^{th}, 25^{th}, 75^{th} and 95^{th} centiles.
Maintenance therapy results are presented as the mean +/- 95% confidence interval of the mean and presented graphically compared with results obtained from the group on no antiplatelet therapy.

13.4.2.1 Statistical analysis

Results between groups were analysed with 2 group two tailed t-tests with a p value of <0.05 considered to represent significance. Pearson's correlation was performed between results obtained with the %CIn and those obtained by comparison with a baseline sample.

13.4.3 Results

13.4.3.1. Responses to an Aspirin 300mg loading dose

35 individuals on no antiplatelet therapy received a 300mg Aspirin loading dose. All 35 individuals had blood tests taken at baseline and 6 hours after Aspirin. 10 of the 35 had additional blood tests taken 2 and 24 hours after Aspirin.

13.4.3.1.1 Platelet reactivity after Aspirin

Mean short TEG AUC15 of AA channel was at 983 +/- 80 at baseline, 175 +/- 66 at 2 hours, 242 +/- 69 at 6 hours and 195 +/- 71 at 24 hours. There were highly significant changes at all timepoints from baseline (all $p<0.0001$).

13.4.3.1.2 Response to therapy

The calculated percentage change in short TEG AUC15 of AA channel from baseline was 83.2 +/- 4.9% at 2 hours, 73.7 +/- 8.5% at 6 hours and 81.6 +/- 5.4% at 24 hours. All changes from baseline were highly significant (all $p<0.0001$).

13.4.3.1.3 Percentage clotting inhibition

The %CIn calculated from short TEG AUC15 results from a "snapshot" sample provided comparable results (86.1 +/- 4.6% at 2 hours, 71.9 +/- 8.3% at 6 hours and 84.7 +/- 4.3% at 24 hours) (Figure 18).

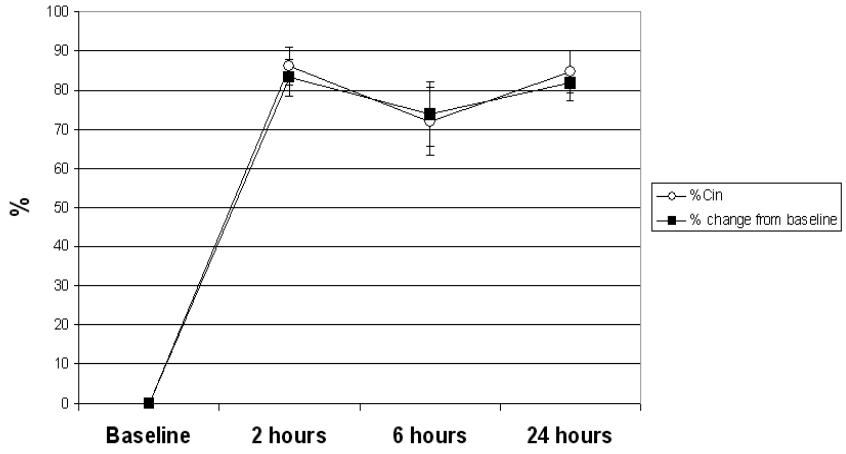

Figure 18 TEG: Comparison of the %CIn and % change from baseline after Aspirin (35 volunteers, 300mg Aspirin)

13.4.3.1.4 Correlation between response to Aspirin and %CIn

There was close correlation between the %CIn and the response to therapy (R=0.93, p<0.001, Figure 19 below)

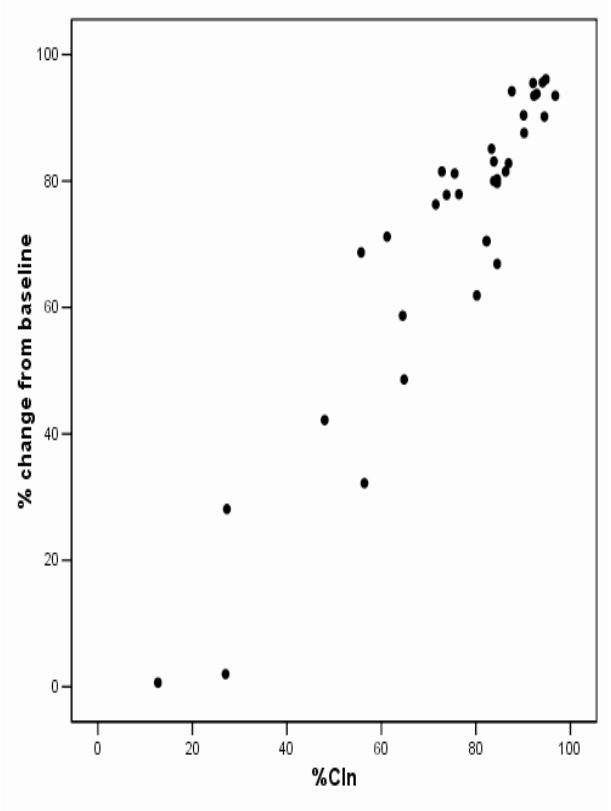

Figure 19 TEG: Correlation between %CIn and % change from baseline after Aspirin

13.4.3.2 Responses to maintenance therapy with Aspirin 75mg daily

65 patients with known coronary disease taking chronic maintenance therapy with Aspirin 75mg daily.

13.4.3.2.1 Platelet reactivity whilst on Aspirin

The short TEG AUC15 of the AA channel was 400 +/- 64 compared to 922 +/- 74 in the baseline samples obtained from volunteers ($p<0.0001$).

Results are presented graphically compared with results obtained from volunteers on no antiplatelet therapy. (Figure 20 below)

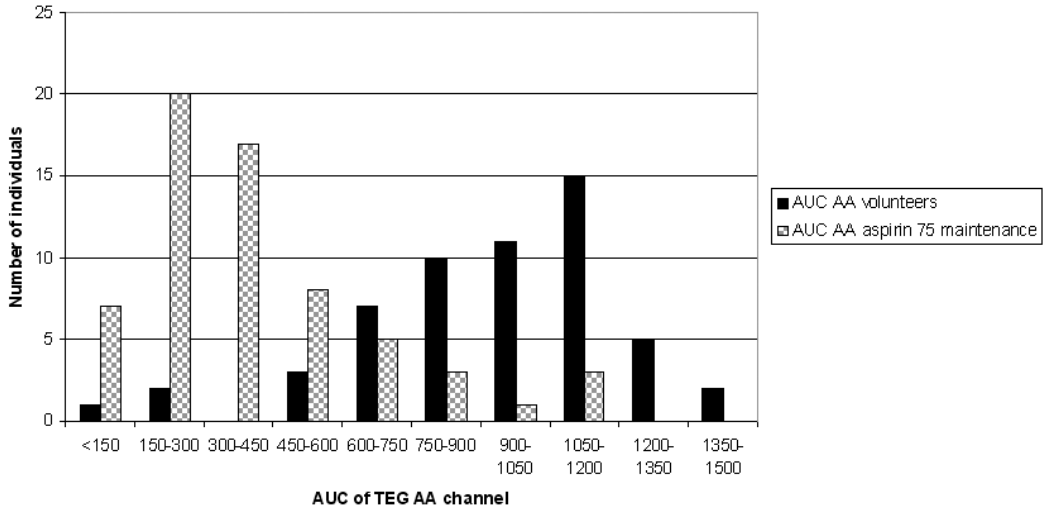

Figure 20 short TEG: AUC15 of the AA channel on and off Aspirin
(Volunteers on no medication vs. patients on Aspirin 75mg daily maintenance therapy)

13.4.3.2.2 Percentage clotting inhibition

%CIn was 56.7+/- 6.9% compared to 6.5+/- 9.7 in Group 1 (Figure 21). There were highly significant differences between those on and off aspirin with both the AUC15 of the AA channel and the %CIn (all $p<0.0001$).

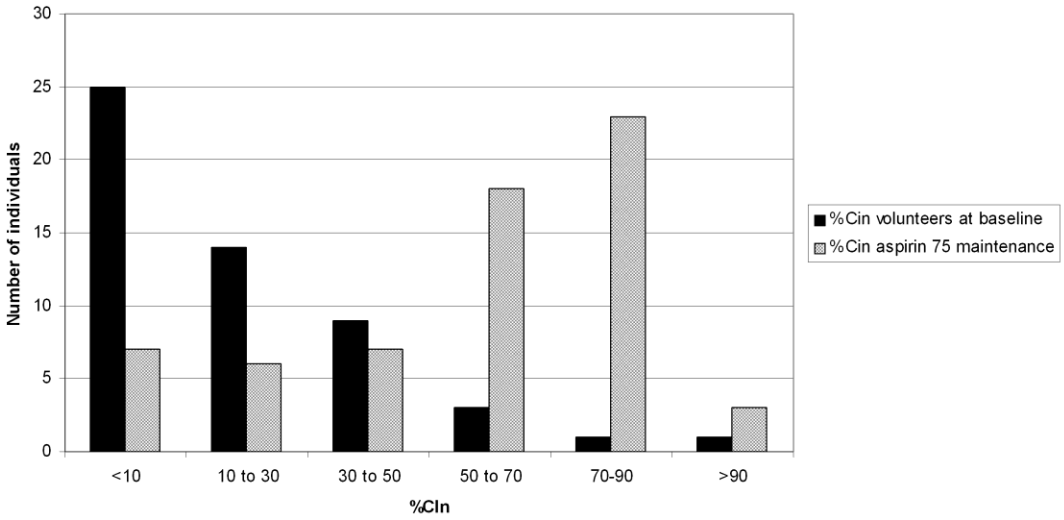

Figure 21 short TEG: %CIn on and off Aspirin
(patients on Aspirin 75mg daily maintenance vs. volunteers on no medication)

13.4.3.3 Responses to a Clopidogrel 600mg loading dose on background of Aspirin maintenance therapy

38 patients on Aspirin 75mg daily being loaded with Clopidogrel prior to planned invasive coronary investigation for symptoms suggestive of angina or known coronary disease. Patients were excluded if they had had a coronary event within 28 days. Blood tests were taken as previously described at baseline and 1,2, 6 and 24 hours after administration of the Clopidogrel loading dose. Results were excluded (7%) if additional antiplatelet or antithrombotic medication not specified in this study (Abciximab or Bivalirudin) was administered. 93% of all datapoints were therefore included in analysis. 23 of 38 patients (61%) receiving Clopidogrel loading underwent successful percutaneous intervention. The remainder were treated medically or with surgical revascularisation.

13.4.3.3.1 Platelet reactivity whilst on treatment

The mean short TEG AUC15 of ADP channel was 1061 +/- 67 at baseline, 930 +/- 87 at 1 hour, 721 +/- 116 at 2 hours, 686 +/- 113 at 6 hours and 707 +/- 102 at 24 hours. All

changes from baseline were significant (p=0.02 at 1 hour, p<0.0001 at all other timepoints) as were changes from1 hour to all subsequent timepoints (all p<0.01).

13.4.3.3.2 Response to therapy

The percentage change in short TEG AUC15 of ADP channel compared to baseline was 9 +/- 9% at 1 hour, 31 +/- 11% at 2 hours, 36 +/- 11% at 6 hours and 32 +/- 11% at 24 hours (Figure 22).

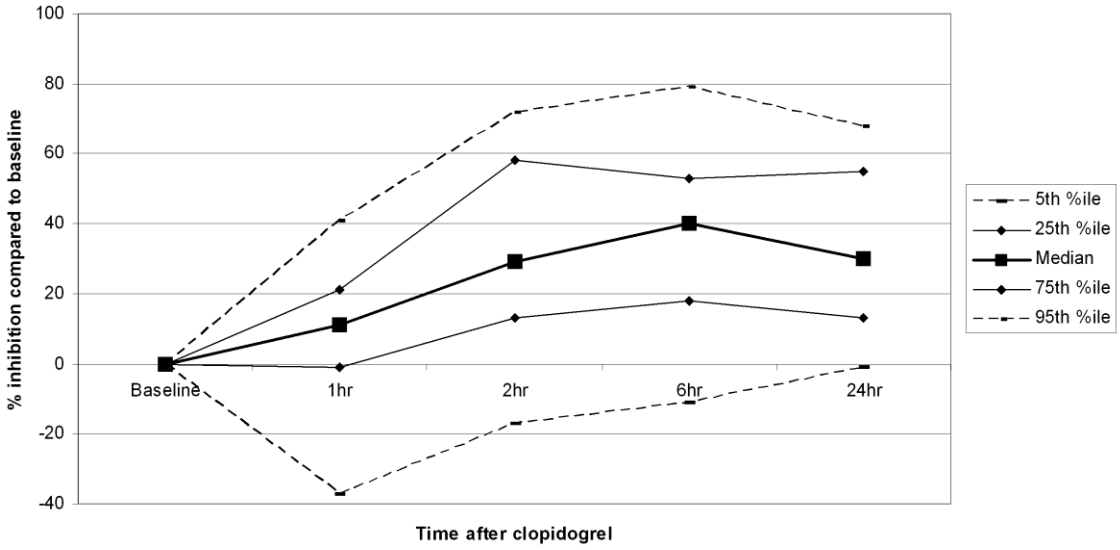

Figure 22 short TEG: % change in the AUC15 of ADP channel after Clopidogrel (post 600mg compared to baseline)

13.4.3.3.3 Percentage clotting inhibition

The %CIn was 5 +/- 9% at 1 hour, 25+/- 12% at 2 hours, 34 +/- 9% at 6 hours and 33 +/- 9% at 24 hours (Figure 23). The change from baseline to 1 hour was not significant (p=0.052). All other changes from baseline were significant as were changes from 1 hour to all subsequent timepoints.

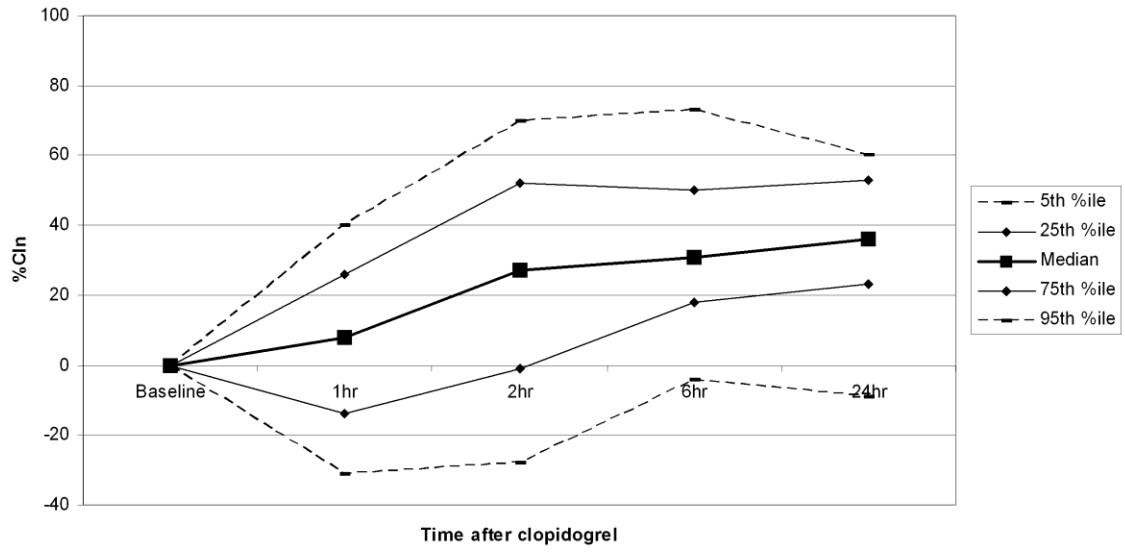

Figure 23 short TEG: %CIn post Clopidogrel (600mg loading dose)

13.4.3.3.4 Correlation between response to Clopidogrel and %CIn

There was a good correlation between results obtained with the %CIn and those obtained by comparison with baseline (R=0.83, p<0.001,Figure 24).

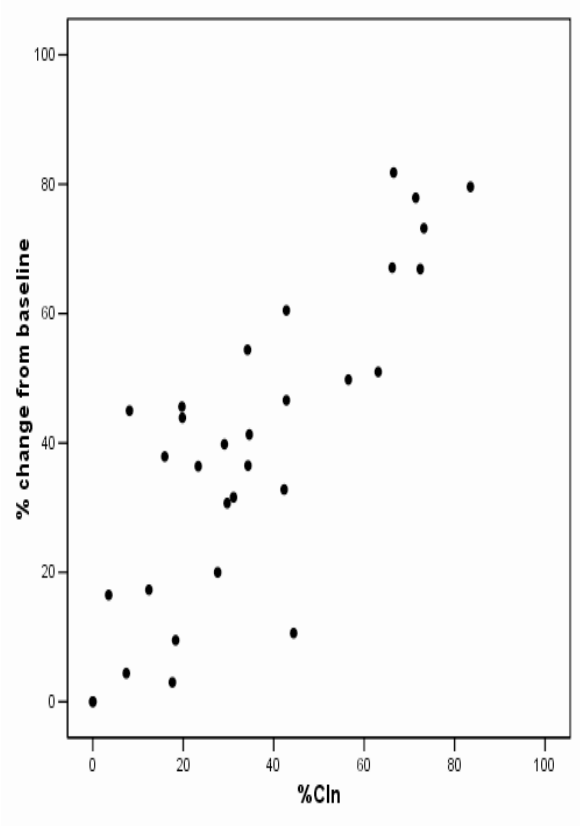

Figure 24 TEG: Correlation between %CIn and % change from baseline (in assessment of response to Clopidogrel)

13.4.3.4. Responses to maintenance therapy with Aspirin and Clopidogrel

20 patients on Aspirin 150mg and Clopidogrel 75mg daily after percutaneous coronary intervention and stent insertion. Patients were included if they had had dual antiplatelet therapy for at least 28 days and excluded if they had had complications or recent thrombotic event**.**

Results are presented as the mean +/- 95% confidence interval of the mean and presented graphically compared with results obtained from the volunteers on no antiplatelet therapy.

13.4.3.4.1 Responses to Aspirin

Platelet reactivity whilst on treatment

The AUC15 of the AA channel was 198 +/- 45. Responses to Aspirin were significantly greater than in the group with Aspirin 75mg monotherapy, (AUC15 of AA channel 198 +/- 45 vs. 400 +/- 64; $p<0.001$). However there was no significant difference compared to the Aspirin responses 6 hours after a loading dose of Clopidogrel in patients on 75mg of aspirin (AUC15 of AA channel 198 +/- 45 vs. 231 +/- 33, $p=0.25$).

Percentage clotting inhibition

%CIn due to Aspirin was 77.4 +/- 5.3%. In keeping with the response to treatment the %CIn was significantly higher than in the Aspirin monotherapy group (%CIn 77.4 +/- 5.3 vs. 56 +/- 7.0%; $p<0.001$) but there was no difference compared to values obtained 6 hours after a Clopidogrel loading dose in patients on Aspirin 75mg maintenance therapy (%CIn 77.4 +/- 5.3 vs. 77.3 +/- 3.4, $p=0.99$).

13.4.3.4.2 Responses to Clopidogrel

Platelet reactivity whilst on treatment

The AUC15 of the ADP channel was 611 +/- 106. There was no significant difference in platelet reactivity whilst on maintenance Clopidogrel compared to results obtained 2, 6 and 24 hours after Clopidogrel loading in Group 3. However, responses were significantly greater than at the 1 hour timepoint ($p=0.002$).

Percentage clotting inhibition

%CIn due to Clopidogrel was 30.4 +/- 11.0. In keeping with the results obtained from platelet reactivity whilst on treatment there was no significant difference in %CIn due to Clopidogrel compared to results obtained 2, 6 and 24 hours after Clopidogrel loading in Group 3. Again however, responses were significantly greater than at the 1 hour timepoint (p=0.002).

13.4.4 Discussion

Given the documented heterogeneity of antiplatelet responses, it is perhaps not surprising that administering standard doses of Aspirin and Clopidogrel to all PCI patients may render some of them at increased risk of adverse clinical events.
I employed short TEG to assess responses of both healthy volunteers and PCI patients to Aspirin and Clopidogrel with the aim of assessing its ability to detect variability of response and build up "reference ranges". The aim was thus to help determine the suitability of TEG as a point-of-care assay in clinical practice.

This study has established that short TEG, through the use of the %CIn, can detect responses to antiplatelet therapy within 15 minutes from a single sample taken whilst on antiplatelet therapy and that these results correlate well with responses to therapy obtained by comparison with a baseline sample. In keeping with other studies, this study has shown marked heterogeneity in response to Clopidogrel.

These results contribute to the validation of TEG as a useful point-of-care assay that can be used independent of laboratory based tests. Short TEG could potentially be used to screen all patients scheduled to undergo PCI in order to detect those who are relatively hyporesponsive. This individualised information could, if borne out by subsequent investigation, then be used to manipulate subsequent risk, possibly by increasing the dose and then re-assessing the response.

This study has some limitations. First, I have not correlated short TEG data with isolated platelet function tests, although this has been done for standard TEG by other groups

(25,127,138). Second, although the administration of all loading doses was witnessed we do not have biochemical validation of compliance with maintenance doses of medication, or indeed with the absence of over-the-counter Aspirin ingestion in baseline samples.

13.4.5 Summary

This study provides reference ranges for responses to Aspirin and Clopidogrel as used in PCI. As a quick, point-of-care test short TEG may provide a clinically relevant solution to the need to assess individual responses to Aspirin and Clopidogrel in patients in order to ensure adequate response to antiplatelet therapies and perhaps reduce the risk of complications, including ST, in some patients. Further data is required on whether short TEG responses correlate with adverse events.

13.5 The influence of gender on TEG responses

Abstract

Background

There is significant variability in both baseline clotting tendency and response to antiplatelet therapy. Responses are associated with outcome. We have investigated gender-dependent differences in baseline clotting and response to antiplatelet therapy using TEG that may be associated with the increased risk observed in women presenting with coronary artery disease.

Methods

We have utilised short TEG to assess (i) baseline clotting responses, (ii) response to Aspirin and Clopidogrel and (iii) post-treatment platelet reactivity in 48 young volunteers, 22 older patients and 18 patients with previous ST.

Results

Baseline clotting responses by TEG (prior to administration of antiplatelet therapy) were significantly higher in young healthy women than in men. Whilst there was no difference in response to Aspirin, platelet reactivity on Aspirin remained higher in women (AUC15 of AA channel 332 ± 122 vs. 172 ± 80, $p=0.04$). Young women had less response to Clopidogrel (% reduction in AUC15 of ADP channel 36.4 ± 12.4 vs. 64.0 ± 13.2, $p<0.01$) in addition to higher post-treatment reactivity (AUC15 of ADP 714 ± 161 vs. 311 ± 146, $p<0.01$) compared to men. There were no such differences between male and female patients over 50. However, young women with previous ST had amongst the highest platelet reactivity observed.

Conclusions

Compared to men, young women have greater baseline clotting tendency, reduced response to Clopidogrel and greater post-treatment reactivity whilst on both Aspirin and Clopidogrel. Differences in clotting tendency and response to antiplatelet therapy may contribute to the excess risk observed in young women but are not observed in older female patients.

13.5.1 Introduction

There are well-established gender differences in both clinical presentation and outcome in ischaemic heart disease. For example, women, whilst less likely to suffer from myocardial infarction (MI), have higher in-hospital mortality and are more likely to sustain complications than men (216,217). Whilst there are differences in presentation, anatomy, co-morbidities and provision of evidence-based therapies between the sexes (218,219) significant clinical differences remain even when these factors are taken into account, particularly in women under the age of 50 who have double the mortality rate of men with the same risk profile (220,221).

It is possible that differences in propensity to blood clotting and response to antiplatelet therapy explain some of the observed gender differences. Baseline platelet hyper-reactivity is more common in women, and mean levels of platelet aggregation in response to stimulation with platelet agonists are consistently greater in women than in men (222,223). Responses to antiplatelet therapy may also differ between men and women. For example, Aspirin therapy appears to be less effective in women. A randomised controlled trial of low dose Aspirin therapy as primary prevention in 39,876 high risk women showed no significant reduction in cardiovascular events after 10 years when compared to placebo (224) in contrast to data from predominantly male study populations, which show an average 32% reduction in risk of MI (225). This could be partly explained by the finding that female gender is associated with higher ADP- and collagen-induced platelet aggregation whilst on Aspirin (226). A strong association has also been shown between Clopidogrel resistance and female gender in patients with coronary artery disease using ADP-induced aggregometry (227).

It is unclear, however, whether these observed gender differences are due to differences in baseline reactivity or due to differences in response to antiplatelet agents. We have sought to elucidate if there are gender differences in platelet reactivity in young women volunteers and in female patients undergoing PCI that could potentially contribute to our understanding of these gender differences in clinical outcome using short TEG.

TEG is uniquely suited to this role. Unmodified TEG provides a global assessment of coagulation incorporating the effects of platelets, thrombin, fibrin and coagulation factors. Used in this way TEG has previously demonstrated greater coagulability in female trauma patients (228). In addition short TEG can assess responses to both Aspirin and Clopidogrel in whole blood. A single TEG assay can therefore provide information on both the overall tendency to thrombosis and the response to antiplatelet therapy.

The aim of this study was therefore to utilise short TEG to establish if there are gender differences in (i) clotting tendency, (ii) response to antiplatelet therapy and (iii) post-treatment platelet reactivity in young volunteers and in patients undergoing PCI which could contribute to our understanding of the reasons for the high risk of mortality observed in young women post MI.

13.5.2 Methods

Data was pooled for both the volunteer and patient groups studied. Power calculations determined that group sizes of 17 would detect a 10% difference in platelet function between men and women with 80% power.

13.5.2.1 Study Groups

The following groups were studied:

Healthy Volunteers

From the cohort of volunteers 51 individuals (23 Males and 28 Females) suitable for analysis were identified. They were all under the age of 50, non-smokers, had not taken antiplatelet medication or non-steroidal anti-inflammatory medication within 14 days and had no history of peptic ulceration, bronchial asthma, or bleeding. All 51 individuals had a baseline blood test. 33 had blood tests immediately before and 6 hours after a witnessed administration of 300mg of Aspirin and 32 had blood tests immediately before and 6 hours after 600mg of Clopidogrel.

Patients Admitted for Elective PCI

For comparison 22 patients (12 males and 10 females) were identified from those recruited attending for routine PCI. All were non-smokers, not diabetic, on statin therapy, known to have CAD and between the ages of 50 and 80 years. All 22 patients reported compliance with Aspirin maintenance therapy and received a 600mg loading dose of Clopidogrel prior to PCI. Blood tests were taken prior to, and 6 hours after Clopidogrel loading.

Patients with a history of ST and matched controls

Data were also obtained from survivors of non-acute ST whilst on maintenance Aspirin and Clopidogrel therapy from a consecutive cohort of 3004 PCI patients at this centre and matched PCI patients without ST (229,230). We studied responses in 18 patients with previous ST reporting compliance with dual antiplatelet therapy including Aspirin and Clopidogrel 75mg daily. Blood tests were taken from all patients at least 10 days after the latest coronary event or intervention.

13.5.2.2 Sample Analysis

Venesection and sample analysis was performed as specified in the Methods (Section 12) and TEG analysis sections (Section 13.2.3). If volunteers had more than one baseline sample only the first was included in analysis of baseline responses. In order to calculate the effect of treatment the percentage change from baseline in the MA and AUC15 (of the AA for Aspirin, and the ADP channel for Clopidogrel) were calculated by comparing the results obtained from the post treatment sample with those from the baseline sample taken immediately prior to drug administration.

From the post treatment samples the %PIn and %CIn were calculated as specified in Section 13.2.

The number of non-responders in each gender group was calculated for both Clopidogrel and Aspirin. A ‘non-responder’ to Clopidogrel was defined using the AUC15 (less than 30% reduction in the ADP channel compared to baseline) and by the %PIn (less than

30% inhibition). Non-response to Aspirin was defined as less than 50% reduction in the AUC15 and by %PIn of less than 50%. As patients were all established on Aspirin it is not possible to examine baseline responses (prior to antiplatelet therapy) in the patient group.

Data are presented as the mean ± 95% confidence interval of the mean. Significance between groups was determined using two-tailed, two group t-tests with a p-value of <0·05 considered to represent significance. Two tail Fisher's Exact tests were used to determine differences between numbers of responders.

13.5.3 Results

13.5.3.1 Healthy Volunteers

13.5.3.1.1 Demographics

There were no important differences between the gender groups. Mean age was 28.8 ± 2.6 years in men and 26.2 ± 2.7 years in women.

13.5.3.1.2 Baseline responses

At baseline, prior to the administration of antiplatelet therapy both the MA and the AUC15 (Table IX) were significantly greater in females than in males in all 4 channels.

	Female	Male	*P*
Thrombin	1141±93	983±94	<0.05
ADP	1090±55	812±112	0.0001
AA	1016±99	809±106.	0.01
Fibrin	210±38	83±21	<0.0001

Table IX short TEG: Baseline responses in male and female volunteers (AUC15; mean ± 95% CI)

13.5.3.1.3 Responses to Aspirin

In females the MA and AUC15 of the AA channel were significantly greater after Aspirin administration (Table X). However there were no significant differences between men and women in the percentage change from baseline (Figure 25) or in the %PIn or %CIn. Two of 17 men (12%) and two of 15 women (13%) were non-responders to Aspirin assessed by change in the AUC15 of the AA channel, and one (6%) man and three (20%) women when assessed using the %PIn.

Aspirin 300mg	***Female***	***Male***	***P***
MA at 6 hours	28.9+/-10.5	14.8+/-5.6	0.04
AUC15 at 6 hours	350+/-122	193+/-80	0.04
% change in MA	57.6+/-14.6	74.8+/-14.9	0.11
% change in AUC15	67.0+/-10.8	74.5+/-14.9	0.44
%CIn at 6 hours	63.9+/-15.5	78.6+/-10.2	0.13
%Pin at 6 hours	71.8+/-22.7	82.5+/-13.2	0.43
Non-responders (AUC15)	2 (13%)	3 (17%)	1.0
Non-responders (%PIn)	3 (20%)	2 (11%)	0.64
Clopidogrel 600mg			
MA at 6 hours	43.1+/-10.0	25.3+/-7.0	0.008
AUC15 at 6 hours	663+/-187	353+/-112	0.01
% change in MA	35.1+/-13.7	56.2+/-11.0	0.03
% change in AUC15	40.7+/-14.2	61.0+/-10.1	0.02
%CIn at 6 hours	37.5+/-18.3	61.4+/-12.3	0.02
%Pin at 6 hours	40.6+/-16.0	65.3+/-11.7	0.04
Non-responders (AUC15)	10 (63%)	1 (6%)	0.002
Non-responders (%PIn)	8 (50%)	0 (0%)	0.01

Table X TEG: Results in male and female volunteers after Aspirin and Clopidogrel (300mg Aspirin, assessed with the TEG AA channel and 600mg Clopidogrel assessed with the TEG ADP channel; Mean ± 95% CI)

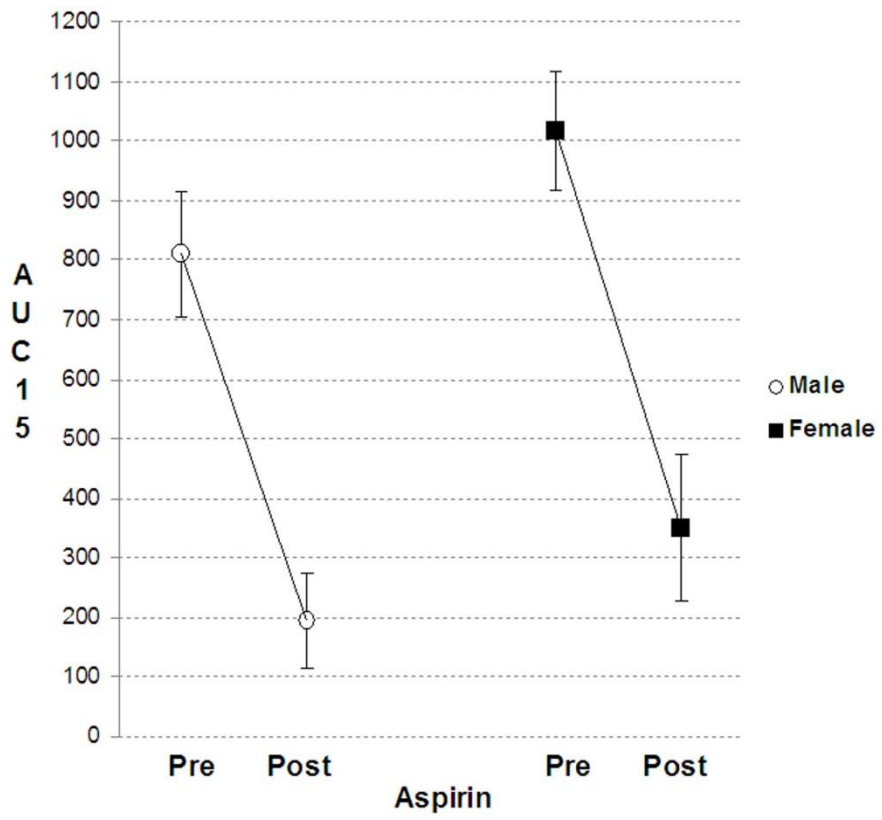

Figure 25 short TEG: Responses to Aspirin in Male and Female Volunteers. (AUC15 of AA channel)

13.5.3.1.4 Responses to Clopidogrel

The MA and the AUC15 of the ADP channel were significantly greater in females after Clopidogrel administration (Table X). In addition, the percentage change from baseline was significantly less in females (Figure 26) as was the %PIn and %CIn calculated from the post treatment sample.

10 of 16 females (63%) and one of 16 males (6%) met the criteria for non-response to Clopidogrel using the change in the AUC15 of the ADP channel (p=0.002). Eight women (50%) and no men (0%) were non-responders as assessed by the %Pin (p=0.01).

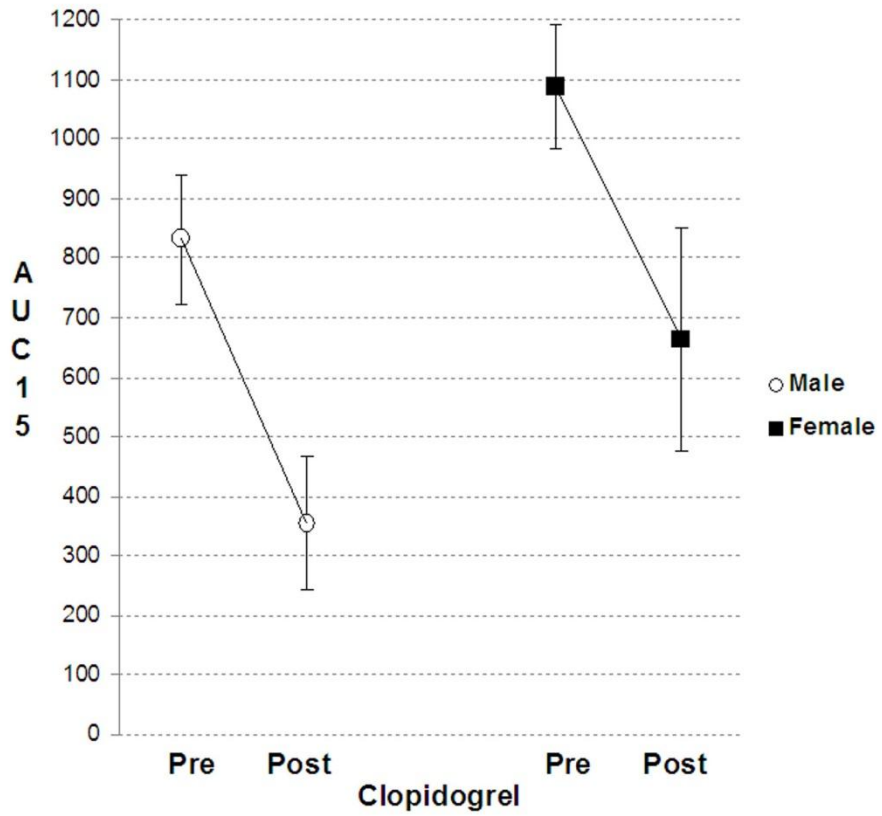

Figure 26 short TEG: Responses to Clopidogrel in male and female volunteers. (ACU15 of ADP channel)

13.5.3.2 Patients Undergoing Elective PCI

13.5.3.2.1 Demographics

There were no significant differences between the two groups in terms of age (68 ± 5 in men and 64 ± 5 in women, p=0.27), medication (all were on statins, one in each group on Atorvastatin), or baseline haematological parameters. Males were significantly heavier (90 ± 8kg vs. 73 ± 7kg, p=0.004). .

13.5.3.2.2 Responses to Clopidogrel

There were no significant differences in the MA or the AUC15 between the gender groups after Clopidogrel administration (Table XI overleaf). There were also no

significant differences in the percentage change from baseline or in the number of non-responders to Clopidogrel therapy.

	Female	**Male**	***P***
MA at 6 hours	41.8±8.3	43.5+/-10.0	0.80
AUC15 at 6 hours	674+/-156	719+/-160	0.70
% change in MA	35.0+/-11.1	27.0+/-18.6	0.47
% change in AUC15	40.1+/-10.6	37.7+/-17.6	0.34
%CIn	40.4+/-10.2	32.4+/-11.8	0.33
%PIn	43.3+/-11.4	36.8+/-16.7	0.53
Non-responders (AUC15)	2 (20%)	6 (50%)	0.07
Non-responders (%PIn)	4 (40%)	5 (42%)	1.00

Table XI short TEG: Responses of male and female patients to Clopidogrel (600mg loading dose, AUC15 of ADP channel, mean ± 95% CI.)

13.5.3.3 Patients with History of Stent Thrombosis

13.5.3.3.1 Demographics

There were 14 ST cases in men and four cases in women. Three of the four cases in women occurred in women under the age of 52. The groups were closely matched. All patients had DES. There were no significant differences in duration and dose of antiplatelet therapy (dose of Clopidogrel 80.4 ± 10 mg in men vs. 75 ± 0 in women, duration of Clopidogrel 287 ± 163 days vs. 276 ± 77 days, coronary risk factors (14% of men were diabetic vs. 0% of women; 21% of men vs. 25% of women were smokers) or procedure undertaken (1.5 ± 0.3 vs. 2 ± 2 stents; minimum stent diameter 2.75 ± 0.2 vs. 2.75 ± 0.2mm; 14% vs. 25 % emergency procedures) (Investigation into ST is covered in more detail in Section 13.7).

13.5.3.3.2 Responses to Clopidogrel

The AUC15 of the ADP channel was 849 ± 188 in the ST group and 640 ± 128 in the control group. Two of the three young women with previous ST have amongst the highest platelet reactivity despite Clopidogrel (AUC15 1159 and 1056 respectively).

13.5.4 Discussion

This study, employing short TEG, demonstrates that there is both an elevated baseline clotting tendency and a reduced response to clopidogrel in young healthy females compared to equivalent males. Whilst these gender differences are not observed in a typical population of older and post-menopausal female patients, it is of interest that the small number of young women from our registry who survived ST did indeed exhibit reduced responsiveness to Clopidogrel.

It is now well established that there is significant variability in responses to antiplatelet therapy (particularly Clopidogrel) between individuals and that poor response is associated with adverse outcomes (Section 11.4). In addition, overall tendency to thrombosis (measured by unmodified TEG) has been associated with adverse outcome in PCI (209). These observations stimulate speculation that such inter-individual variability could be a potential target for therapeutic intervention and manipulation. However, there is as yet no universally accepted, rapid and reliable near-patient test of the effects of antiplatelet therapy and no widely accepted definition of resistance or poor response. Furthermore, currently available assays are limited by their inability to differentiate poor response to therapy from the effects of high baseline platelet reactivity. As a result identifying individuals at risk of ST after PCI either (a) by virtue of an increased overall tendency to thrombosis or (b) through resistance to antiplatelet therapy is still not possible in routine clinical practice. A universal "one size fits all" strategy is still used for the administration of oral antiplatelet therapies to patients undergoing PCI.

In this study we have used short TEG to determine (i) overall clotting tendency, (ii) response to platelet agonists at baseline and (iii) as a 15 minute test of response to antiplatelet therapy. In healthy volunteers we have shown that young (premenopausal) women have a greater clotting tendency and increased response to platelet agonists (AA

and ADP) at baseline. Whilst men and women in this cohort of volunteers respond equally to Aspirin (in terms of percentage reduction from baseline, %CIn, %PIn and the percentage of "non-responders"), post treatment platelet reactivity remains higher in women. With Clopidogrel, baseline and post treatment platelet reactivity is higher in women but, in contrast to Aspirin, the percentage reduction from baseline, %CIn and %PIn are also significantly reduced in women compared to men, and there are also more women demonstrating hyporesponsiveness (or resistance). This suggests that the diminished response to Aspirin observed in young female volunteers is explained by increased baseline platelet reactivity alone, whereas for Clopidogrel the diminished response in women is due to both increased baseline platelet reactivity and a diminished response to the drug.

The gender differences in volunteers are interesting but we found no such important differences between male and (postmenopausal) female patients (all over the age of 50). This age group represents the vast majority of female patients in clinical practice. Thus, it is clearly not possible to extrapolate from the physiological differences observed in our healthy volunteers to the female patient. However, it is possible that a high baseline clotting tendency and a diminished response to Clopidogrel may render younger women treated with PCI and stents at increased risk of ST. Certainly, the data from the few survivors of ST who were younger women did seem to provide some support to this hypothesis.

The findings from this study highlight the importance of understanding the differences between assays of clotting tendency, assays of platelet reactivity and assessment of response to antiplatelet therapy. Specifically, whilst comparison with a baseline sample demonstrates the effect of drug therapy it may not most accurately reflect residual risk whilst on treatment. Similarly an assay of platelet reactivity whilst on antiplatelet therapy may not be able to differentiate treatment failure from high baseline (and therefore post treatment) platelet reactivity. In this regard, TEG Pmap has potential advantages as (i) the thrombin channel gives additional information on overall clotting tendency, itself a marker of risk and (ii) allows assessment of the mechanism of treatment failure, allowing differentiation between "true" poor response to treatment (as we found in young women administered Clopidogrel) and an adequate response to treatment but residual high

platelet reactivity due to high baseline clotting tendency (as we found in young women administered aspirin).

This study has some limitations. First, whilst the administration of all medication was witnessed we cannot exclude the use of other medications not reported by study participants that could have influenced baseline results. Secondly, there is no specific investigation of the effects of hormonal contraceptives in women.

13.5.5 Summary

This study demonstrates higher post treatment platelet reactivity in young female volunteers after the administration of both Aspirin and Clopidogrel. There was also a reduced response to Clopidogrel, but not Aspirin, in women. As both high post treatment reactivity and reduced response to Clopidogrel have been correlated with outcome this may at least in part explain the worse outcome observed in young women. Consistent with this concept we have found poor response to Clopidogrel in two out of three young women with previous ST. This effect however does seem limited to young women and we found no differences in clotting tendency or response to antiplatelet therapy in older women who make up the vast majority of the patient population,

CAD is rare in pre-menopausal women. However, given the significantly increased risk associated with CAD in this group and the current data indicating an increased clotting tendency and diminished response to Clopidogrel in young women volunteers we feel that this area warrants further study.

The strategy of giving standard doses of Aspirin and Clopidogrel to all PCI patients is almost certainly flawed. This study also highlights how understanding the mechanisms of "poor response" could help guide treatment modification in these high risk individuals.

13.6 Effects of Clopidogrel on "Aspirin specific" pathways of platelet inhibition.

Abstract

Background

The most widely accepted methods of assessing response to Clopidogrel involve isolated ADP-induced platelet aggregation. Whilst poor response determined by these assays does correlate with adverse clinical events, the number of "poor responders" is far higher than the number of events attributed to treatment failure. It is possible that Clopidogrel may have independent effects, which cannot be assessed using isolated ADP-induced aggregation, which could contribute to this apparent discrepancy. We have investigated the effect of Clopidogrel on AA-induced platelet activation, an "Aspirin specific" pathway, using short TEG.

Methods and Results

(a) 34 volunteers on no medication and (b) 36 patients, on maintenance therapy with Aspirin 75mg daily, were recruited. Blood tests for TEG Pmap were taken immediately prior to and 6 hours after administration of a 600mg Clopidogrel loading dose. Changes in the AUC15 with both ADP- and AA-stimulation were calculated as were the corresponding %PIn and %CIn.

There were predictable and significant changes in the AUC15 of the ADP channel in response to Clopidogrel and the corresponding %PIn and %CIn in both volunteers and patients. There were also significant reductions in the AUC15 of the AA channel (presented as Mean +/- 95%CI), by 27.2+/-11.8%, $p=0.005$ in volunteers and 35.0+/-8.2%, $p<0.001$ in patients) and increases in the %PIn and %CIn calculated using the AA channel in volunteers (by 20.0+/-11.4%, $p=0.02$ and 32.3+/-12.8%, $p<0.001$ respectively) and patients (by 24.2+/-8.6%, $p<0.001$ and by 18.0+/-8.6, $p<0.001$ respectively).

Conclusions

Clopidogrel has both independent and Aspirin-synergistic effects on AA-induced platelet activation suggesting potentiation of the antiplatelet activity of Aspirin. This effect may be clinically important and is not detected by current "gold standard" methods of assessing response to Clopidogrel.

13.6.1 Introduction

Clopidogrel, a thienopyridine antiplatelet agent, is widely used in patients with cardiovascular disease. There is a sound evidence base to support its use (a) instead of, or in addition to, Aspirin in the prevention of vascular events in high-risk individuals, (b) in addition to Aspirin in patients with acute coronary syndrome, (c) in ST elevation myocardial infarction, and (d) in patients treated with coronary stents (Section 11.2.3). Despite these data there remains concern about responses of individual patients to standard dose Clopidogrel therapy. These uncertainties stem from (i) the diversity of methods available for monitoring its effects (ii) the marked heterogeneity in observed responses to Clopidogrel using these methods and (iii) an incomplete understanding of how Clopidogrel exerts its full clinical effect.

Clopidogrel is a pro-drug that is metabolized by hepatic CYP3A4 to its active metabolite. The latter is thought to predominantly exert its effect by irreversibly binding platelet P2Y12 receptors, thereby inhibiting (a) ADP-induced platelet aggregation and (b) the conformational change that allows fibrinogen to bind to the platelet glycoprotein IIb/IIIa receptor. The most widely accepted method of assessing responses to Clopidogrel is light transmission aggregometry using 10 mmol ADP-stimulation, with residual aggregation of >50% taken to demonstrate lack of therapeutic efficacy. Aggregation however, is just one aspect of platelet function. For example, changes in platelet adhesion and the release of platelet granules are not directly assessed using this technique. Importantly, there are large disparities between apparent rates of "resistance" as assessed by isolated platelet function tests such as aggregometry and the subsequent occurrence of adverse clinical outcomes. Thus, some aggregometry studies show far higher numbers of poor responders than clinical treatment failure would suggest and others, in which the vast majority of participants would be "resistant" using current criteria, show important benefits of clopidogrel on outcome (46). It is therefore likely that there are clinically important effects of Clopidogrel that cannot be assessed using ADP-induced platelet aggregation. Indeed there is now substantial evidence that Clopidogrel has other properties including an anti-inflammatory effect (231), and effects on enzymatic components of coagulation (232).

It is likely that Clopidogrel plays an important role in preventing the amplification of platelet activation by other platelet activators by inhibiting the effect of ADP released in dense platelet granules. There is some indirect evidence for a clinically important action of Clopidogrel on AA-induced platelet activation. In a small study Dropinski et al showed, using aggregometry, that in four of five patients initially labelled as 'non-responders' to Aspirin, Clopidogrel therapy increases inhibition of AA-induced platelet activation enough to convert them to responders (233). However, another study using aggregometry showed that in a group of 36 patients on Aspirin therapy, Clopidogrel therapy resulted in no significant change in AA-induced platelet aggregation (234). The observed interaction may be dependent on the dose of Aspirin. Serebruany et al showed using aggregometry that whilst raising the dose of Aspirin from 81mg to 325mg increases the antiplatelet effect of Aspirin monotherapy, the same dose adjustment in patients concurrently treated with Clopidogrel did not increase its antiplatelet effect further (235).

We have used short TEG to investigate the hypothesis that Clopidogrel has important independent and Aspirin synergistic effects, through inhibition of AA-induced platelet activation. We have investigated the activity of Clopidogrel on both ADP-induced platelet activation and AA-induced platelet activation (previously considered as specific for detecting the effects of Aspirin), in the context of both Clopidogrel monotherapy and in patients already established on Aspirin therapy.

Elucidating the precise mechanisms by which Clopidogrel exerts its full antiplatelet effect could have important implications in determining the optimal methods of assessing response to Clopidogrel and Aspirin, and potentially in guiding therapeutic manipulation and identifying individuals at risk from Clopidogrel withdrawal.

13.6.2 Methods

Procedures followed are consistent with those described in the Methods section (Section 12).

13.6.2.1 Study Participants

34 volunteers on no regular medication and 36 patients on maintenance therapy with Aspirin were recruited. Volunteers were excluded if they had taken antiplatelet medication or non-steroidal anti-inflammatory medication within 14 days or if they had a history of peptic ulceration, bronchial asthma, or bleeding. Patients were all non-smokers with angiographically proven coronary artery disease and were on maintenance therapy with Aspirin 75mg daily.

13.6.2.2 TEG analysis

In all subjects, venesection was performed immediately prior to and 6 hours after witnessed administration of a Clopidogrel 600mg loading dose. TEG analysis was performed as laid out in the methods section. For each sample the MA and AUC15 were recorded for all channels. The percentage change from baseline in the AUC15 of the AA and ADP channels were calculated as were the %PIn and %CIn (i) calculated from the AA channel and considered to represent response to Aspirin and (ii) calculated from the ADP channel and considered to represent response to Clopidogrel.

13.6.2.3 Statistical analysis

"Non-response" to Aspirin (defined as a %PIn or a %CIn of <50%) was calculated in the patient group for both the pre-clopidogrel and post-clopidogrel samples. Data are presented as the mean +/- 95% confidence interval of the mean. Significance was determined using paired two-tailed, t-tests with a p value of <0·05 considered to represent significance. For comparison of the number of non-responders significance was determined using Fisher's Exact test with a p value of <0·05 considered to represent significance.

13.6.3 Results

13.6.3.1 Effects of Clopidogrel loading dose in healthy volunteers

There were no significant differences in the AUC15 of the Thrombin or Fibrin Channels after Clopidogrel compared to baseline.

13.6.3.1.1 Effects of Clopidogrel on ADP-induced platelet activation

There was a significant reduction in the AUC15 of the ADP channel following Clopidogrel (by 47·2±9·7%, from 970±87 to 553±126, p<0·001). There were significant increases in both the %PIn (from -11.7±13.7 to 39.6±14.7, p<0.001), and the %CIn (from 0.11± 8.1 to 49.1±11.3, p<0.001, Figure 27) due to Clopidogrel.

13.6.3.1.2 Effects of Clopidogrel on AA-induced platelet activation

There was a significant reduction in the AUC15 of the AA channel following Clopidogrel (by 27·2±11·8%, from 854±103 to 619±117, p=0·005). There were significant increases in the %PIn (from 3.5±11.6 to 16.5±11.3, p=0.02) and %CIn (from 2.2±13.8 to 34.5±11.7, p<0.001, Figure 27) calculated from the AA channel.

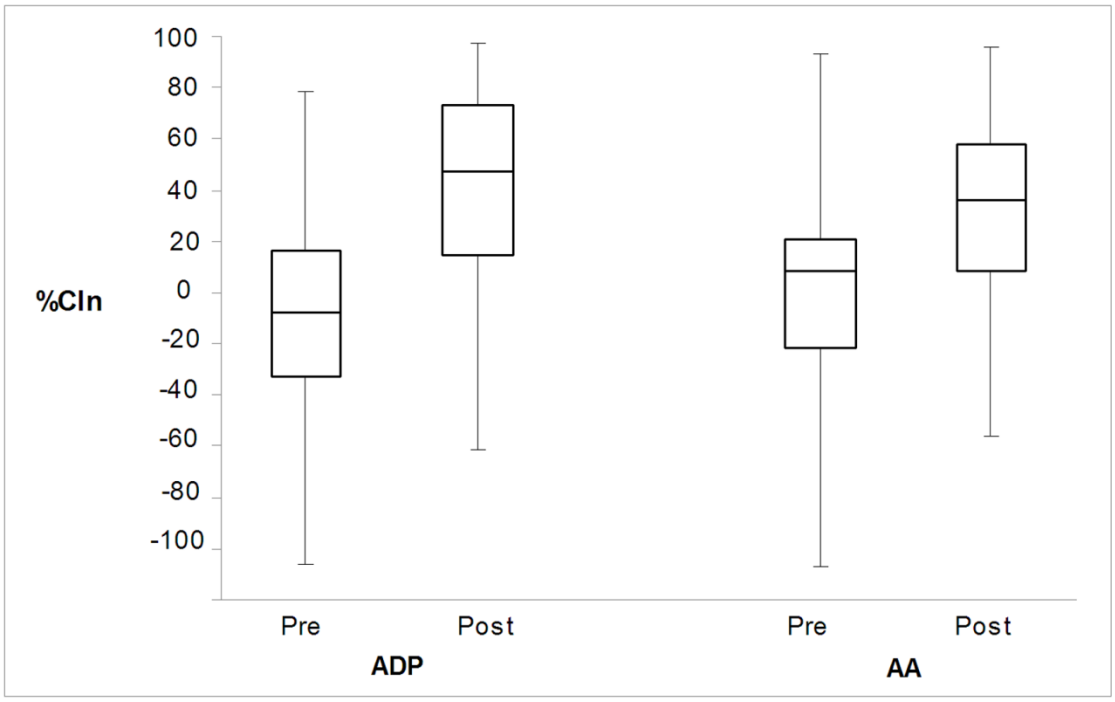

Figure 27 short TEG: Effect of Clopidogrel on %CIn calculated from both ADP and AA channels. ("Due to Aspirin", calculated with AA-stimulation and "due to Clopidogrel", calculated with ADP-stimulation in 34 volunteers on no medication before and 6 hours after the administration of a 600mg Clopidogrel loading dose.)

13.6.3.2 Effects of Clopidogrel loading dose in patients on maintenance therapy with Aspirin

There were no significant differences in the AUC15 of the Thrombin or Fibrin channels following Clopidogrel compared to baseline.

13.6.3.2.1 Effects of Clopidogrel on ADP-induced platelet activation

There was a 34·2±9·2% reduction in the AUC15 of the ADP channel (from 1074±58 to 700±99, p<0·001). This corresponded to significant increases in %PIn (from 0.58±5.0 to 39.5±9.1, p<0.001) and %CIn (from -10.1±27.4 to 31.9±8.3, p<0.001, Figure 28) due to Clopidogrel.

13.6.3.2.2 Effects of Clopidogrel on AA-induced platelet activation

There was a 35·0±8·2% decrease in the AUC15 of the AA channel (from 401±71 to 234±33 p<0·001,). The %PIn and %CIn calculated from the AA channel increased significantly (from 66.0±10.1 to 90.2±5.5, p<0.001, and from 58.4±8.2 to 76.4±4.1, p<0.001, Figure 28).

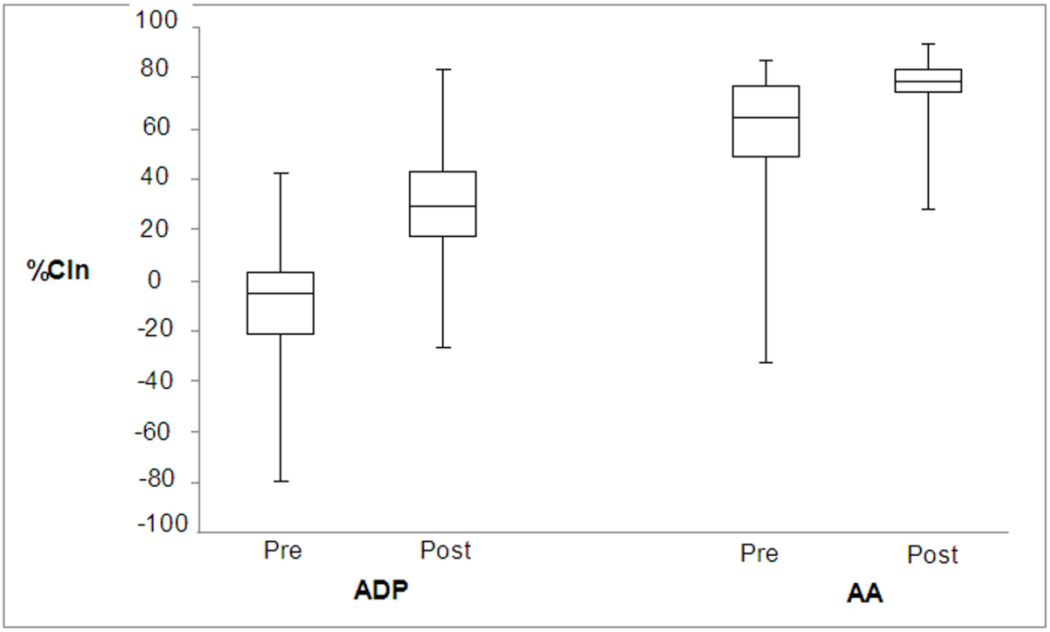

Figure 28 short TEG: Effect of Clopidogrel on %CIn calculated from both ADP and AA channels in patients. ("Due to Aspirin", calculated with AA-stimulation and "due to Clopidogrel", calculated with ADP-stimulation in 36 patients on maintenance therapy with Aspirin before and 6 hours after the administration of a 600mg Clopidogrel loading dose.)

13.6.3.2.3 Effects of Clopidogrel on non-responders to Aspirin

At baseline, 10 of 36 patients (by %CIn) were "non-responders" to Aspirin, compared to 2 of 36 after Clopidogrel administration (p=0.02). By %PIn 4 of 6 non-responders to Aspirin were converted to responders by the addition of Clopidogrel therapy. All initial non-responders by %PIn were also non-responders by %CIn.

13.6.4 Discussion

It is well established that there is significant variability in patient responses to Clopidogrel and furthermore that "poor response" is associated with adverse clinical outcomes (Section 11.2.3 and 11.4). However, there is as yet no generally accepted clinically relevant technique for assessing patient responses to Clopidogrel, particularly in the context that estimates of poor response or "resistance" using isolated platelet function tests are much higher than clinical treatment failure would suggest. Furthermore, there are concerns about an increase in clinical events such as stent thrombosis upon Clopidogrel withdrawal, even in the ongoing presence of Aspirin.
In this study we have used short TEG to determine the effect of Clopidogrel on ADP- and AA-induced platelet activation, in volunteers on no other medication, and in patients on Aspirin maintenance therapy. We have demonstrated a significant effect of Clopidogrel on AA-induced platelet aggregation as well as the predictable inhibition of ADP-induced clot formation. Furthermore, the effect on AA-induced platelet aggregation significantly potentiates the effect of Aspirin, to the extent that 8 out of 10 Aspirin non-responders by %CIn (and 4 out of 6 Aspirin non-responders by %PIn) are converted to responding normally to Aspirin as a result of additional Clopidogrel therapy. This result is in keeping with a previous study using optical aggregation (233).

The ability of Clopidogrel to inhibit AA-induced platelet activation is as yet unexplained but it may relate at least in part to platelet granule release (containing ADP) during platelet activation. Platelet activation by diverse stimuli, including AA, results in platelet granule release and the subsequent action of released ADP on P2Y12 receptors is then blocked by Clopidogrel, hence perhaps potentiating the antiplatelet activity of Aspirin.

However, the mechanism of action of Clopidogrel is undoubtedly complex. Clopidogrel is likely to have an anti-inflammatory action (231), inhibit multiple platelet agonists in addition to ADP, and have an effect on enzymatic components of coagulation (232).

Recent studies have suggested that Clopidogrel may even have important clinical effects months after its cessation (237).

The effects of Clopidogrel that we have demonstrated on AA-induced platelet activation may have important clinical implications. Clopidogrel withdrawal can precipitate adverse events despite ongoing maintenance therapy with Aspirin (238). For example; a temporal relationship has been reported between cessation of Clopidogrel therapy and the onset of ST (239). These findings suggest that one possible mechanism would be that removal of Clopidogrel leads to a rebound attenuation of the antiplatelet effect of Aspirin. This study raises questions about how to best assess patient responses to Aspirin and Clopidogrel therapy. Isolated tests of platelet function, even the current gold standard of ADP-induced light transmission aggregation, potentially ignore other such clinically relevant effects of Clopidogrel. Short TEG has potential advantages in that it is (a) rapid, (b) provides information on the effects of both aspirin and clopidogrel from a single sample, (c) is a whole blood assay incorporating the effects of platelets, other cellular components, thrombin and clotting factors and (d) provides information on maximal clot formation due to thrombin stimulation, itself a marker of risk for adverse thrombotic events (209).

This study has some limitations. Whilst the administration of Clopidogrel was witnessed we cannot exclude the use of other medications not reported by study participants that could have influenced baseline results, or Clopidogrel efficacy. In the patient group, although only those that reported compliance with Aspirin therapy were included, the presence of Aspirin was not assessed objectively.

13.6.5 Summary

Short TEG demonstrates that clopidogrel has both independent and Aspirin-synergistic effects, on AA-induced platelet activation. These data may have clinical relevance. The current gold standard method of assessing response to Clopidogrel, ADP-induced platelet aggregation, is unlikely to accurately determine the true clinical effect of Clopidogrel therapy, particularly in individuals administered additional antiplatelet therapies.

13.7 Investigating Stent Thrombosis

Abstract

Background: To test the hypothesis that point-of-care assays of platelet reactivity would demonstrate reduced response to antiplatelet therapy in patients who experienced DES ST whilst on dual antiplatelet therapy compared to matched DES controls. Whilst the aetiology of ST is multifactorial there is increasing evidence from laboratory-based assays that hyporesponsiveness to antiplatelet therapy is a factor in some cases.

Methods: From 3004 PCI patients, seven survivors of DES ST whilst on dual antiplatelet therapy were identified and each matched with two patients without ST. Analysis was performed using (a) short TEG and (b) VerifyNow Aspirin and P2Y12 assays.

Results: There were no differences in responses to Aspirin. There was significantly greater platelet reactivity on Clopidogrel in the ST group using VerifyNow (PRU 183 ± 51 vs. 108 ± 31, p=0.02) and a trend towards greater reactivity using short TEG AUC15 (910 ± 328 vs. 618 ± 129, p=0.07). 57% of the ST group by TEG and 43% of the ST cases by VerifyNow PRU had results > two standard deviations above the expected mean in the control group.

Conclusions: This study demonstrates reduced platelet response to Clopidogrel in some patients with DES ST compared to matched controls. The availability of point-of-care assays that can detect these responses raises the possibility of prospectively identifying DES patients at risk of ST and manipulating their subsequent risk.

13.7.1 Introduction

Robust evidence demonstrating the ability of DES technology to reduce restenosis in comparison to BMS has led to widespread DES uptake. Balanced against this significant clinical benefit, however, is concern about the incidence of ST in DES patients. Observational and randomised trial data suggest that there is a cumulative incidence of ST in DES patients of between 0.5%-1% per year (240,241), correlating in some series with a similar rate of death and myocardial infarction (242).

There are well established procedural risk factors for ST such as stent under-deployment (243), length of stented segment (244), and idiosyncratic factors including a form of hypersensitivity (171). However, the inappropriate termination of Aspirin and Clopidogrel therapy appears to be particularly hazardous (245). In DES patients there is therefore an important reliance for some (as yet undetermined) period of time on dual antiplatelet therapy (246,247), possibly as a result delayed stent endothelialisation (168). This requirement for ongoing dual antiplatelet therapy, together with evidence of considerable biological variability in the response of individuals to antiplatelet therapies, particularly Clopidogrel, and association between poor response and adverse cardiovascular outcome (section 11.4) has raised the important question: can poor responses to these agents render some individuals at risk of DES ST? There is now growing evidence from laboratory based assays that variability in the response to antiplatelet agents, particularly Clopidogrel, can contribute to ST in DES patients (section 11.4.3.3).

Typically, Clopidogrel is given in standard doses to patients receiving DES, despite both experimental and clinical data demonstrating important biological variation in response (Section 11.2.3). However, clinical detection of reduced responsiveness to Clopidogrel and/or Aspirin has been hampered both by a lack of point-of-care assays and by an appropriate definition of what constitutes “resistance”.

The purpose of this study was to test the hypothesis that platelet reactivity whilst on Aspirin and Clopidogrel, assessed using short TEG and VerifyNow, would be significantly greater in a consecutive group of patients who survived DES ST than in matched DES controls.

13.7.2 Methods

General methods, venesection and sample analysis are consistent with those described in Section 12.

13.7.2.1 Study Population

Twenty-two patients with DES ST were identified from a consecutive series of 3004 patients, 90% of whom received DES, over a 2 year period at this centre (229). Seven cases (four subacute and three late) were identified where ST occurred in the context of dual antiplatelet therapy with both Aspirin and Clopidogrel and in whom dual antiplatelet therapy was on-going. None of the cases were taking additional antiplatelet therapy, anticoagulants or non-steroidal anti inflammatory medication.

For each case two control patients were identified from the interventional database, and individually matched according to duration and dose of antiplatelet therapy, gender, age, smoking, diabetes, initial presentation and procedure undertaken.

13.7.2.2 Result analysis

For TEG samples comparisons were made between the MA and AUC15 for each individual channel in the 2 groups. The %PIn and %CIn were calculated for both Aspirin (using the AA channel) and Clopidogrel (using the ADP channel) from each sample VerifyNow comparisons were made between the ARU and PRU.

13.7.2.3 Statistical analysis

Data are presented as the mean and 95% confidence interval of the mean. Significance between the groups was determined using two group t-tests for continuous variables and χ^2 (chi squared) tests for categorical data with a p value of <0.05 (2-tailed) considered to represent significance.

13.7.3 Results

Baseline demographics are shown in Table XII. There were no significant differences between the 2 groups which were well matched, nor were there any significant differences in baseline haematological variables between the 2 groups (Table XIII).

	ST cases	**Individual Controls**	**p value**
Age	61.9±5.7	61.8±4.3	N/S
Sex (% Male)	86	86	N/S
Smokers (%)	42.9	35.7	N/S
Diabetes (%)	14.3	14.3	N/S
Emergency cases (%)	14.3	14.3	N/S
Elective cases (%)	14.3	33.3	N/S
ACS cases (%)	71.4	52.4	N/S
Stent length (mm)	26.7±11.3	27.9±6.3	N/S
Minimum stent diameter (mm)	2.8±0.3	2.9±0.3	N/S
Aspirin dose (mg)	140±21.0	140±13.4	N/S
Clopidogrel dose (mg)	86±21	75	N/S
Duration of clopidogrel (days)	161±163	77±28	N/S
Time from latest event / intervention (days)	145±84	76±28	N/S
Statin (% total / % Atorvastatin)	100/14	100/7	N/S

Table XII Demographics. ST cases vs. Controls. (7 ST cases, 14 controls matched 2:1, there are no significant differences between the groups.)

	ST cases	Controls	p value
Hb conc. (g/l)	130±13	137±10	0.30
Haematocrit (%)	0.39±0.03	0.40±0.03	0.44
Platelet count	262±42	229±25	0.35
INR	1.1±0.1	1.0±0.0	0.15
eGFR (mls/min)	67±8	64±4	0.64

Table XIII Haematological variables, ST cases vs. controls. (There were no significant differences between the two groups.)

13.7.3.1 Responses to Aspirin

TEG: There was no significant difference in responses assessed with AA-stimulation between the ST group and control group in (i) %PIn (79·6 ± 20·8 vs. 89·9 ± 7·6, p=0·39); %CIn (75·2 ± 9·4 vs. 75·9 ± 6·0, p=0·90); (ii) MA of the AA channel (25·3 ± 17·6 vs. 14·0 ± 4·5, p=0·26) or (iii) AUC15 of the AA channel (244 ± 113 vs. 216 ± 56, p=0·67).
VerifyNow: There was no significant difference between ARU measurements in the ST group and controls (453 ± 50 vs. 410 ±29, p=0·18).

13.7.3.2 Responses to Clopidogrel

TEG: There was no significant difference between the ST group and controls in the %PIn due to Clopidogrel (18·2 ± 33·2 vs. 31·8 ± 13·2, p=0·38); the %CIn (5·6 ± 27·7 vs. 30·6 ± 13·4, p=0·09 or the MA of the ADP channel (50·5 ± 15·8 vs. 46·0 ± 7·5, p=0·62). There was trend towards significance with the AUC of the ADP channel (910 ± 329 vs. 618 ± 130, p=0·07) with 57% (4 of 7) of the ST group compared to 0% of controls having an AUC >1100 (AUC > 2 standard deviations above the mean of the control group). (Figure 29).

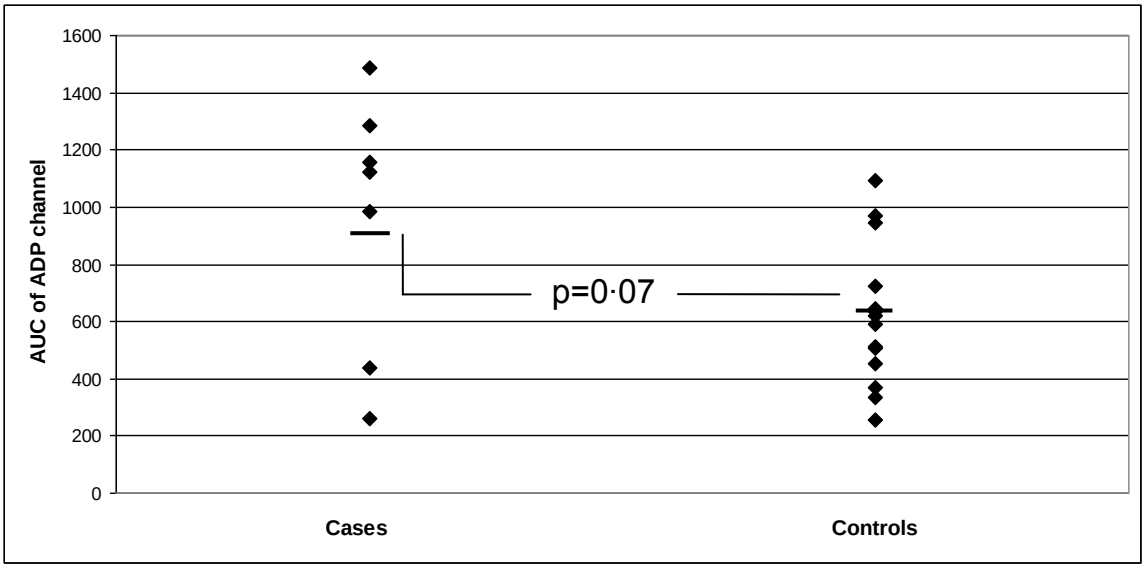

Figure 29 short TEG: AUC15 of the ADP channel in the ST group and in matched controls.

Other TEG variables: There were no significant differences between cases and controls in TEG variables which have been previously identified as predictive of ischaemic events after PCI (i.e. MA of the Thrombin channel (61·6 ± 2·9 vs. 64·1 ± 2·7, p=0·21) and the R time of the Thrombin channel (6·9 ± 1·5 vs. 7·1 ± 2·0, p=0·84)) (209).

VerifyNow: The PRU in the ST group was significantly higher than in controls (183 ± 51 vs. 108 ± 31, p=0·02). 43% (3 of 7) of the ST cases compared to 0% of controls had PRU >225 (PRU > 2 standard deviations above the mean of the control group). (Figure 30)

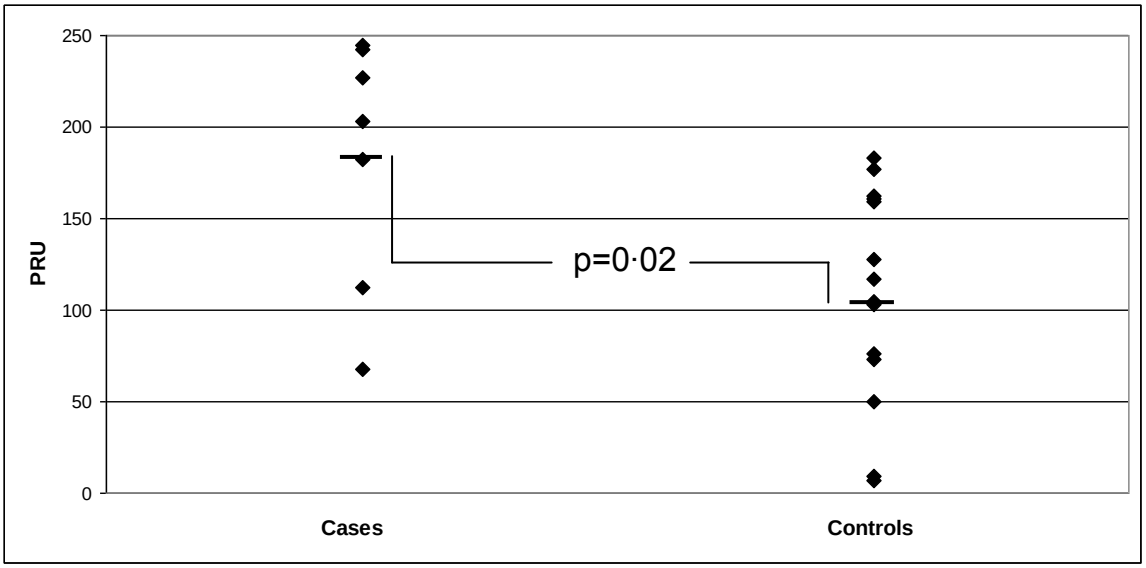

Figure 30 VerifyNow: PRU in the ST group and in matched controls.

13.7.4 Discussion

The hypothesis for this study was that these two point-of-care tests (VerifyNow and short TEG) could detect reduced responsiveness to antiplatelet therapy in survivors of ST whilst on dual antiplatelet therapy compared to matched controls. This hypothesis has been disproved in the case of Aspirin but proved for Clopidogrel using VerifyNow with a trend towards significance using short TEG. Our results suggest that Clopidogrel, but not Aspirin, resistance contributes to ST in some cases.

The implications of this study are clinically relevant. Firstly, they add to the growing concern that some patients receiving DES may be intrinsically at risk of ST because of their relative lack of response to Clopidogrel. Secondly, there are now two point-of-care assays available that allow rapid detection of the response of prospective DES patients to Clopidogrel, raising the possibility that such "at risk" individuals could be detected in routine clinical practice, before they are exposed to the potentially suboptimal combination of DES and standard doses of antiplatelet drugs. We found that approximately 50% of patients with previous ST whilst on Clopidogrel had abnormally

high reactivity whilst on Clopidogrel with both VerifyNow and TEG (PRU and TEG AUC15 of ADP channel > 2 standard deviations above the expected means). Further data are now required to determine (a) whether the responses of such individuals to antiplatelet therapy can be normalised by interventions such as increasing doses of Clopidogrel (as we will go on to do in Section 13.8), or through the use of alternative or additional antiplatelet agents, and (b) whether prospectively screening large populations of patients undergoing PCI using such tests to guide therapy can decrease ST rates.

This study has important limitations. Firstly, the absolute number of ST patients is low. Nevertheless, the ST population in this study was derived from a consecutive series of 3004 DES patients at a single centre. Secondly, as this is a retrospective study we do not have baseline samples prior to the initiation of antiplatelet therapy for calculation of a response to antiplatelet therapy compared to baseline. Instead we utilised the clinically important measure of platelet reactivity whilst on antiplatelet treatment. Thirdly, studies recruiting patients who have survived ST will inevitably be weaker for the absence of data relating to patients who died as a result of ST. Lastly we did not test for evidence of drug compliance in this study.

13.7.5 Summary

Using assays suitable for point-of-care use in routine clinical practice, this study demonstrates an association between reduced responses to Clopidogrel, but not Aspirin, in a proportion of DES ST patients when compared to matched DES patients without ST. These findings are in keeping with recent studies using laboratory based research tools (Section 11.4.3.3). The availability of two point-of-care assays that can be employed to detect such responses raises the possibility of detecting Clopidogrel hypo-responsiveness prior to DES implantation and possibly manipulating the risk of subsequent ST. Further data are required.

13.8 Comparison of 600mg and 900mg Clopidogrel loading doses.

Abstract

Background

Whilst poor response to Clopidogrel is associated with adverse outcomes after PCI uncertainty exists as to how (a) response should be assessed and (b) how initial poor responders should be managed. Individualised assessment of platelet function would provide clinically valuable information enabling Clopidogrel therapy to be tailored where response is deemed to be suboptimal. We utilised short TEG and VerifyNow to assess high Clopidogrel doses (900mg) in (i) initial poor responders to a 600mg dose and (ii) in a randomised comparison with 600mg doses in patients.

Methods

Blood was taken before and six hours post Clopidogrel and analysed using VerifyNow P2Y12 and short TEG. (i) 30 volunteers received 600mg Clopidogrel. Initial poor responders (%CIn <30%, or PRU reduction<30%) were administered 900mg Clopidogrel more than two weeks later.

(ii) 60 patients were randomized 1:1 to 600mg or 900mg Clopidogrel prior to PCI.

Results

(i) Poor responders to 600mg had greater PRU reduction (45.0 vs. 20.1%, p=0.03) and greater %CIn (22.9 vs. -15.1%, p=0.01) after 900mg. 4 of 5 (PRU) and 4 of 9 (%CIn) poor responders were classified as responding normally after the 900mg.

(ii) There were no differences in %CIn or %PRU reduction between the 900mg and 600mg patient groups (%PRU reduction 55.3 ± 9.1 vs. 37.2 ± 15.8, p=0.06, %CIn 35.7 ± 8.7 vs. 25.8 ± 8.6, p=0.13).

Conclusions

Whilst not producing significant differences in response on a population basis, 900mg doses do significantly increase the antiplatelet effect of Clopidogrel in initial poor responders. Near patient assessment of response to antiplatelet therapy is therefore feasible and provides clinically useful information, allowing therapy to be altered where responses are deemed to be inadequate.

13.8.1 Introduction

In the field of PCI in particular, a large and expanding body of evidence indicates that peri-procedural complication rates can be reduced by loading doses of Clopidogrel given at least 6 hours before planned stenting. The superiority of 600mg loading doses of Clopidogrel over 300mg is now widely accepted (54,55,68,69). There remain several areas of uncertainty in relation to optimal Clopidogrel therapy in PCI patients, however. First, whether the risk of peri-procedural and 30 day events could be further reduced by a 900mg loading dose. The current data are discrepant in this regard (70,71,72). Second, it is clear that there is heterogeneity in the response of individual patients to Clopidogrel and that poor responders are susceptible to both early events and later ST (Section 11.4.3.3). Taking these factors into account, there is a logical case to be made that all patients being treated with Clopidogrel should have their platelet function assessed to ensure a therapeutic response with the intention of reducing their risk. This concept is undermined by the limitations of current options for platelet function testing. As a result global testing of patient responses to Clopidogrel still does not occur, despite the knowledge that if it did it has the potential to reduce the incidence of both periprocedural myocardial infarction and stent thrombosis.

The aims of this group of investigations were:
1) To investigate the incidence of poor response to Clopidogrel 600mg in healthy volunteers and to assess whether the response of these individuals could be modified by 900mg loading.
2) To compare the response of patients being considered for elective PCI to 600mg and 900mg loading doses of Clopidogrel in a randomised fashion.

I employed both VerifyNow and short TEG. The overall hypothesis for these experiments was that near patient assessment of platelet function in the context of whole blood clotting in individual patients is feasible and provides clinically valuable information that could be used to tailor Clopidogrel therapy where response was deemed to be inadequate.

13.8.2 Methods

General methods are consistent with those described in detail in Section 12.

13.8.2.1 Study Population

Group A

30 volunteers were recruited and received a 600mg loading dose of Clopidogrel. Poor responders to this initial loading dose as defined by previously published criteria (138,248) received a 900mg loading dose of Clopidogrel at least two weeks later. Individuals were excluded if they had taken antiplatelet medication or non-steroidal anti-inflammatory medication within 14 days or if they had a history of peptic ulceration, bronchial asthma, or bleeding.

Group B

60 patients receiving a loading dose of Clopidogrel prior to either elective PCI or coronary angiography with a view to proceed were recruited. They were randomised 1:1 to 600mg or 900mg loading doses of Clopidogrel. All patients were established on Aspirin 75mg maintenance therapy for >28 days. Exclusion criteria were: use of antiplatelet or anticoagulant medication other than Aspirin in the preceding 28 days, known intolerance to Clopidogrel, planned use of glycoprotein IIb/IIIa inhibitor, recent bleeding, major haematological disturbance and known malignancy.

13.8.2.2 Sample analysis

Blood tests were taken immediately before and six hours after drug administration in Group A and prior to and one, two, six and 20-24 hours after drug administration (before administration of maintenance doses of clopidogrel) in Group B. The percentage reduction in VerifyNow PRU from baseline was calculated at each timepoint, with reduction of <30% considered to represent poor response to Clopidogrel (142). For TEG samples the %PIn and %CIn were calculated as previously described with %PIn and

%CIn of <30% considered to represent poor response (138,248). In Group B analysis of results was performed by an individual blinded to the dose administered.
Some patients received unplanned use of glycoprotein IIb/IIIa inhibitors (all Abciximab) or Bivalirudin during PCI. In these cases blood tests were not performed at any time after administration of Abciximab or within six hours of cessation of Bivalirudin infusion.

13.8.2.3 Clinical Endpoints

For Group B rates of major and minor bleeding, peri-procedural myocardial infarction, stent thrombosis and death were compared between the two groups.

13.8.2.4 Statistical analysis

For Group A previous data suggest that a group size of 30 was required to identify 10 poor responders (212,248). Ten poor responders would be required to detect a 10% difference in platelet function with the higher dose with 80% power using paired, two-tailed t-tests. For Group B the study was powered to detect a 25% relative difference in platelet inhibition with 80% power. Significance between the groups was determined using two-tailed, two group t-tests with a p value of <0·05 considered to represent significance. Data are presented as the mean ± 95% confidence interval of the mean. Fisher's exact tests were used to determine differences between numbers of responders.

13.8.3 Results

13.8.3.1 Volunteers

13.8.3.1.1 Baseline demographics

15 males and 15 females were recruited, age 33 ± 4 years. Two volunteers were smokers. Two individuals were withdrawn prior to study completion as they developed a pre-specified exclusion criteria. Data from 28 volunteers was therefore analysed.

13.8.3.1.2 VerifyNow

Baseline mean PRU was 264 ± 16 compared to 106 ± 28 six hours after a 600mg loading dose of Clopidogrel. Mean % reduction in PRU was 59.9 ± 10.1%.

Five volunteers (17%) were classified as non-responders by VerifyNow. Initial non-responders had significantly greater reduction in PRU after a 900mg loading dose of Clopidogrel (45.0 vs. 20.1%, p=0.03). Four of the five (80%) non-responders to a 600mg dose were classified as responders after the 900mg dose. (Figure 31, Table XIV.)

	All Volunteers	Initial Poor Responders (to 600mg)		
		Response to 600mg	Response to 900mg	P
%PIn	47.8±12.2	9.9±9	32.2±16.3	0.01
%CIn	37.3±16.7	-15.1±21.6	22.9±15.9	0.01
% Change in PRU	61.2±10.6	20.1±8.2	45.0±16.3	0.03

Table XIV TEG and VerifyNow: Results in volunteers 6 hours after 600mg or 900mg Clopidogrel ((a) all volunteers post 600mg (b) poor responders to 600mg post 600mg and (c) poor responders to 600mg post 900mg; Mean +/- 95% confidence interval of the mean.)

13.8.3.1.3 TEG

Six hours after the initial 600mg loading dose of Clopidogrel %PIn was 48.1 ± 11.6, %CIn was 37.7 ± 15.9%. Nine volunteers (31%) were classified as non-responders by both %PIn and %CIn. In non-responders to the 600mg dose %PIn (32.0 vs. 9.9%, p=0.01) and %CIn (22.9 vs. -15.1%, p=0.01) were significantly greater after 900mg of Clopidogrel. Of the nine non-responders to 600mg Clopidogrel four (44%) were classified as responders after a 900mg loading dose by both %PIn and %CIn. (Figure 31, Table XIV.)

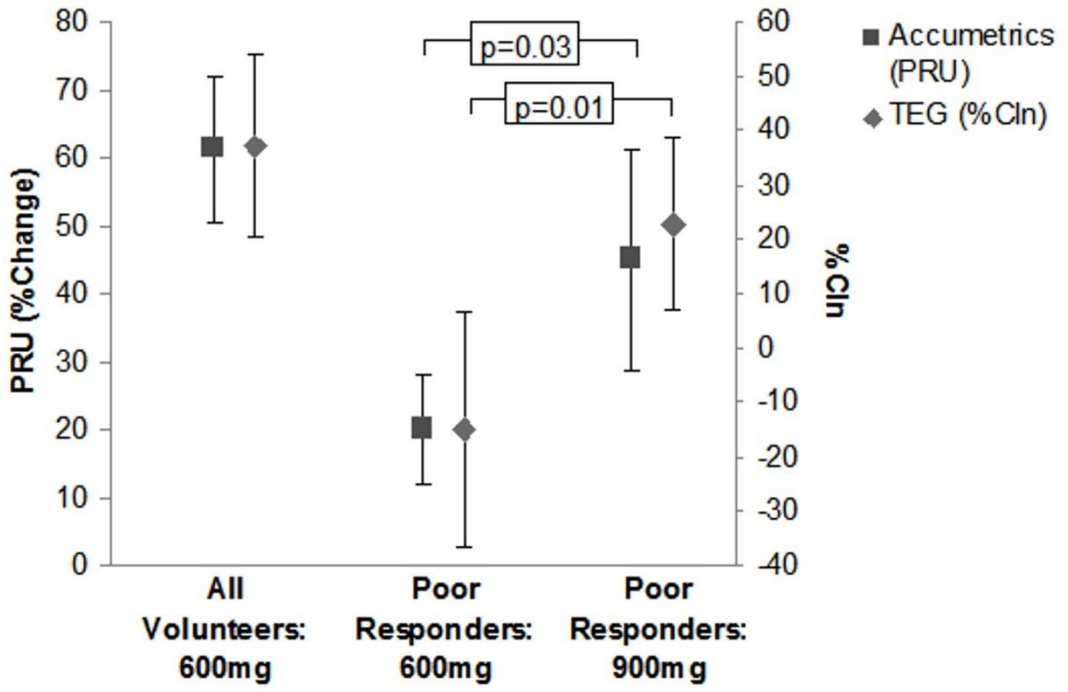

Figure 31 short TEG and VerifyNow: Change in PRU and %CIn after 600mg or 900mg Clopidogrel (6 hours after Clopidogrel in (i) all volunteers taking 600mg (ii) poor responders taking 600mg and (iii) poor responders taking 900mg.)

13.8.3.2 Patients

13.8.3.2.1 Baseline Demographics

There were no significant differences between the 2 groups in terms of baseline demographics, haematological parameters or in procedure undertaken (Table XV). Four patients received Abciximab, one patient received Bivalirudin and 18 patients were discharged prior to the 20-24 hour timepoint. Data was therefore analysed from 87% of the planned datapoints (100% at baseline, one and two hours, 92% at the six hour timepoint and 70% at the 20-24 hour timepoint).

	600mg group	**900mg group**	**p value**
Age	65 +/- 3.8	65.2 +/- 3.7	0.92
% male	70%	63.30%	0.78
Weight	81.8 +/- 5.6	81.3 +/- 5.5	0.86
Aspirin dose	80.4 +/- 11.3	81.3 +/- 11.4	0.88
PCI performed	16 (53%)	19 (63%)	1.00
No sig. coronary disease	4 (13%)	1 (3%)	0.35
Smokers	6 (20%	1 (3%)	0.10
Diabetics	6 (20%)	2 (7%)	0.25
Haematoma	0	1 (3%)	1.00
Enzyme rise	5 (17%)	6 (20%)	0.72
Haemoglobin conc.	140 +/- 5.7	139 +/- 5.7	0.86
Platelet count	235 +/- 23.7	240 +/- 23.7	0.68
Haematocrit	0.41 +/- 0.01	0.41 +/- 0.01	0.84
estimated GFR	70.4 +/- 5.4	70 +/- 5.3	0.88
INR	0.99 +/- 0.0	0.99 +/- 0.0	0.94

Table XV Baseline demographics and haematological parameters.
(PCI patients randomized to 600mg and 900mg Clopidogrel loading doses.)

13.8.3.2.2 Clinical Endpoints

There were no significant differences between the groups in terms of procedural complications. There were no major bleeds and no acute stent thromboses. There was one access site haematoma (in the 900mg group). There was no difference in peri-procedural myocardial infarction (17% in the 600mg group and 20% in the 900mg group).

13.8.3.2.3 VerifyNow

Whilst there was no difference in % reduction in PRU between the two groups at the one and two hour timepoints, there was a trend towards greater reduction at six hours (55·3 ± 9·1 vs. 37·2 ±15·8, p=0.06) and significantly greater reduction by 24 hours (60·9 ± 9·1 vs. 37·9 ± 17·0, p=0.04) in the 900mg group. (Table XVI)

There were significantly less poor responders in the 900mg group at the six hour timepoint. (Table XVII)

13.8.3.2.4 TEG

Whilst the %PIn and %CIn were greater in the 900mg group at all timepoints there were no significant differences between the two groups. There was a trend towards greater %PIn in the 900mg group at six hours (45.9 ± 8.4 vs. 33.2 ± 8.9, p=0.06) and at 24 hours (37.8 ± 7.7 vs. 24.7 ± 7.1, p=0.06). (Table XVI) There were no differences between the two groups in the number of poor responders at any timepoint. (Table XVII)

	600mg group	900mg group	p value
PRU (% reduction compared to baseline)			
1 hour	10.4 +/- 10.3	22.3 +/- 9.1	0.09
2 hour	35.9 +/- 13.3	44.5 +/- 9.5	0.31
6 hour	37.2 +/- 15.8	55.3 +/- 9.1	0.06
24 hour	37.9 +/- 17.0	60.9 +/- 9.1	0.04
%PIn			
1 hour	13.0 ± 7.2	22.0 ± 7.6	0.10
2 hours	26.5 ± 9.5	33.2 ± 8.5	0.31
6 hours	33.2 ± 8.9	45.9 ± 8.4	0.06
24 hours	24.7 ± 7.1	37.8 ± 7.7	0.06
%CIn			
1 hour	4.0 ± 11.2	4.4 ± 12.4	0.95
2 hours	19.8 ± 11.7	23.3 ± 12.1	0.68
6 hours	25.8 ± 8.6	35.7 ± 8.7	0.13
24 hours	18.1 ± 11.5	31.2 ± 10.2	0.19

Table XVI TEG and VerifyNow: Response to 600mg or 900mg Clopidogrel in PCI patients. (60 patients randomised 1:1 to 600mg or 900mg loading doses.

	600mg group	900mg group	p value
PRU			
1 hour	77	67	0.57
2 hour	40	27	0.29
6 hour	41	11	0.02
24 hour	41	15	0.07
%PIn			
1 hour	70	77	0.77
hours	60	47	0.44
6 hours	43	30	0.40
24 hours	61	47	0.51
%CIn			
1 hour	77	80	0.77
2 hours	53	43	0.45
6 hours	36	33	1.00
24 hours	55	37	0.34

Table XVII TEG and VerifyNow: % of 'poor responders' to 600mg or 900mg Clopidogrel. (60 PCI patients randomized 1:1 to 600mg and 900mg Clopidogrel loading doses, poor response defined as: PRU: <30% reduction from baseline; %PIn<30%; %CIn<30%.)

13.8.4 Discussion

There is increasing evidence that poor response to Clopidogrel post PCI correlates with subsequent ischaemic events, ST and even death (Section 11.4). However, at present responses are not routinely monitored. This is largely due to (a) the expense and complexity of laboratory based assays of platelet function; (b) difficulties in determining the threshold for poor response with poor positive predictive values and (c) uncertainty in how to manage poor response.

There is now evidence that (i) repeated loading doses (249); (ii) increased maintenance doses of Clopidogrel (76,249) and (iii) changing to Ticlopidine (249) or Prasugrel (250) improve the observed antiplatelet response and can decrease the rate of poor responders to Clopidogrel by 60 (76) to 78.9% (249) but as yet little direct evidence that these strategies improve clinical outcomes post PCI. However, recently Bonnello et al, using a strategy of VASP-guided clopidogrel loading prior to PCI with up to 3 further 600mg Clopidogrel loading doses administered to obtain adequate response did, in addition to improving response to Clopidogrel, achieve significantly improved MACE rates at 30 days without increased bleeding (251).

900mg loading doses of Clopidogrel could increase both the speed and extent of the antiplatelet effect and decrease the number of poor responders but previous studies have shown discrepant results. The ALBION study suggested an increased effect with 900mg doses (70). However ISAR-CHOICE found no differences in effect and no differences in levels of the active metabolite of clopidogrel suggesting that absorption or metabolism of Clopidogrel may be a limiting factor (71). Price et al also showed no difference in the magnitude of response or the time to maximal response with VerifyNow P2Y12 assays (72).

We have compared the antiplatelet effects of 600mg and 900mg loading doses of Clopidogrel using VerifyNow and TEG, two rapid and reliable tests of response to Clopidogrel and platelet reactivity whilst on clopidogrel that are suitable for near patient clinical use. Using these techniques we have demonstrated that near patient testing is feasible and provides individual patient data on response to clopidogrel in a time dependent manner. Further, these tests can both detect poor responders and also reassess responses after increased dose to determine if the response has become adequate. These data also demonstrate that whilst 900mg Clopidogrel loading doses do not significantly enhance platelet inhibition compared to 600mg doses in the general patient population, selective and individualised dose adjustment in poor responders improves the level of response and reduces the number of poor responders.

This study has some limitations. We did not correlate the results of our two near patient assays with laboratory based assays. In emergencies such as primary angioplasty this strategy would also be inappropriate and the role of 'top up' doses in this context should be explored. In addition, this study was not designed to investigate long term clinical outcomes.

If these techniques and this approach of individualized therapy are to be incorporated into routine clinical practice then large clinical outcome trials are obviously required. In order to maximize the chances of the concept of tailored antiplatelet therapy becoming widespread these trials should be performed using rapid, simple, near patient assays.

13.8.5 Summary

We have shown that near patient assessment of response to antiplatelet therapy with both VerifyNow and short TEG is feasible and provides clinically useful information that could be used to tailor Clopidogrel therapy where responses are deemed to be inadequate. Whilst there remain contentious issues, particularly in determining the threshold for "poor response", we believe that there is a clear role for universal near patient assessment of response to antiplatelet therapy and that soon it may become unethical to manage high risk individuals without it.

14 Discussion

14.1 Introduction

The pivotal role of the platelet in the pathophysiology of acute coronary syndromes and in the development of thrombotic complications after PCI and stenting is now well understood. Dual antiplatelet therapy with Aspirin and a thienopyridine, usually Clopidogrel, has contributed to the dramatic improvements in outcomes that have led to the emergence of PCI and stenting as the preferred treatment strategy in many patients with ACS and as a viable alternative to coronary bypass surgery in many patients with stable angina. The success of PCI in terms of (a) relief of angina symptoms in stable patients and (b) improved prognosis in patients with STEMI or NSTEMI and (c) the range and complexity of coronary stenoses that can be treated with stents with an acceptably low restenosis rate has ensured its rapid expansion.

Aspirin and Clopidogrel therapy is now standard for all patients following PCI involving stents.

However, whilst this combination unequivocally reduces thrombotic complications after stent placement thrombotic complications, including the potentially catastrophic complication of ST, continue to occur. As a result there has been a trend to increasing intensity of antiplatelet therapy. However, our desire to minimise thrombotic complications through administration of more aggressive and prolonged dual anti-platelet therapy has to be tempered by the risk of inducing bleeding. Indeed, it is noteworthy that in some populations increasing intensity of antiplatelet therapy is associated with worse outcome (10,11).

Therefore key areas of uncertainty about optimal antiplatelet therapy in the context of PCI persist. These questions would be considerably easier to answer if convenient tests for the assessment of the response of individual patients to Aspirin and Thienopyridines were available.

14.2 The current situation

There are a wide variety of assays of response to antiplatelet therapy (summarised in Table I). Several different assays have shown that (i) there is marked heterogeneity in observed responses to antiplatelet therapy (ii) levels of response to antiplatelet therapy correlate with clinical outcome (evidence summarised in Table II) and (iii) that therapeutic manipulation can improve the level of observed response.

However, whilst interventionalists are sensitive to the well described heterogeneity of responses to Clopidogrel and to a lesser extent Aspirin, the universal response is to prescribe standard loading and maintenance doses of these agents! The evidence, by contrast, clearly implies that we could minimise individual risk if we were to tailor doses for individual patients in order to obtain therapeutic levels according to the observed responses to antiplatelet therapy. This approach is unlikely to happen however until there is a rapid, easy to use, reproducible, intuitive, and inexpensive test with which to make the assessment. These requirements cannot be fulfilled by current laboratory tests of isolated platelet function, even though these are considered the scientific "gold standard" for platelet function assessment. In addition there is a large range of assays available and important variability in definitions of poor response. An ideal assay would be (i) cheap, quick and reliable (ii) be able to assess response to both Aspirin and Clopidogrel (both alone and in combination) (iii) provide useful data in individuals already established on antiplatelet therapy (i.e. not need a baseline sample for comparison) (iv) provide data on the overall tendency to thrombosis, not just on response to a specific therapy and (v) must have a clear evidence base supporting its use to improve clinical outcome.

Whilst several current assays have correlated response to therapy with adverse outcome they all have low positive predictive values (252) and it would therefore currently be necessary to modify therapy in large numbers of patients, most of whom would not have

gone on to suffer an adverse event to try to improve outcomes. In addition, it is currently unclear whether assays determining response to antiplatelet therapy by comparison with a baseline sample or assays assessing platelet reactivity whilst on treatment provide superior risk stratification or prognostic information. It also remains unclear whether highly specific assays (such as VASP phosphorylation for Clopidogrel) or non-specific assays (eg PFA-100 for Aspirin) are preferable. As there are significant differences in baseline platelet aggregation, with higher levels of aggregation correlating with total mortality at long-term follow up (77) post treatment platelet reactivity rather than a percentage change from baseline may prove to be a more appropriate measure. A whole blood assay may also be preferable as platelet count and fibrinogen levels have also been associated with outcome and it avoids the need for sample preparation (253).

In these studies we have investigated the utility of two assays of response to antiplatelet therapy potentially suitable for widespread clinical use, TEG Pmap and VerifyNow. TEG Pmap, in particular, has potential advantages in comparison with other methods of assessment. First, TEG is a whole blood assay incorporating the interaction of all of the components of coagulation including platelets, fibrin, clotting factors, and thrombin. In addition as platelet counts are not standardized (in contrast to many other assays) TEG incorporates both platelet count and platelet function, giving an overall picture of platelet effect. This is potentially important as platelet count is a powerful and independent predictor of re-infarction and mortality after PCI (253,254). TEG Pmap is importantly also capable of assessing responses to both Aspirin and Clopidogrel and is suitable for use near to the point of care. However, there is little existing data on its use and conventional methods of assessment are time-consuming and cumbersome.

14.3 Fulfilment of initial study aims

With regard to the specific aims of this study that set out in Chapter 11:
We initially set out to establish a novel approach to TEG PlateletMapping analysis "short TEG" with a view to (i) speeding up result acquisition; (ii) incorporating clot kinetics; (iii) abolishing the need to use 4 channels as part of the "PlateletMap" and (iv) make the technique faster, easier and therefore more suitable for use in routine clinical practice.

We have established that by using the AUC15 and the %CIn, as a novel approach to TEG analysis we can speed up result acquisition, with results obtained after only 15 minutes. We have shown that results obtained with short TEG correlate well with standard methods of assessment taking far longer. This technique also incorporates clot kinetics and abolishes the need to use 4 channels as part of the "PlateletMap." We believe that these modifications make TEG more suitable for use in routine clinical practice.

In addition we set out to provide a framework for the potential use of this method of analysis by:

(i) assessing baseline variability in short TEG responses.

We have established that baseline variability is 5-8% for the individual short TEG channels. It is noteworthy that there appears to be greater variability in the Fibrin channel which is excluded from short TEG analysis.

(ii) assessing the ability of short TEG to detect differences due to antiplatelet therapy with both Aspirin and Clopidogrel.

We have shown that short TEG can assess differences due to antiplatelet therapy with both Aspirin and Clopidogrel.

(iii) assessing the reproducibility of short TEG in determining responses to antiplatelet therapy.

Short TEG appears to reliably and reproducibly detect responses to both Aspirin and Clopidogrel.

(iv) determining "normal" ranges of response to antiplatelet therapy using short TEG.

In further studies we have established reference "normal" ranges for short TEG at baseline and in response to routinely utilised doses of Aspirin, Clopidogrel and dual antiplatelet therapy

(v) identifying the specificity of the assay for responses to Clopidogrel and to Aspirin.

We have also demonstrated using short TEG that Clopidogrel appears to have both independent and aspirin-synergistic effects on AA-induced platelet activation.

(vi) comparing this novel method of assessment with currently accepted methods of TEG analysis.

We have shown that observed responses with short TEG correlate well with conventional methods of TEG analysis. In addition we have shown that "snapshot" samples after the administration of antiplatelet function using short TEG %CIn correlate well with responses assessed with comparison with a baseline sample.

(vii) identifying if there are important differences due to baseline demographics in short TEG responses.

In studies on volunteers using short TEG we have demonstrated higher post-treatment platelet reactivity in young females after the administration of both Aspirin and Clopidogrel and a reduced response to Clopidogrel, but not Aspirin, in women.

(viii) identifying if levels of response differ in high risk patients.

In addition to an ability to determine normal responses we have also established, in a cohort of patients with previous ST, that abnormal responses by short TEG may be associated with increased clinical risk.

(ix) establishing whether dose adjustment may improve response both on a population basis and specifically in individuals identified as initial poor responders.

Furthermore we have established that selective and individualised dose adjustment in poor responders to Clopidogrel (as detected by short TEG and VerifyNow) improves the level of response and reduces the number of poor responders. This potentially enables short TEG to be utilised to provide a clinically relevant solution to the need to assess individual responses to Aspirin and Clopidogrel in patients in order to ensure optimal antiplatelet activity and perhaps reduce the risk of complications.

14.4 Summary

These studies have shown that near patient assessment of response to antiplatelet therapy with both VerifyNow and short TEG is feasible and provides clinically useful information that could be used to tailor antiplatelet therapy where responses are deemed to be inadequate. Short TEG in particular appears to fulfil many of the necessary criteria for an ideal assay of thrombotic risk whilst on antiplatelet therapy. Importantly TEG analysis, through assessment of simultaneous thrombin-, AA- and ADP-induced platelet activation, allows an in-depth analysis of the aetiology of poor responses to therapy and importantly an assessment of the overall effects of combinations of antiplatelet agents. These data may have clinical relevance in (i) determining how to best assess responses to antiplatelet therapy, particularly in the context of combination therapy and (ii) how best to manage poor response to therapy.

The results of these studies demonstrate that the current gold standard method of assessing response to Clopidogrel, ADP-induced platelet aggregation, is unlikely to accurately determine the true clinical effect of Clopidogrel therapy, particularly in individuals administered additional antiplatelet therapies. Furthermore they demonstrate that the strategy of giving standard doses of antiplatelet agents to all is almost certainly flawed. Unfortunately there remains with these assays, in keeping with all other assays of response to antiplatelet therapy, a large disparity between observed responses to therapy and clinical outcome. The fact that there is a 30 fold increased risk of ST on

premature Clopidogrel cessation (245) clearly shows that Clopidogrel must have important effects, even in the 30% found to respond poorly by all current assays. If these assays have an important role at present it is therefore more likely to be in that they are useful for their high negative predictive values for adverse events when platelet reactivity on treatment is found to be low (252).

Thrombotic complications after PCI, including ST, are undoubtedly multifactorial with procedural factors also important. Greater use of intravascular ultrasound and high pressure balloon post-dilatation to reduce incomplete stent apposition, a risk factor of ST (243), may also help reduce the incidence of ST as may developments in stent technology, such as endothelial progenitor cell capture by encouraging rapid endothelialisation (255). Identification of cohorts of patients at increased risk of ST, possibly including patients with malignancy (256), and those at increased risk of poor response to antiplatelet therapy, such as diabetics (257) could also help guide the choice of antiplatelet therapy and procedure undertaken without recourse to assessment of response to antiplatelet therapy. In addition, whilst the widespread use of Clopidogrel has led to dramatic improvements in outcome following PCI its relatively slow onset of action and the marked heterogeneity in observed responses undoubtedly contributes to some of the residual risk post PCI. It may be that future antiplatelet therapy is delivered using agents in whom the response is less heterogeneous. Several novel antiplatelet therapies such as Prasugrel, Cangrelor and Ticagrelor show promise. For the foreseeable future, however, we continue to administer standard doses of drugs to stent patients despite the knowledge that variations in individual responses make it inevitable that we are not achieving therapeutic effect in some patients.

Whilst there remain contentious issues, particularly in determining the threshold for "poor response", I believe that there is a clear role for near patient assessment of response to antiplatelet therapy and that soon it may become unethical to manage high risk individuals without it. VerifyNow, and in particular short TEG, may provide solutions to the urgent clinical need for a rapid and reliable assay of response to antiplatelet therapies

to enable individualised antiplatelet therapy with a view to minimising both haemorrhagic and thrombotic complications after PCI.

15 Publications arising from this project

Hobson AR, Agarwala RA, Swallow RA, Dawkins KD, Curzen NP. Thrombelastography: Current clinical applications and its potential role in interventional cardiology. Platelets 2006;17:509-18

Hobson AR, Petley G, Dawkins K, Curzen N. A Novel Fifteen Minute Test for Assessment of Individual Time-dependent Clotting Responses to Aspirin and Clopidogrel using Modified Thrombelastography. Platelets 2007;18:497-505

Hobson AR, Dawkins KD, Curzen NP. Antiplatelet effects of licking an aspirin tablet can be detected by thrombelastography. Acute Card Care 2008;10:62-3

Hobson AR, McKenzie D, Kunadian V, Purcell I, Zaman A, Dawkins KD, Curzen NP. Malignancy: An Unrecognised Risk Factor for Coronary Stent Thrombosis? J Inv Cardiol 2008;20:E120-123

Hobson AR, Petley G, Morton G, Dawkins KD, Curzen NP. Point-of-care platelet function assays demonstrate reduced responsiveness to clopidogrel, but not aspirin, in patients with Drug-Eluting Stent Thrombosis whilst on dual antiplatelet therapy. Thromb J 2008;6:1

Hobson AR, Curzen NP. Improving outcomes with antiplatelet therapies in percutaneous intervention. Thromb Haemost 2009;101:23-30

Hobson AR, Qureshi Z, Banks P, Curzen NP. Effects of clopidogrel on "aspirin specific" pathways of platelet activation. Platelets 2009;20:386-90.

Hobson AR, Qureshi Z, Banks P, Petley G, Dawkins KD, Curzen NP. Gender and responses to aspirin and clopidogrel: insights using short thrombelastography. Cardiovascular therapeutics 2009;27:246-52.

Hobson AR, Qureshi Z, Banks P, Curzen NP. The potential value of near patient platelet function testing in PCI: Randomised comparison of 600mg versus 900mg Clopidogrel loading doses. Thrombosis 2010 (in press).

Hobson AR, Curzen NP. (2009) Current Status of Oral antiplatelet therapy. In: Redwood S, Curzen N, eds. Oxford Textbook of Interventional Cardiology. Oxford University Press, Oxford. (In press)

16 Presented abstracts relating to this project

Hobson AR, Dawkins KD, Curzen N. Individual Responses to Aspirin Using a Novel Method of Thrombelastogram analysis: Hyporesponsiveness or poor compliance? Oral presentation at British Cardiovascular Society 2007. Heart 2007;93(Suppl 1);A51

Hobson AR, Dawkins KD, Curzen N. Marked Heterogeneity of Individual responses to Loading Dose Clopidogrel Employing a Novel Point of Care Test. Oral presentation at British Cardiovascular Society 2007. Heart 2007;93(Suppl 1);A51-2

Hobson AR, Morton G, Dawkins KD, Curzen N. Point-of-care assays demonstrate resistance to clopidogrel, but not aspirin, in patients with previous stent thrombosis. Presented at Transcatheter Cardiovascular Theraputics (TCT), Washington, USA. Am J Cardiol 2007;100(Suppl 8A);195L.

Hobson AR, Qureshi Z, Banks P, Curzen NP. Anti-platelet effects of aspirin are modified by clopidogrel: Assessment of a clinically relevant interaction using short thrombelastography. Poster presentation at British Cardiovascular Society 2008. Heart 2008; 94 (Suppl II):A125

Qureshi Z, Hobson AR, Curzen NP. A comparison of two rapid assays of clopidogrel responsiveness, short TEG and VerifyNow P2Y12, in the context of Percutaneous Coronary Intervention (PCI). Poster presentation at Acute Cardiac Care Conference, Versailles, France 2008. Acute Cardiac Care 2008,10 (Suppl 3) A569

Hobson AR, Qureshi Z, Banks P, Curzen NP. Do higher loading doses of clopidogrel increase the observed antiplatelet effect in initial poor responders to a 600mg dose? Oral presentation at the International Congress of Medical Sciences, Sofia, Bulgaria 2008. Awarded second prize for excellence in a project relating to therapy.

Qureshi Z, Hobson AR, Curzen NP. The addition of clopidogrel enhances the effect of aspirin, particularly in poor responders to aspirin. Poster presentation at Canadian Cardiovascular Congress, Toronto, Canada 2008. Canadian Journal of Cardiology 2008;24. (Suppl E), A98

Hobson AR, Qureshi Z, Banks P, Curzen NP. Platelet function testing can be used to optimise the use of 900mg Clopidogrel loading doses in the setting of percutaneous intervention. Poster presentation at American College of Cardiology, Orlando, USA 2009. J Am Coll Cardiol 2009;53(Suppl A);A394

Qureshi Z, Hobson AR, Banks P, Curzen N. Gender differences in baseline platelet reactivity and response to clopidogrel, but not aspirin, may contribute to the increased risk in young women with cardiovascular disease. Oral presentation at the European Association for Clinical Pharmacology and Therapeutics, Edinburgh 2009. Basic Clin Pharmacol Toxicol 2009;105 (Suppl 1). O33

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18 Appendices

18.1 Loading dose study

18.1.1 Patient information

Cardiothoracic Directorate
Department of Cardiology
E Level, East Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Patient Information Sheet

Comparison of 900mg and 600mg loading doses of clopidogrel.

You are being invited to take part in a clinical study taking part in the Wessex Cardiothoracic unit at Southampton General Hospital. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- *Part 1 tells you the purpose of this study and what will happen to you if you take part.*
- *Part 2 gives you more detailed information about the conduct of the study.*

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Medication (usually including aspirin and clopidogrel) is given at and around the time of angioplasty and stent insertion to prevent the formation of blood clots inside the stents. These medications have dramatically reduced the risk of blood clots forming but it can still occur and this can lead to blockage of the stent. At present all patients undergoing stent implantation in this hospital receive 600mg of clopidogrel. It is possible however that 900mg of clopidogrel may have an extra benefit in reducing blood clots. We aim to study whether there is a difference in blood clotting between patients receiving 600mg and 900mg of clopidogrel.

Why have I been chosen?

You have been chosen because you are having an angioplasty and stent insertion and will receive clopidogrel treatment.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

If you agree to take part you will be asked to sign a consent form. You will receive either 600mg or 900mg of clopidogrel. When we don't know which way of treating patients is best in order to find out, we need to make comparisons between different treatments. We put people into groups and give each group a different treatment; the results are compared to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). The results are then compared. You will have a blood test before taking the clopidogrel and further blood tests 20 and 60 minutes and 2 and 6 hours after taking the clopidogrel. If you were not in this study you would only require one blood test before and one blood test afterwards, so there will be 3 extra blood tests taken. The blood tests will be analysed within one hour to look at blood clotting and then discarded. They will not be stored. The rest of your treatment including angioplasty and stent insertion, subsequent drug treatment, discharge plans and follow up will be according to current hospital practice.

Taking part in the study will not delay discharge from hospital. No additional follow up will be required if you agree to take part.

What do I have to do?

If you agree to take part in this study you will be randomised to receive either 600mg or 900mg of clopidogrel and will have up to 3 more blood tests than if you do not agree to take part.

What is the drug that is being tested?

Clopidogrel is an antiplatelet medication that reduces blood clotting and is routinely given to everyone having an angioplasty and stent inserted.

What are the alternatives for treatment?

Clopidogrel is so important in the setting of angioplasty and stent insertion that it is unwise to go ahead with the procedure without taking it. At present there are no comparable medications in widespread use.

What are the side effects of the treatment received when taking part?

If you take part there is a 50% chance that you will receive our current standard dose of clopidogrel. There is a 50% chance that you will receive a higher dose. There is no evidence that this is associated with a higher risk of side effects. Clopidogrel does reduce blood clotting and it is possible that a higher dose may reduce blood clotting more (this is what we are trying to detect in this study). If it were to reduce blood clotting more it could increase the risk of bruising and bleeding, particularly in the very occasional patient (less than 1 in 100) who requires a bypass operation shortly following angioplasty. We anticipate that the risk of increased bleeding is slight.

What are the other possible disadvantages and risks of taking part?

There are the risks associated with venesection (blood tests). There are small risks of bruising and bleeding and a very small risk of infection (1 in 100,000 or less).

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get might help improve the treatment of people undergoing angioplasty and stent insertion in the future.

What happens when the research study stops?

There is no long-term follow up and no blood samples will be stored.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

We hope to publish the results of this study once completed, however you will not be identified in any report or publications. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

Principal Investigator: Dr Alex Hobson. Alex.Hobson@suht.swest.nhs.uk
Cardiology Study Co-ordinator: Sue Kitt. Tel: 023 8079 8538

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What will happen if I don't want to carry on with the study?

It is up to you to decide whether you wish to take part or not. You can withdraw from the study at any time but we will need to use the data collected up to the time of your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Tel: 023 8079 8538). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (or Private Institution). Details can be obtained from the hospital. In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against Southampton University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

We hope to publish the reports of this study once completed, however you will not be identified in any report or publication. All information that is collected about you during the course of the research will be kept strictly confidential. All procedures for handling, processing, storage and destruction of data collected during the course of this study are compliant with the Data Protection Act 1998.

Involvement of the General Practitioner/Family doctor (GP)

You will be asked if you are happy for us to inform your GP that you are taking part in this study.

What will happen to any samples I give?

Samples will be analysed within 1 hour of collection. Samples will then be discarded. No samples will be stored. No genetic tests will be done

Who is organising and funding the research?
The Wessex cardiology research group is organising this research. It is funded by the Wessex Cardiology Intervention Fund.

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the Southampton and South West Hampshire Research Ethics Committee B.

Thank you for taking the time to read this sheet.

If you agree to take part in this study you will be given a copy of the information sheet and a signed consent form to keep.

18.1.2 Consent form

Cardiothoracic Directorate
Department of Cardiology
E Level, East Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Study Number: RHM CAR0321

Patient Identification Number for this trial:

CONSENT FORM

Title of Project:
Comparison of 900mg and 600mg loading doses of clopidogrel.

Name of Researcher: Dr A Hobson

Please initial box

1. I confirm that I have read and understand the information sheet dated 27/01/2006 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher Signature		Date

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

18.2 Stent thrombosis study

18.2.1 Patient information

Cardiothoracic Directorate
Department of Cardiology
E Level, East Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Patient Information Sheet

Investigating a patient population with previous stent thrombosis

You are being invited to take part in a clinical study taking part in the Wessex Cardiothoracic unit at Southampton General Hospital. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- *Part 1 tells you the purpose of this study and what will happen to you if you take part.*
- *Part 2 gives you more detailed information about the conduct of the study.*

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Unfortunately, despite the best treatment available at present, a few patients still suffer from stent thrombosis following stent insertion. This is where a blood clot forms inside the stent. We know that this can be prevented in the majority of patients by using antiplatelet medication (such as aspirin and clopidogrel). Despite this it still occurs in a few individuals. This is something that we are trying hard to prevent.
We aim to see if there is a difference in the way blood clots in patients who have had a stent thrombosis. If there is it could give us clues as to how it is best avoided in the future.

Why have I been chosen?

You have been chosen because you have **either** had a stent thrombosis **or** are a close match (in terms of age, sex procedure performed and medication) to a patient who has had a stent thrombosis following angioplasty and stent insertion.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

If you agree to take part you will be asked to sign a consent form. We will ask you to come to the hospital for a single blood test. This blood test will be analysed within 1 hour. The sample will then be discarded. No samples will be stored.

What do I have to do?

You will be asked to attend for a single blood test as above.

What are the possible disadvantages and risks of taking part?

There are the risks associated with venesection (blood tests). There are small risks of bruising and bleeding and a very small risk of infection (1 in 100,000 or less).

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get might help improve the treatment of people undergoing angioplasty and stent insertion in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

We hope to publish the results of this study once completed, however you will not be identified in any report or publications. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

Principal Investigator: Dr Alex Hobson. Alex.Hobson@suht.swest.nhs.uk
Cardiology Study Co-ordinator: Sue Kitt. Tel: 023 8079 8538

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What will happen if I don't want to carry on with the study?

It is up to you to decide whether you wish to take part or not. You can withdraw from the study at any time but we will need to use the data collected up to the time of your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Tel: 023 8079 8538). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.
In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against Southampton University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

We hope to publish the reports of this study once completed, however you will not be identified in any report or publication. All information that is collected about you during the course of the research will be kept strictly confidential. All procedures for handling, processing, storage and destruction of data collected during the course of this study are compliant with the Data Protection Act 1998.

What will happen to any samples I give?

Samples will be analysed within 1 hour of collection. Samples will then be discarded. No samples will be stored. No genetic tests will be done

Who is organising and funding the research?
The Wessex cardiology research group is organising this research. It is funded by the Wessex Cardiology Intervention Fund.

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the .....

Thank you for taking the time to read this sheet.

If you agree to take part in this study you will be given a copy of the information sheet and a signed consent form to keep.

18.2.2 Consent form

Cardiothoracic Directorate
Department of Cardiology
E Level, East Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Study Number: RHM CAR0322

Patient Identification Number for this trial:

CONSENT FORM

Title of Project:
Investigating a patient population with previous stent thrombosis.

Name of Researcher: Dr A Hobson

Please initial box

1. I confirm that I have read and understand the information sheet dated 27/03/2006 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher Signature		Date

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

18.3 Response of non-responders to higher doses

18.3. 1 Information sheet

Cardiothoracic Directorate
Department of Cardiology
E Level, East Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Patient Information Sheet

Response of "non-responders" to higher doses of antiplatelet agents.

You are being invited to take part in a research study taking part in the Wessex Cardiothoracic unit at Southampton General Hospital. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- *Part 1 tells you the purpose of this study and what will happen to you if you take part.*
- *Part 2 gives you more detailed information about the conduct of the study.*

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

It is increasingly apparent that the varied response to antiplatelet therapies (blood thinning medication such as aspirin and clopidogrel) can contribute to complications such as stent thrombosis (blood clot formation inside the stent) following angioplasty and stent implantation. We have already shown that we can detect people who appear to respond poorly to these drugs. We aim to give volunteers the doses of aspirin and clopidogrel currently given to patients at the time of angioplasty. We will take blood tests to establish which volunteers appear to respond poorly to the medication.
We will then give these volunteers (shown to respond poorly to standard doses) a higher dose of medication to see if this improves their response.
If the response improves it may mean that we can decrease the risk of complications in patients following angioplasty who appear to initially respond poorly to antiplatelet medications.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason

Who will not be allowed to take part?

Some people will be excluded from the study for their own safety.

- *If you are allergic to aspirin, clopidogrel or a related medication you will not be allowed to take part.*
- *You will not be allowed to take part if you are or may be pregnant.*
- *You will not be allowed to take part if you are asthmatic or have a history of gastro-oesophageal reflux, stomach or duodenal ulcers*
- *You will not be allowed to take part if you are already taking aspirin, other blood thinning medication or non-steroidal anti-inflammatory drugs such as ibuprofen.*
- *You will not be allowed to take part if you have had bleeding problems in the past or if it is likely that you will require an operation in the next 2 weeks.*

What will happen to me if I take part?

If you agree to take part you will be asked to sign a consent form.

A blood test will be taken before and 6-24 hours after a 300mg dose of aspirin (this is the amount in one normal aspirin tablet). We will use the blood samples to assess the effect on platelets (a component of blood involved in blood clotting). Some people appear to respond more than others. If there is a low response (this will occur in up to 40%) we will ask you to reattend on a subsequent occasion at least 2 weeks later when we will take blood tests before and 6-24 hours after 600mg of aspirin (the amount you might take for a headache).

At least 2 weeks after the last time you took aspirin we will take a blood test before and a blood test 6 to 24 hours after receiving the standard dose (600mg) of clopidogrel (another tablet that acts on platelets). If this shows a low response to clopidogrel (there is about a 30% chance of this) you will be asked reattend on another occasion at least 2 weeks later. On this occasion you will have a blood test before and after receiving 900mg of clopidogrel (a higher than standard dose). Blood tests will again be taken between 6 and 24 hours after the medication is given.

What is the drug that is being tested?

Aspirin is widely used as a painkiller and as an antiplatelet medication. Clopidogrel is an antiplatelet medication that reduces blood clotting and is routinely given to everyone

having an angioplasty and stent inserted and large numbers of other patients with cardiovascular disease.

What are the side effects of the treatment received when taking part?

The side effects of aspirin are generally mild and infrequent and much more common when it is taken long-term.
Aspirin can cause: gastro-intestinal irritation, skin rashes and bronchospasm (like asthma).

Side effects of clopidogrel are also very rare and unlikely after one dose but include: rash, tummy pain and diarrhoea.

Both aspirin and clopidogrel act as blood thinning medication (that is why they are usually given). They may therefore increase the risk of bruising and bleeding. However following just one dose the risk of significant bleeding is extremely small. There may however be a slightly increased risk of bleeding if you were to need an operation within a few days of taking the medication.

What are the other possible disadvantages and risks of taking part?

There are the risks associated with venesection (blood tests). There are small risks of bruising and bleeding and a very small risk of infection (1 in 100,000 or less). A maximum of 80mls of blood will be taken over the period of a month, which is inconsequential.

What are the possible benefits of taking part?

The study will help you but the information we get might help improve treatments in the future.

What happens when the research study stops?

There is no long-term follow up and no blood samples will be stored.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

We hope to publish the results of this study once completed, however you will not be identified in any report or publications. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

Principal Investigator: Dr Alex Hobson. Alex.Hobson@suht.swest.nhs.uk
Cardiology Study Co-ordinator: Sue Kitt. Tel: 023 8079 8538

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What will happen if I don't want to carry on with the study?

It is up to you to decide whether you wish to take part or not. You can withdraw from the study at any time but we may need to use the data collected up to the time of your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Tel: 023 8079 8538). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.
In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against Southampton University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

We hope to publish the reports of this study once completed, however you will not be identified in any report or publication. All information that is collected about you during the course of the research will be kept strictly confidential. All procedures for handling,

processing, storage and destruction of data collected during the course of this study are compliant with the Data Protection Act 1998.

What will happen to any samples I give?

Samples will be analysed within 1 hour of collection. Samples will then be discarded. No samples will be stored. No genetic tests will be done

Who is organising and funding the research?
The Wessex cardiology research group is organising this research. It is funded by the Wessex Cardiology Intervention Fund.

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the Southampton and South West Hampshire Research Ethics Committee B.

Thank you for taking the time to read this sheet.

If you agree to take part in this study you will be given a copy of the information sheet and a signed consent form to keep.

18.3.2 Consent form

Cardiothoracic Directorate
Department of Cardiology
E Level, East Wing, Mailpoint 46
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Study Number: RHM CAR0323

Patient Identification Number for this trial:

CONSENT FORM

Title of Project:
Response of non-responders to higher doses of antiplatelet agents.

Name of Researcher: Dr A Hobson

Please initial box

1. I confirm that I have read and understand the information sheet dated 27/03/2006 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes