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Strategies for the Efficacy and Safety of Antiplatelet Drugs

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Strategies for the Efficacy and Safety of Antiplatelet Drugs

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School of Postgraduate Studies, Royal College of Surgeons in Ireland

**A thesis submitted to the National University of Ireland in fulfillment
of the requirements for Doctor of Medicine.**

Submission date November 2nd 2012

Appendix 1: Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a Doctorate of Medicine is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work.

Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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SUMMARY

P2Y12 receptor antagonists including clopidogrel, prasugrel or ticagrelor in addition to aspirin are the standard of care for the treatment in acute coronary syndrome and percutaneous coronary intervention. The benefit of this therapy is based on the balance between the reduction of ischaemic events and minimising bleeding risk. Therefore, it is important to have strategies to both ensure the optimal inhibition of the P2Y12 receptor and the effective prevention and treatment of bleeding events. These form the basis for our three studies.

In the first study, we demonstrated in 241 patients after an acute coronary syndrome, the feasibility of individualised P2Y12 receptor antagonist therapy based on rapid genotyping to identify common CYP2C19 polymorphisms associated with clopidogrel metabolism. Our findings show that this strategy, when combined with follow up point-of-care platelet function testing, almost eliminated high on-treatment platelet reactivity (2.9%) and allowed the more judicious use of prasugrel therapy, thereby minimising both thrombotic and bleeding risk.

In the latter two studies, we examined in an ex-vivo and in-vivo model the biological efficacy of platelet transfusion in the restoration of platelet function in patients on P2Y12 receptor antagonists. In 35 patients receiving loading doses of clopidogrel and prasugrel for acute coronary syndrome (ACS) and/or percutaneous coronary intervention (PCI) we demonstrated that increasing proportions of platelets are needed reverse stronger P2Y12 inhibition. In the ex-vivo model, we showed that in 33 cardiac surgery patients on P2Y12 receptor antagonists that were given platelet transfusion for perioperative bleeding, there was a 30% increase in platelet activation vasodilator stimulated phosphoprotein (VASP) phosphorylation platelet reactivity index (PRI), a sensitive marker of platelet activation.

Platelet transfusion was also associated with changes in the rate and strength of clot formation as assessed by thromboelastograph (TEG).

In conclusion, we demonstrated strategies to best identify patients outside the therapeutic window of P2Y₁₂ receptor antagonism using rapid genetic and point-of-care platelet function platforms, allowing the tailored selection of treatment with the potential to avoid future adverse clinical events. For those presenting with bleeding events, we showed the biological efficacy of the strategy of platelet transfusion for the rapid reversal of P2Y₁₂ therapy in both the ACS/PCI and the cardiac surgery setting.

Chapter 1

INTRODUCTION

STATE OF THE ART: ANTIPLATELET THERAPY IN CORONARY HEART DISEASE PATIENTS

Platelets structure and function

Platelet adhesion, activation and aggregation are fundamental to the initiation of thromboses and play a vital role in the early formation of an acute coronary thrombosis¹. Adenosine diphosphate (ADP) is a central mediator in this process². Atherosclerotic plaque rupture and endothelial activation causes localized adhesion of platelets leading to platelet activation in response to various platelet agonists including thrombin, thromboxane A₂ (TXA₂) and collagen³. ADP is secreted in high concentrations from platelet dense granules and acts to amplify the platelet activation induced by these agents. Its proaggregatory effects are via its interaction with the two G protein-coupled receptors, P2Y₁ and P2Y₁₂. These belong to a family of P2Y receptors whose ligands are purine and pyrimidine nucleotides. They are divided into two distinct subgroups based on structural difference: G_q-coupled subtypes and G_i-coupled subtypes⁴.

The P2Y₁ receptor couples to G_{αq} which, in response to ADP, mediates phospholipase activation and calcium mobilization from internal stores leading to platelet shape change and weak and transient aggregation (**Figure 1.1**). Concomitant ADP activation of the P2Y₁₂ receptor through its G-protein, G_{i2} is essential for complete aggregation⁵.

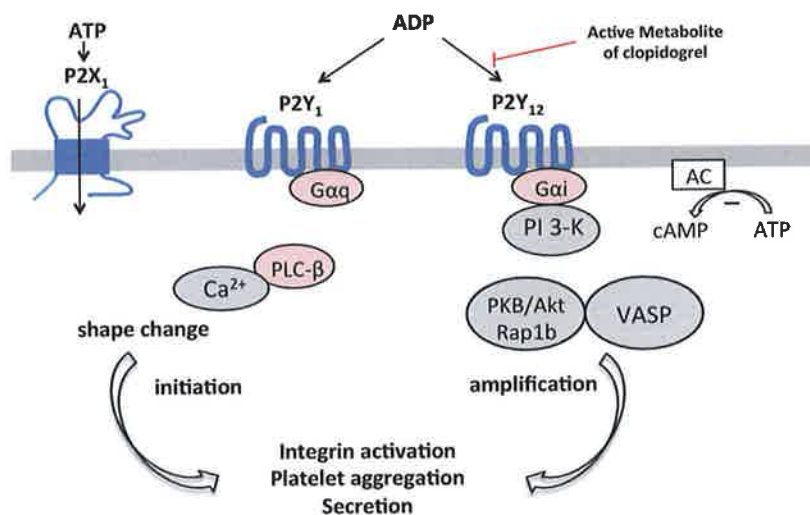


Figure 1.1 Illustration of the activation of the P2Y1 and the P2Y12 receptor by adenosine diphosphate

The P2Y₁₂ receptor, previously named P2T_{AC}, P2C_{YC} and P2Y_{ADP}, was the last receptor of the P2Y family to be cloned in 2001⁶. However, it has been well characterized on the basis of both pharmacological and genetic evidence. Its presence is limited to platelets, endothelial cells, glial cells and smooth muscle cells thus making it an attractive target for antiplatelet drugs. Structurally, it contains 342 aminoacid residues, including four extracellular Cys residues and exists mainly as homo-oligomers within lipid rafts⁷. The P2Y₁₂ receptor is coupled to Gα₁₂ protein and appears to influence platelet activation and aggregation through several intracellular pathways downstream of the receptor. Activation of Gα₁₂ leads to inhibition of cAMP (cyclic adenosine mono-phosphate) which has a facilitating effect on platelet activation by inhibition of the cAMP-dependant protein kinase mediated phosphorylation of the vasodilator-stimulated phosphoprotein (VASP)^{8,9} VASP is an actin regulatory protein and a

negative modulator of the α IIb β 3 integrin activation. Thus, levels of VASP phosphorylation/dephosphorylation reflect P2Y₁₂ inhibition/activation state, which constitutes a sensitive marker to identify the effects of P2Y₁₂ antagonists^{10, 11}. The other pathways by which P2Y₁₂ amplifies platelet response include stimulation of phosphatidylinositol-3 kinase (PI-3K) activity^{12, 13} leading to sustained aggregation; and activation of small GTPase Rap1b through a PI-3K dependant mechanism^{14, 15}.

The important role of P2Y₁₂ in thrombosis has been further demonstrated in studies of patients with congenital selective defects of the receptor and of ADP-induced platelet aggregation having a history of mild to moderate excessive bleeding¹⁶. This is also shown in studies of P2Y₁₂ knock out mice. This underlines its relevance as a key target of efficient antithrombotic therapy. P2Y₁₂ receptor blockade acts early in the cascade of events leading to the formation of platelet thrombus and effectively inhibits platelet aggregation. This occurs due to the prevention of platelet degranulation, thereby inhibiting release of prothrombotic and inflammatory mediators from the platelet. In addition it inhibits the transformation of the glycoprotein (GP) GIIb/IIIa receptor to the form that binds fibrinogen and links platelets¹⁷.

P2Y₁₂ receptor inhibiting drugs

Dual antiplatelet therapy (DAPT) comprising aspirin and a P2Y₁₂ inhibitor is central to the prevention of thrombotic events in patients with acute coronary syndromes and post percutaneous coronary intervention¹⁸⁻²⁰.

Ticlopidine and Clopidogrel

The first- and second generation thienopyridines Ticlopidine and Clopidogrel are prodrugs that require metabolism to their active forms by the hepatic cytochrome P450 (CYP 450) isoenzymes. Ticlopidine is metabolised by at least five main pathways resulting in a minimum of

13, mostly inactive, metabolites^{21,22} One of these has been identified to have antiplatelet activity²³. However, ticlopidine has almost completely been replaced by clopidogrel in clinical practice due its increased pharmacological activity and better safety and tolerability profile. Clopidogrel is a second-generation thienopyridine derivative that binds specifically and irreversibly to the platelet P2Y₁₂ purinergic receptor, inhibiting ADP-mediated platelet activation and aggregation^{24,25}. It is a prodrug that is metabolised to its active form in the liver. In particular, the thiophene ring of clopidogrel is oxidized to form a thiolactone intermediate metabolite, 2-oxoclopidogrel. This is further oxidized, resulting in the opening of the thiolactone ring and the formation of a carboxyl and thiol groups^{24,26}. The reactive thiol group of the active metabolite of clopidogrel forms a disulfide bridge between one or more cysteine residues of the P2Y₁₂ receptor. This interaction is irreversible, accounting for the observation that platelets are inhibited, even if no active metabolite is detectable in plasma. This results in inhibition of the binding of the P2Y₁₂ agonist 2-methylthio-ADP and the ADP-induced down regulation of adenylyl cyclase²⁷. Platelet aggregation is affected not only when triggered by ADP but also by other substances requiring released ADP as an amplifier.

In a landmark analyses, clopidogrel in addition to aspirin was shown to be superior to aspirin alone in preventing cardiovascular events in patients with symptomatic atherosclerosis^{28,29}. The combination of aspirin and clopidogrel or dual antiplatelet therapy has since become the standard of care for patients with acute coronary syndromes and for patients undergoing percutaneous coronary intervention with stenting^{30,31}.

Despite its proven benefit, recurrent clinical adverse cardiovascular events still occur with dual antiplatelet therapy which is largely related to the various limitations of clopidogrel as an antithrombotic agent. Firstly, it has an onset of action that is time and dose dependent with

steady state platelet inhibition of 42-47% reached after eight days of 75mg administration³², 40% reached 4 to 6 hours after 300mg loading dose^{33,34} and 60% reached 2 to 4 hours after 600mg loading dose³⁵, with further and faster platelet inhibition achieved with 900mg loading dose³⁶. This results in sub-optimal platelet inhibition in the acute setting³⁷. Secondly, the inhibition of the P2Y12 receptor is irreversible and there is interindividual variability in the recovery of platelet function leading to higher bleeding risk for patients undergoing surgery including CABG³⁸. Thirdly, there is considerable interindividual variability in the pharmacodynamic response to the drug with some patients being termed clopidogrel resistant or as having high on treatment platelet reactivity (HTPR). This is largely due to interindividual differences in the metabolism of the prodrug and has been correlated with increased risk of atherothrombotic events³⁹⁻⁴².

Mechanisms of the variability of response to clopidogrel

The factors related to variability of response to clopidogrel can broadly be divided into four categories: environmental, cellular, clinical and genetic factors⁴³ (**Table 1.1**).

Environmental factors include diet, age, smoking, and drug-drug interactions. Cellular mechanisms such as the accelerated platelet turnover and up regulation of the ADP platelet receptor pathway may also be important. There are multiple clinical factors such as diabetes, body mass index, renal impairment, compliance and dosing which also play a significant role in clopidogrel response⁴⁴.

Table 1.1 Factors Influencing Platelet Aggregation/Drug Response.

Genetic Polymorphisms	CYP GP 1a P2Y ₁₂ GP IIIa
Cellular Factors	Accelerated platelet turnover Reduced CYP3A metabolic activity Increased ADP exposure Up-regulation of the P2Y ₁₂ pathway Up-regulation of the P2Y-independent pathways (collagen, epinephrine, thromboxane A ₂ , thrombin) Up-regulation of the P2Y ₁₂ pathway
Clinical factors	Failure to prescribe/poor compliance Underdosing Poor absorption Drug-drug interactions involving CYP3A4 ACS Diabetes mellitus/insulin resistance Elevated body mass index

The response to clopidogrel can also be affected by drug-to-drug interactions⁴⁵. Proton pump inhibitors have been shown to interact with the metabolism of clopidogrel thereby reducing the platelet inhibition effect^{46, 47}. Indeed, co-administration of omeprazole with clopidogrel reduced exposure to the active metabolite of clopidogrel by almost half⁴⁶. The clinical significance of this interaction has been much debated with a suggestion that the detrimental effects may only be seen in high-risk cardiovascular patients.^{47, 48}

Similarly, 3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors appear to competitively inhibit the bioactivation of clopidogrel as both are metabolized by the CYP enzymes, particularly CYP3A4⁴⁷. The clinical impact, however, does not appear to be significant as suggested in several studies examining this issue⁴⁹⁻⁵⁴.

Genetic variants and clopidogrel pharmacodynamic response

The influence of various genetic polymorphisms has emerged as one of the major determinants of clopidogrel response. After oral administration, clopidogrel is absorbed from the intestine; this is limited by an intestinal efflux pump P-glycoprotein, which is encoded for by the ABCB1 gene (Figure 1.2). The majority of clopidogrel (85%) is hydrolyzed by carboxyesterases to form inactive metabolites and the remaining 15% of the drug is then metabolised by the cytochrome P450 (CYP) system in two sequential oxidative steps to generate the active metabolite⁵⁵⁻⁵⁷. Given that only an estimated 2% of ingested clopidogrel ends up bound to platelets, it is easy to appreciate that small changes in its metabolism may substantially affect platelet P2Y₁₂ inhibition⁵⁸.

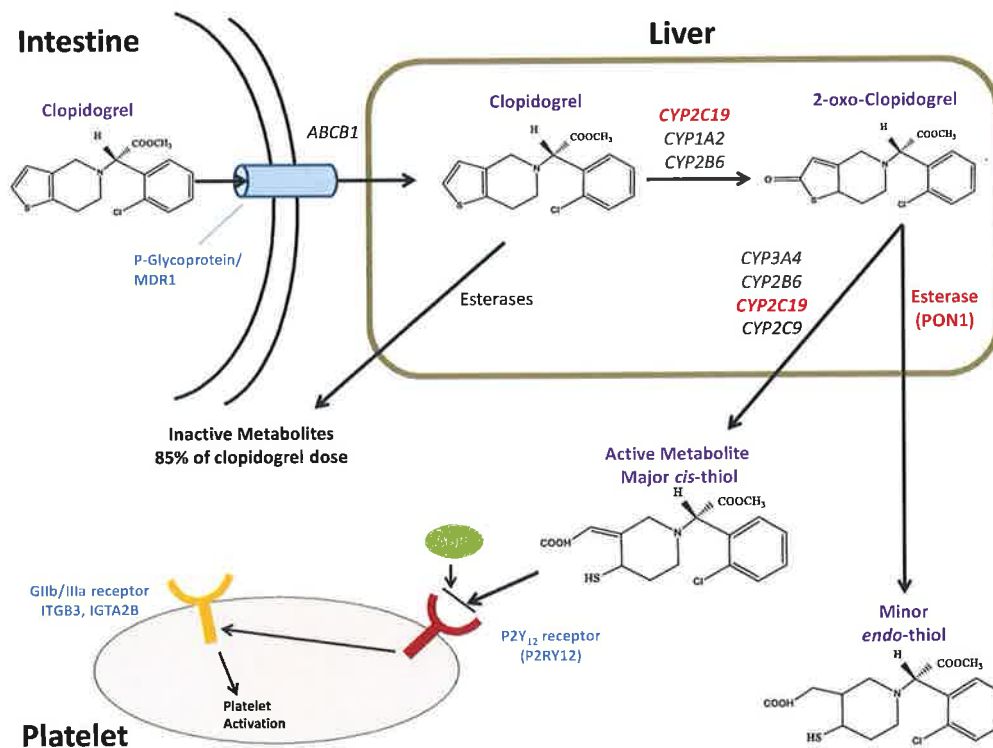


Figure 1.2 Two-step metabolic activation of Clopidogrel

Bioavailability of the prodrug is determined by intestinal absorption, which might be limited by the efflux pump MDR1 (encoded by *ABCB1*). Subsequently, 85% of the prodrug is converted into inactive metabolites by ubiquitous esterases. The remaining 15% is converted into a thiol-containing active metabolite through two-step oxidations that involve several cytochrome P450 enzymes. The first oxidative step is catalyzed by *CYP2C19*, *CYP1A2* and *CYP2B6* enzymes, producing the intermediate 2-oxo-Clopidogrel. The second step is mediated by *CYP3A4*, *CYP2B6*, *CYP2C19*, and *CYP2C9* and yield the bioactive metabolite, the *cis*-thiol isomer which irreversibly binds to P2Y₁₂ receptors (encoded by *P2RY12*) on platelets, and subsequently prevents ADP-induced stimulation of the GPIIb/IIIa receptor (encoded by *ITGB3* and *ITGA2B*) and thus platelet activation.

The cytochrome P450 enzymes (CYP) are a superfamily of microsomal drug-metabolizing enzymes important in oxidative drug metabolism. The liver is the key site for metabolism related to CYP. In humans, 57 CYP genes have been identified, but only an estimated 15 of the encoded proteins have been linked to the metabolism of drugs⁵⁹. They are a highly polymorphic group of enzymes leading to a significant number of variations and subsequent

changes in drug metabolism. The many different alleles are summarized on the Human CYP allele nomenclature committee home page (<http://www.cypalleles.ki.se>). The polymorphisms consist of gene duplications and gene deletions causing frameshift mutations and often altered splice sites.

CYP1A2, CYP2B6, CYP2C9, *CYP2C19*, and CYP3A4/5 are the key cytochrome P450 enzymes in the metabolism of clopidogrel^{56, 60-63}. The relative contribution of each enzyme to each oxidative step is debated in the literature. In one of the first studies, Savi et al. showed, using a rat model, first demonstrated the importance of the cytochrome P450 enzymes in activation of clopidogrel by showing decreased platelet aggregation when using a global cytochrome P450 enzyme inhibitor⁶⁰. Clarke et al. then demonstrated the role of the enzymes CYP3A4 and 3A5 in a human model using genetically engineered microsomes containing a single human P450 isozyme and testing their ability to oxidize clopidogrel while examining the inhibitory effect of atorvastatin on this process⁶⁴. Since, the role of CYP3A4/5 has been further examined in other studies with conflicting results⁶⁵⁻⁶⁷.

Kazui et al. examined the overall hepatic metabolism of clopidogrel. They concluded that three enzymes CYP1A2, CYP2B6 and *CYP2C19* contribute to the first oxidative step where each enzyme is responsible for 45%, 36% and 19% of the conversion respectively; four enzymes (CYP3A4, CYP2C9, *CYP2C19*, and CYP2B6) contribute to the second step of active metabolite formation, where each enzyme is responsible for 40%, 33%, 21% and 7% of the conversion respectively⁶³. The *CYP2C19* isoenzyme is involved in both steps and appears to have the most influence.

The *CYP2C19* polymorphisms

The hepatic *CYP2C19* enzyme contributes to the metabolism of many clinically important drug classes including antidepressants, benzodiazepines, mephenytoin and proton

pump inhibitors. The *CYP2C19* enzyme has been shown to play a dominant role in clopidogrel activation and seems to be the most consistent genetic determinant of differences in response to clopidogrel⁶⁸⁻⁷⁰. Located on chromosome 10 (10q24.1-q24.3), the *CYP2C19* gene consists of 490 amino acid residues and is one of the most polymorphic CYP genes, having more than 25 known variant alleles varying in prevalence among different ethnic groups^{71, 72} (<http://www.cypalleles.ki.se/CYP2C19.htm>). The *CYP2C19**1 allele results in normal metabolism of clopidogrel whereas the *CYP2C19**2 and *CYP2C19**3 alleles are responsible for more than 90% of cases of poor metabolism^{69, 73, 74}. The majority of pharmacogenetic studies for clopidogrel have observed that *CYP2C19*-inactivating variations are associated with decreased clopidogrel activation, a decreased antiplatelet effect, and an increased likelihood of a cardiovascular event. Additional missense mutations in *CYP2C19* that are relevant to defective enzyme function including *3, 4*, 5* and 8*, are rare but also associated with impaired metabolism. The *CYP2C19**17 allele (c.-806C>T; rs12248560) is a regulatory, gain-of-function variant that has been associated with increased expression and enzymatic activity. There are several classification schemes, although a commonly used method classifies patients into one of five categories of metaboliser phenotype on the basis of combinations of alleles^{68, 75} (Table 1.2)

Table 1.2 Patient classification by *CYP2C19* genotype.

Metaboliser type	Genetic variant
Ultra	*17/*17, *1/*17
Extensive	*1/*1
Intermediate	*1/*2, *1/*3
Poor	*2/*2, *2/*3, *3/*3
Unknown	*2/*17, *3/*17

CYP2C19 Loss-of-Function alleles

The most common and widely analysed loss-of-function allele is *CYP2C19*2* with a frequency of around 15% in Caucasians and Africans, and significantly higher at 30% in Asian populations⁷⁶. *CYP2C19*2* is inherited as an autosomal co-dominant trait. The principal defect in carriers of *CYP2C19*2* (rs4244285) is a single base pair mutation (681G>A) in exon 5, which creates an aberrant splice site. This change alters the reading frame of the mRNA starting with amino acid 215 and produces a premature stop to codon 20 amino acids downstream resulting in a truncated, non-functional protein⁷⁷.

Hulot et al first demonstrated the contribution of *CYP2C19* to clopidogrel responsiveness⁶⁹. In a group of 28 young healthy subjects treated with a 7-day course of 75mg/day clopidogrel, carriers of the *2 allelic variant (i.e *CYP2C19* *1/*2 heterozygotes) had impaired responsiveness to clopidogrel compared with the *CYP2C19* wild-type as assessed by light transmission aggregometry in response to ADP. Brandt et al. confirmed this in 74 healthy subjects⁷⁸. The association between *CYP2C19* loss-of-function variants has been confirmed in other studies. In healthy volunteers Mega and colleagues showed a 32.4% relative reduction in plasma exposure to the active clopidogrel metabolite and a relative reduction of approximately 25% in maximal platelet aggregation in carriers with at least one *CYP2C19* reduced-function allele compared with non-carriers⁶⁸. Fontana et al. also demonstrated these findings in a healthy volunteer population but failed to find any influence of this polymorphism on clopidogrel response when studied in patients with coronary artery disease⁷⁴. However, there are numerous studies that have since demonstrated this loss-of-function allele to be associated with poor pharmacodynamic response in ACS/PCI patients treated with clopidogrel (**Table 1.3**).

More recently, in young post myocardial infarction myocardial patients receiving loading doses of clopidogrel, we demonstrated that the *CYP2C19**2 allele was associated with 30% relative reduction in plasma exposure to clopidogrel active isomer H4 (clopi-H4) and a gene-dose effect evident between *1/*1, *1/*2 and *2/*2 genotype ⁷⁹ Based on these studies platelet responsiveness to clopidogrel in heterozygotes (*1/*2) appears to lie somewhere between the responsiveness in individuals with the *1/*1 genotype and that in those with the *2/*2 genotype ^{78,80}. Therefore, individuals can be categorized as extensive metabolisers (e.g., *1/*1), intermediate metabolisers (e.g., *1/*2), or poor metabolisers (e.g., *2/*2)⁷⁶.

Table 1.3 Pharmacodynamic and pharmacokinetic studies of effect of *CYP2C19* variants on clopidogrel response

Study	Number of Participants	Population	<i>CYP2C19</i> allelic variants	Clopidogrel dose	Platelet function test	Findings
Hulot <i>et al.</i> , 2006 ⁶⁹	28	Healthy Subjects	<i>CYP2C19</i> *2, *3, *4, *5, *6	75mg MD	LTA (10 µmol/L ADP) Flow cytometry /VASP-P	<i>CYP2C19</i> *2 is associated with higher on-treatment platelet reactivity
Brandt <i>et al.</i> , 2007 ⁷⁸	74	Healthy Subjects	<i>CYP2C19</i> *2, *3, *4, *5	300mg LD	LTA (20 µmol/L ADP)	<i>CYP2C19</i> *2 is associated with higher on-treatment platelet reactivity
Fontana <i>et al.</i> , 2007 ⁷⁴	94	Health Subjects	<i>CYP2C19</i> *2	300mg LD 75mg MD	LTA (20 µmol/L ADP)	The <i>CYP2C19</i> *2 allele explained 10% of the variability in clopidogrel responsiveness
Giusti <i>et al.</i> , 2007 ⁶⁶	1,419	ACS patients	<i>CYP2C19</i> *2	600mg LD 75mg MD	LTA (2, 10 µmol/L ADP)/Residual Platelet Reactivity	The <i>CYP2C19</i> *2 allele was associated with higher platelet aggregability in high-risk vascular patients (p=0.001).
Frere C <i>et al.</i> , 2008 ⁸¹	603	ACS patients	<i>CYP2C19</i> *2	600mg LD	RPA by LTA (10 µmol/L ADP) VASP-P P-selectin	The <i>CYP2C19</i> *2 allele was associated with high on treatment platelet reactivity
Kim <i>et al.</i> , 2008 ⁸²	24	Healthy Subjects	<i>CYP2C19</i> *2, *3	300mg LD 75mg MD	LTA (5 µmol/L ADP) Plasma concentration of clopidogrel	The <i>CYP2C19</i> genotype affects the plasma levels of clopidogrel and is associated with high on-treatment platelet reactivity.
Umemura <i>et al.</i> , 2008 ⁸³	47	Healthy Subjects	<i>CYP2C19</i> *2, *3	300mg LD	LTA (20 µmol/L ADP) VASP-P Plasma concentration of the active metabolite	Poor and intermediate metabolisers had higher on-treatment platelet reactivity by VASP PRI than extensive metabolisers. The <i>CYP2C19</i> genotype is a determinant for the formation of the active clopidogrel metabolite.
Mega <i>et al.</i> , 2009 ⁸⁴	162	Healthy subjects and ACS/PCI	<i>CYP2C19</i> *2, *3, *4, *5, *8	300mg or 600mg LD 75mg MD	MPA by LTA (20 µmol/L ADP)	Carriers of at least one <i>CYP2C19</i> reduced-function allele had less change in MPA
Frere <i>et al.</i> , 2009 ⁸⁵	598	ACS/P CI	<i>CYP2C19</i> *4, *5, *6, *17	600mg LD	MPA by LTA (10 µmol/L ADP) VASP-P	The <i>CYP2C19</i> *17 genotype was significantly associated with lower PRI VASP values The <i>CYP2C19</i> *17 genotype was not significantly associated with ADP-induced platelet aggregation
Shuldiner <i>et al.</i> , 2009 ⁸⁶	656	ACS/P CI	Genome-wide association study	300mg LD 75mg MD	LTA (20 µmol/L ADP)	The <i>CYP2C19</i> *2 allele was associated with a reduced clopidogrel response, accounting for 12% of the variation in platelet aggregation to ADP
Sibbling <i>et al.</i> , 2010 ⁸⁷	1,524	ACS/P CI	<i>CYP2C19</i> *17	600mg LD 75mg MD	RPA by LTA (6.4 µmol/L ADP)	The <i>CYP2C19</i> *17 allele was associated with decreased ADP-induced platelet aggregation

The importance of *CYP2C19* was further emphasized by a genome-wide association study (GWAS) that examined the marked heterogeneity in ADP- stimulated platelet aggregation in response to clopidogrel among healthy Amish subjects⁸⁸. The researchers genotyped single nucleotide polymorphisms (SNPs) across the genome of the participants covering about 400,000 single nucleotide polymorphism (SNP)s and identified a cluster of 13 SNPs within and flanking the *CYP2C18-2C19-2C9-2C8* cluster on chromosome 10q24 that was strongly associated with clopidogrel response. Fine-mapping revealed that the *CYP2C19**2 variant accounted for almost the entire associated signal.

With regard to the other loss of function *CYP2C19* genotypes such as *CYP2C19**4 and 5*, there is limited data on the pharmacokinetics and pharmacodynamics of clopidogrel owing to the rarity of their occurrence in all ethnicities⁸⁹.

CYP2C19 gain-of-function alleles

The influence of the gain-of-function allele *CYP2C19**17 on the biological response of clopidogrel has not been as extensively investigated as the *2 allele, but it has been reported in numerous studies. Discovered in early 2006, *CYP2C19**17 has an average multi-ethnic allele frequencies of ~3–21%^{76,90} and is characterized by two SNPs in the 5'-flanking region (g.-3402C > T and g.-806C > T) of the gene. The two polymorphisms are in complete linkage disequilibrium with each other. The apparently faster activity of the encoded enzyme was ascribed to the recruitment of transcription factors to the mutated g.-806C > T site⁹¹. Such increased transcriptional activity results in a more pronounced and rapid metabolism of clopidogrel.

Geisler *et al* showed a trend toward lower levels of ADP-induced platelet aggregation in carriers of the *CYP2C19**17 allele in a PCI cohort. Frere *et al.* then evaluated the impact of clopidogrel extensive metabolism in the presence of *CYP2C19**17 allelic variant in 598 patients suffering from non-ST elevation ACS⁹². The *CYP2C19**17 allele carriers exhibited the lowest vasodilator stimulated phosphoprotein phosphorylation index PRI VASP (mean \pm SD 49.7 \pm 23.7 vs. 55.9 \pm 22.8), demonstrating a better platelet response to a 600 mg loading dose of clopidogrel. Contrary to these findings, the previously mentioned GWAS study did not find any influence of *17 on platelet aggregation values in 268 patients treated with clopidogrel. However in a larger cohort of 1524 patients undergoing PCI after pretreatment with 600 mg clopidogrel by Sibbing *et al.*, *CYP2C19**17 gain-of-function allele carriers had significantly lower ADP-induced platelet aggregation⁸⁷. The authors also reported an interaction effect between the *2 and *17 alleles on platelet function, with a gradual increase from (+)*17/(-)*2 patients, who exhibited the lowest to (-)*17/(+)*2 patients, who exhibited the highest aggregation values. However, the overall effect of the *2 allele appears to have the strongest impact owing to higher platelet reactivity seen in *CYP2C19**2/*17 patients when compared to the *CYP2C19**1/*1 genotype. This may be explained by the fact that *2 represents a complete loss-of-function of the enzyme, whereas *17 only enhances existing enzyme activity.

Other genetic influences

There are other genetic factors that have less influence on the clopidogrel biological response. Although the *CYP3A4* enzyme has been shown to be central within the CYP system, data regarding its effect on clopidogrel response are conflicting^{65, 66, 93}. *CYP3A5* has also been investigated, yielding no significant demonstrable effect on clopidogrel response⁹⁴⁻⁹⁶.

ABCB1 has also been implicated in variability in clopidogrel responsiveness by affecting the oral bioavailability of clopidogrel. The *ABCB1* gene (cytogenetic located on chromosome 7 q21.12) encodes a permeability glycoprotein called MDR1 or P-glycoprotein (P-GP) that is located on the intestinal cell membrane. The plasma concentration time curve of clopidogrel and its active metabolite have been demonstrated to be significantly lower among homozygous variant allele carriers compared with hetero- or homo-zygous wild-type carriers⁹⁷.

More recently, paraoxonase-1 (*PON1*), an esterase synthesized in the liver and linked to high density lipoprotein in the blood was proposed as a more crucial enzyme than *CYP2C19* for clopidogrel metabolic activation⁹⁸. While these findings were initially exciting, several studies have since shown no influence of *PON1* in the pharmacodynamics or the pharmacokinetics of clopidogrel response⁹⁹⁻¹⁰³.

Finally, there are some SNPs of the *P2RY12* gene encoding the P2Y₁₂ ADP receptor and also the *ITGB3* gene encoding the beta subunit of glycoprotein IIb/IIIa receptor that have been studied with some showing a degree of influence on the pharmacokinetic and pharmacodynamic response to clopidogrel^{66, 104-107} and some showing none^{27, 66, 108}. Further research in this area is needed.

Genetic variants and cardiovascular outcome

There is ample evidence to suggest that genotype has a significant impact on biological response to clopidogrel but the important question is whether this has any clinical significance.

There is a significant body of research exploring this issue in patients with coronary artery disease.

CYP2C19 loss-of-function alleles

Several large-scale studies have explored the clinical ramifications of the *CYP2C19**2 and the other *CYP2C19* loss-of-function alleles genotype in populations ranging from non-CAD or low risk CAD patients to high risk ACS patients undergoing PCI(**Table 1.4**)

Table 1.4 Studies of cardiovascular outcome and *CYP2C19* loss-of-function

References	Type of Study	N	Population	Clopidogrel Dose	Primary Endpoint	OR (95% CI) Carriers versus Non-Carriers
Trenk <i>et al.</i>, 2008¹⁰⁹	Retrospective cohort	797	ACS = 27% PCI = 100%	600mg LD	Death, MI	0.58 (0.22–1.58)
Giusti <i>et al.</i>, 2009¹¹⁰	Prospective cohort	772	ACS = 66% PCI = 100%	600 mg LD 75 mg MD	Death, MI Stent thrombosis	2.73 (1.06–7.00) 2.73 (0.78–8.57)
Sibbling <i>et al.</i>, 2009¹¹¹	Prospective Randomized study	2,485	ACS = 34% PCI = 100%	600 mg LD 75 mg MD	Death, MI, stroke Stent thrombosis	1.15 (0.82–1.62) 3.83 (1.45–10.11)
Mega <i>et al.</i>, 2009⁸⁴	Post hoc analysis of RCT (TRITON)	1,459	ACS = 100% PCI = 95%	300 mg LD 75 mg MD	Death, MI, stroke Stent thrombosis	1.56 (1.06–2.28) 3.09 (1.18–8.07)
Simon <i>et al.</i>, 2009²⁷	Prospective cohort study	2,178	MI = 100% PCI = 69%	300 mg LD 5 mg MD	Death, MI, stroke	0.85 (0.64–1.12)
Shuldiner <i>et al.</i>⁸⁸	Prospective cohort study	227	ACS= NA PCI= 100%	300-600mg LD 75mg	Death, MI, Stroke, UTVR	2.38 (1.086-5.205)
Collet <i>et al.</i>, 2009⁸⁰	Prospective cohort study	259	MI = 100% PCI = 73%	MD 75 mg	Death, MI, revasc Stent thrombosis	5.03 (1.89–13.36) 5.95 (1.72–20.60)
Paré <i>et al.</i>, 2010⁷⁵	Post hoc analysis of RCT (CURE)	2,530	ACS = 100% PCI = 15.5%	300 mg LD	Death, MI, stroke	0.83 (0.60–1.15)
Wallentin <i>et al.</i>¹¹²	Post hoc analysis of RCT (PLATO)	4904	ACS = 100% PCI = 61%	300–600 mg LD	Death, MI, stroke Stent thrombosis	1.15 (0.94–1.41) 1.49 (0.86–2.57)
Sawada <i>et al.</i>, 2010¹¹³	Prospective cohort	100	ACS = 9% PCI = 100%	75 mg MD	Death, MI	1.40 (0.19–10.36)
Bouman <i>et al.</i>, 2010⁹⁹	Prospective cohort	1,982	ACS = 100% PCI = 100%	600 mg LD	Death, MI, stroke Stent thrombosis	1.17 (0.88–1.57) 1.10 (0.59–2.05)
Tiroch <i>et al.</i>, 2010¹¹⁴	Retrospective cohort	928	ACS = 100% PCI = 90%	600 mg LD	Death, MI Stent thrombosis	0.94 (0.30–0.98) 1.18 (0.30–4.59)
Harmsze <i>et al.</i>, 2011¹¹⁵	Retrospective cohort	606	ACS = 77.3% in cases PCI = 100%	Unknown	Stent thrombosis	1.1 (1–2.6)
Oh <i>et al.</i>¹¹⁶	Retrospective Cohort	1011	ACS = 100% PCI = 100%	300mg or 600mg	Death, MI, Stent Thrombosis	2.58 (1.22-5.45)

MD, maintenance dose; LD, loading dose; PCI, percutaneous coronary intervention; ACS, acute coronary syndromes

High-risk ACS/PCI patients

The *CYP2C19* genotype appears to have the greatest influence in patients presenting with ACS that have high risk factors for recurrent ischemic events that undergo stent implantation. In the AFIJ study, our group demonstrated that in 259 prospectively selected patients of less than 45 years of age that had survived a myocardial infarction, there was a strong relationship between the presence of the *CYP2C19**2 allelic variant and recurrent thrombotic coronary events⁸⁹. Compared to wild-type *CYP2C19* homozygotes (72%), patients with the *CYP2C19**2 allele (28%) had a 3.7-fold increased risk of cardiovascular death, nonfatal MI, or urgent revascularization at one year, mainly due to a significant increase in the rate of non-fatal MI. This increased risk was demonstrated soon after clopidogrel treatment was initiated and persisted throughout the study period. The risk of stent thrombosis was also significantly increased 6-fold in carriers. A multivariable analysis suggested that the *CYP2C19* genotype was the only independent predictor of cardiovascular events in this population (HR 4.04 [1.81–9.02], $p = 0.0006$) suggesting a substantial effect of the *CYP2C19**2 genetic variant on the prognosis of young patients receiving clopidogrel treatment after MI.

These findings were supported by studies in the general ACS cohorts undergoing PCI. In the genetic substudy of the TRITON–TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction 38) trial, the researchers tested the association between functional genetic variants in cytochrome P450 genes and cardiovascular outcomes among 1477 clopidogrel-treated subjects^{68, 84}. *CYP2C19* reduced function allele carriers had a more than 50% increased risk in the primary end point of death from cardiovascular causes, MI, or stroke compared with noncarriers, as well as a threefold increase in the risk of stent thrombosis. This association between loss-of-function genotype and poor cardiovascular outcome was also shown in the

FAST-MI (French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction) study²⁷.

Low risk ACS and non-CAD patients

Numerous studies in low risk patients treated with clopidogrel have shown a far less convincing association between *CYP2C19* status and cardiovascular outcomes. One such study was a genetic analysis of two large randomized trials that compared clopidogrel to placebo, CURE and ACTIVE-A, in patients with unstable angina and in those with atrial fibrillation (AF) who were unsuitable for treatment with warfarin, respectively¹¹⁷. The effect of clopidogrel on the primary outcome was similar in patients with loss-of-function alleles (heterozygous or homozygous) and in non-carriers. Among the 1156 genotyped AF patients in the ACTIVE A trial, there was no evidence of an interaction with respect to either efficacy or bleeding between the study treatment and the metaboliser phenotype, loss-of-function carrier status, or gain-of-function carrier status. The possible explanation for lack of increased risk in patients on clopidogrel with the *CYP2C19**2 allele may be that, not only were they in a low risk cohort, but in the CURE study, only 14.5% underwent PCI and thus the lack of use of stents may account for the neutral outcomes. In comparison, in previous studies the majority of patients had PCI and the endpoint most impacted was stent thrombosis. Another trial reporting a neutral effect of the *CYP2C19* allele was a genetic substudy of the CHARISMA trial¹¹⁸. Again the analysis was in a stable, non-PCI population.

Meta-analyses of loss-of-function alleles

Several meta-analyses have been recently published examining the association between *CYP2C19* loss-of-function variants and cardiovascular outcomes (Table 1.5). Due to differences in design and end points assessed, conflicting conclusions were reached which has lead to a considerable amount of debate on the issue. Three successive early meta-analyses were performed pooling the results of the studies of high-risk ACS patients undergoing PCI; all demonstrated the significant influence of *CYP2C19* loss-of-function genotype on cardiovascular outcome.

Three more recent meta-analyses showed less of an association between *CYP2C19* loss-of-function alleles and ischemic events when small study bias was considered and studies in low risk, non PCI populations were included^{112, 117, 119}. After adjustments for small study effect bias and replication diversity, Bauer *et al.* reported a reduced effect on MACE for 12 studies included¹¹⁹. Another meta-analysis published around the same time also demonstrated the effect of small study bias when reporting cardiovascular events with substantial heterogeneity observed between studies¹²⁰. The pooled HR was higher among studies with a sample size <500 patients (HR =3.55; 95% CI 1.66 to 7.56) and lower among studies with a sample size ≥500 (HR =1.06; 95% CI 0.89 to 1.26). However, even with this adjustment, *CYP2C19**2 was still associated with over a 2-fold increased risk of a stent thrombosis.

Table 1.5 Meta-analyses of CYP2C19 loss-of-function genotype and cardiovascular outcome.

Author	Year	Population	CYP2C19 allelic variants	MACE definition	Follow up	Adjustment for small study bias	Carriers versus Non-Carriers OR/HR/RR (95% CI)	MACE Stent Thrombosis
Huilot <i>et al.</i> ^{1,21}	2010	ACS/PCI	*2	Death, MI, Stroke, Urgent revascularization	Longest available	No	OR 1.29 (1.12-1.49)	OR 3.45 (2.14-5.57)
Mega <i>et al.</i> ^{1,22}	2010	ACS:54.5% PCI: 91.3%	*2, *3,*4,*5, *8	CV Death, MI, Stroke.	30 day 31 days to end of follow up	No	HR 1.57 (1.13-2.16)	HR 2.81 (1.81-4.37)
Sofi <i>et al.</i> ^{1,23}	2010	ACS/PCI	*2	CV Death, MI, Stroke, UA, ST	Longest	No	RR 1.96 (1.14-3.37)	RR 3.82 (2.23-6.54)
Bauer <i>et al.</i> ^{1,19}	2011	ACS/PCI	*2, *3,*4,*5, *6,*7,*8,*17	CV Death, MI, Stroke	Longest	Yes	OR 1.11 (0.89-1.39)	OR 1.77 (1.31-2.40)
Zabalza <i>et al.</i> ^{1,24}	2012	ACS/PCI/ Non CAD	*2, *3,*4,*5, *17	CV and all cause Death, MI, Stroke, Recurrent Ischemia,	Longest	Yes	HR 1.23 (0.97-1.55)	HR 2.24 (1.52-3.30)
Holmes <i>et al.</i> ^{1,25}	2011	ACS/PCI/ Non CD	*2, *3,*4,*5, *6,*7,*8,*17	All-cause mortality, fatal and nonfatal CHD, Stroke, stent thrombosis, TVR and hospitalization for ACS	Longest	Yes	HR 1.18 (1.09-1.28)	HR 1.75 (1.50-2.03)

ACS, acute coronary syndrome; PCI, Percutaneous Coronary Intervention; UA: Unstable angina; CV, Cardiovascular; ST, Stent Thrombosis; TVR, Target vessel revascularization, CHD; Coronary Heart Disease; MI, Myocardial Infarction; OR, Odds Ratio; HR, Hazard Ratio; RR, Relative Risk.

The most recent and controversial meta-analysis to date by Holmes *et al.* included 32 studies that assessed the *CYP2C19* genotype and its association with clopidogrel response and cardiovascular events¹²⁵. Again, when the authors considered small study bias and restricted the analysis to studies with >200 events, the point estimate was attenuated to non significance, RR 0.97 (95% CI 0.86-1.09). The conclusion reached was that there was no clinically important association of genotype with cardiovascular outcomes.

There were numerous criticisms of this meta-analysis. Firstly, many of the studies in the analysis included either non-coronary patients or low risk coronary patients that were medically treated and not with PCI; these are patients where the *CYP2C19* loss-of-function alleles are already known not to have an impact on cardiovascular events. At the same time, trials that had stent thrombosis as the primary end point were not included in the analysis, thus excluding the extensive body of research that demonstrates the strongest link between genotype and this dramatic event. Secondly, the outcomes assessed in this meta-analysis were not pertinent to clopidogrel and the cardiovascular adverse events it is known to impact. Softer outcomes such as rehospitalization for ACS and target vessel revascularization were incorporated. Finally, the authors conclusion that *CYP2C19* genotype did not have an overall clinically significant impact on outcome was directly contradicted by the data that demonstrated a significant 37% increase in fatal and nonfatal MI, RR 1.37 (95% CI 1.13-1.65), and a 1.5 to twofold increase in stent thrombosis in patients with the loss-of-function variant.

CYP2C19*17 Gain of function allele

There are several clinical outcome studies which examine the potential double edged sword effect of the *CYP2C19*17* variant, namely a reduced ischemic event rate due to improved metabolism of clopidogrel, balanced by increased bleeding risk. Sibbing *et al.* identified allele carriage as an independent risk factor for bleeding in patients undergoing PCI (odds ratio for carriers of at least 1 allele vs. noncarriers 1.85; 95% CI 1.19–2.86; $P = 0.006$). In a similar elective coronary stenting cohort, Harmsze *et al.* reported a 2.7-fold increase in bleeding events¹²⁶ and in the PLATO trial, higher bleeding events were reported in ACS presenting carriers of the gain-of-function allele¹²⁷. However, this association is not consistent as was demonstrated in the CURE, ACTIVE-A, FAST-MI and the TRITON-TIMI 38 trials where the *CYP2C19*17* allele had no influence on bleeding events^{27, 68, 117}. Furthermore, it is uncertain whether the *CYP2C19*17* allele is a protective factor against ischemic events. The CURE trial and a study by Tiroch *et al.* showed greater benefit of clopidogrel therapy among *CYP2C19*17* carriers, an effect not mirrored in other studies^{27, 87}. When the data were pooled in a meta-analysis by Zabalta *et al.*, the gain-of-function allele was associated with a higher risk of major bleeding (HR =1.26; 95% CI 1.05 to 1.50) and a lower risk of cardiovascular events (HR =0.75; 95% CI 0.66 to 0.87). However, there were too many biases in the study selection and variation in the bleeding endpoint definition, particularly with regard to early versus late events, whereby early or acute bleeding is influenced greatly by other confounding factors such as concomitant use of other antithrombotics. In summary, further studies are needed to clarify the clinical impact of this allele⁷⁶.

ABCB1

The clinical effects of this variant in patients treated with clopidogrel are conflicting, even though they have been evaluated in several large studies. The FAST-MI registry showed that

carriers of the *ABCB1* TT and CT genotypes at Cys3435Thr had a higher risk of cardiovascular events than those with the CC genotype ²⁷. Specifically, the TT genotype patients demonstrated a 72% increased risk of death, nonfatal MI or stroke at 1 year compared with the CC genotype group. However, in a subanalysis of patients undergoing coronary angioplasty, the *ABCB1* polymorphism was not an independent predictor of outcome. The TRITON-TIMI 38 pharmacogenetic analysis also addressed this question and found that only TT homozygotes have a higher risk of cardiovascular events probably due to less platelet inhibition ¹²⁸. However, in contrast, Wallentin *et al.* reported the reverse in the PLATO genetic substudy, where patients homozygous for CC (high expression group) had numerically higher rates of ischemic events than patients with other forms of the *ABCB1* polymorphism. In view of this uncertainty of effect, combining the *ABCB1* polymorphism with the *CYP2C19* genotypes might improve the ability to predict poor clopidogrel response and ischemic events as some studies suggest ^{27, 97,}

129

Other gene variants

Similar to the pharmacodynamic and pharmacokinetic studies that contradicted the findings of Bouman *et al.* with respect to *PON-1*, there are several clinical outcome studies which failed to replicate the findings of the clinical studies performed by this group ^{100, 102, 130}. Overall, the evidence demonstrates that PON1 has likely no major role in clopidogrel response.

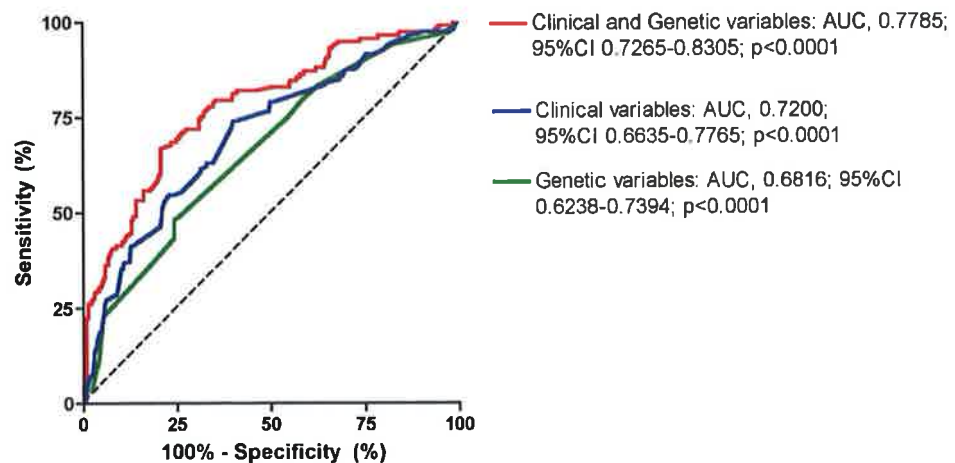
P2RY12 and *ITGB3* have been extensively assessed in clinical studies, but no specific variant has been consistently associated with increased cardiovascular event rate ^{27, 66, 85, 108}. In fact, the ONASSIST study found the PLA2 isoform of the *ITGB3* gene less frequent in a group of patients with stent thrombosis than controls whereas previous studies suggested a trend toward increased cardiovascular event rate

Genotype and stent thrombosis

Overall genotype appears to have the most significant impact on the incidence of stent thrombosis. Almost all patients with stent thrombosis will either die or have a heart attack as a result so it is vital to prevent these events. The risk of a stent thrombosis with even one loss-of-function variant is high, consistently two- to threefold across all of the studies that have focused on this end point. Giusti *et al.*, in the genetic analysis of the RECLOSE study, specifically evaluated the role of the *CYP2C19**2 polymorphism on the occurrence of stent thrombosis or the composite end point of stent thrombosis and cardiac mortality within a 6-month follow-up in 772 patients undergoing percutaneous coronary interventions with drug-eluting stent implantation on dual-antiplatelet treatment¹¹⁰. Patients carrying at least one variant *CYP2C19**2 allele were at increased risk of stent thrombosis (OR 3.43, 95% CI 1.01-12.78, $P = 0.047$) or the composite of stent thrombosis and cardiac mortality (OR 2.70, 95% CI 1.00-8.42, $P = 0.049$). The RECLOSE study also showed that the *CYP2C19**2 polymorphism was an independent risk factor for stent thrombosis or the composite end point of cardiac mortality and stent thrombosis. disease, bifurcation lesion, myocardial infarction, total stent length, left ventricular ejection fraction). These data were further supported by a case-control study by Sibbing *et al.* that assessed 2,485 consecutive patients undergoing PCI who were all pretreated with a clopidogrel 600-mg loading dose demonstrating that *CYP2C19**2 carriers had a significantly higher cumulative 30-day incidence of stent thrombosis compared with wild-type *CYP2C19* carriers (HR 3.81 95% CI 1.45–10.02, $P = 0.007$). There was also a gene-dose effect with the risk of stent thrombosis highest in *CYP2C19* *2/*2 homozygotes¹³¹.

More recently, Cayla *et al.* clinical, angiographic and genetic variants independently associated with early stent thrombosis in the aforementioned ONASSIST study which included 123 patients undergoing PCI who had definite stent thrombosis and DNA samples available and 246

matched controls without stent thrombosis¹²⁹. The accuracy of early stent thrombosis prediction was measured in 23 genetic variants of 15 different genes. *CYP2C19*, *ABCB1* and *ITGB3PLA2* were significant determinants. Patients who had an adverse event were twice as likely to carry the loss-of-function *CYP2C19**2 allele and seven times more likely to carry the *2/*2 homozygous allele. Risk models were created using several clinical, angiographic, genetic factors to identify patients at risk for stent thrombosis. Combining the clinical and genetic models together provided the greatest power to discriminate stent-thrombosis cases when compared with the clinical-only model (area under the curve 0.78 vs 0.73; $p=0.004$) (Figure 1.3)



Cayla G et al JAMA. 2011 Oct 26;306(16):1765-74.

Figure 1.3 Receiver Operator Character Curve for association with Early Stent Thrombosis in the ONASSIST registry.

The clinical model is based on nongenetic factors (type C lesion, proton pump inhibitor use, diabetes mellitus, left ventricular dysfunction <40%, percutaneous coronary intervention in acute setting, and clopidogrel loading dose), with sensitivity of 60% and specificity of 70%, for a

positive likelihood ratio of 2.1 (area under the curve, 0.73; 95% CI, 0.67-0.78; $P < .001$). The genetic model contains *CYP2C19* metabolic status, *ABCB1* 3435 TT genotype, and *ITGB3* PLA2 polymorphism, with a sensitivity of 48% and specificity of 78%, for a positive likelihood ratio of 2.0 (area under the curve, 0.68; 95% CI, 0.62-0.74; $P < .001$). The combined model contains all clinical, angiographic, and genetic predictors, with a sensitivity of 67% and specificity of 79%, for a positive likelihood ratio of 3.4 (area under the curve, 0.78; 95% CI, 0.73-0.83; $P < .001$). The diagonal dotted line is the expected receiver operating characteristic curve for a totally random classifier.

Genetic Testing in Routine Practice

The use of genetic profile testing to guide antiplatelet therapy appears to be an appealing strategy although there has been considerable debate about this potential form of 'tailored therapy' with divided opinion due to unresolved key questions such as: Should we perform this testing routinely in all patients on clopidogrel? Are the forms of testing available accurate? How should the results be interpreted and acted upon and is there evidence to support the safety and efficacy of tailored strategies?

Who should we genotype?

The US FDA added a boxed warning to the clopidogrel label which suggests that individuals with poor metaboliser genotypes (i.e., *CYP2C19**2 and *3 carriers) may be at increased risk for adverse cardiovascular outcomes and should consider other antiplatelet medications or alternative dosing strategies²⁵ Recently, the American College of Cardiology Foundation/American Heart Association outlined possible actions to be taken by clinicians in response to the FDA's boxed warning¹³². Routine genetic testing was not recommended due to the lack of prospective randomized clinical outcome trials of genotype-directed antiplatelet therapy, leaving the decision with the physician acting on an individual patient basis. So in light of the current trial data, in whom should we perform the testing? The most definitive studies showing a response between genotype and clopidogrel response have been performed in the ACS population and there is no evidence to support genetic testing in non-ACS patients such as

those with atrial fibrillation, peripheral artery disease, chronic stable angina and stroke. The strongest association between *CYP2C19* genotype and adverse cardiovascular outcomes appears to be in ACS patients that undergo stenting. This is highlighted by the interaction between genotyping and treatment effect that is only observed in patients with a stent. This effect is more pronounced in patients with other factors associated with recurrent ischemic events such as the complex CAD, diabetes, age, renal dysfunction and heart failure. It seems reasonable, therefore, that these patients be selected for genetic testing.

A bedside approach

For genetic testing to be feasible, it needs to be accurate and rapid so that the information can be acted on in the urgent setting and appropriate therapy commenced immediately. Numerous laboratory based genotyping assays are currently available and are straightforward, highly accurate and reproducible. These tests differ in their genotyping methodology, specimen required (e.g. whole blood, saliva, buccal swab) and availability. Most of the tests cover the most common alleles (*1,*2,*3, *17). However these tests typically take 3-5 days to perform. As a result, point-of-care rapid genotyping platforms have been developed that are currently undergoing validation in clinical trials^{133, 134}. These 'bed-side' tests can give a result in as little as one hour which allows for immediate modification of treatment in the acute setting.

Interpretation of the results

It is important to note that according to previous papers, *CYP2C19* loss-of-function polymorphism is estimated to explain only 5 to 12% of the observed variation in pharmacodynamic response to clopidogrel^{88, 135}. It is one of many factors, both clinical and genetic, that influence the antiplatelet response to clopidogrel and thus should not be used in isolation to distinguish clopidogrel responders from non-responders. The *CYP2C19* loss-of-function polymorphism should therefore be seen as a risk factor for poor response to

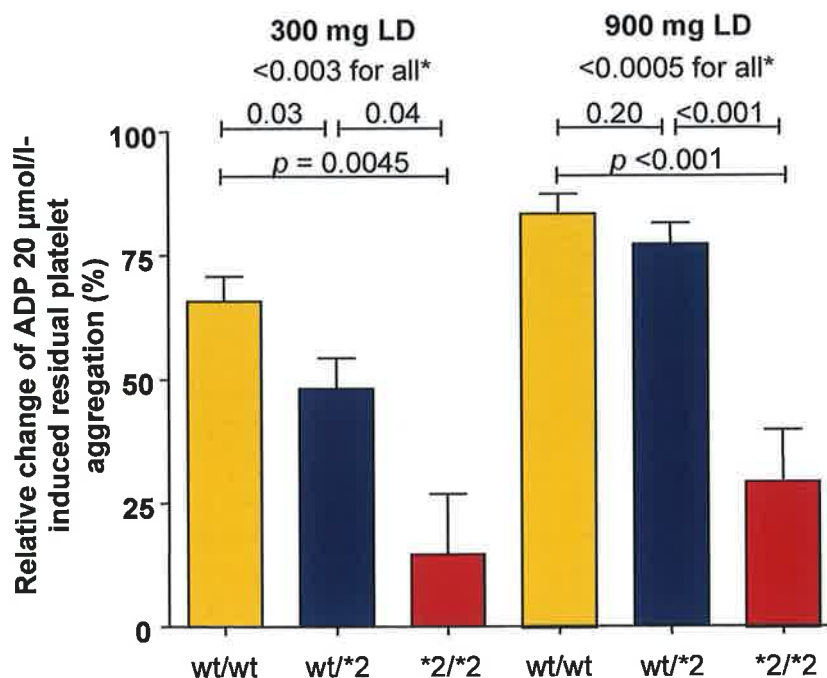
clopidogrel and subsequent recurrent cardiovascular events. Most of the clinical outcome studies have shown a gene dose effect, whereby homozygotes (poor metabolisers) are at higher risk than heterozygotes (intermediate metabolisers), who in turn have higher risk than non-carriers (extensive metabolisers)^{121, 122}. To further improve the efficacy of *CYP2C19* genetic testing in identifying high-risk patients, it has been suggested to combine it with platelet function testing^{136, 137}. Platelet function assays determine high on-treatment platelet reactivity and potentially capture the complex phenotype of platelet reactivity. Finally, the concept of a global clinico-genetic model has been proposed as a practical aid to both the interpretation of genotype in the clinical setting and risk stratification of the patient. This would integrate *CYP2C19* profile and other gene candidates with known clinical factors associated with recurrent events¹³⁸. This strategy requires further validation but may represent a realistic solution to this complex issue.

Possible treatment strategies

There are two main proposed options when considering modifying therapy in poor metaboliser of clopidogrel: 1) Dose escalation of clopidogrel or 2) switching to novel P2Y₁₂ receptor antagonists.

Increasing clopidogrel dose

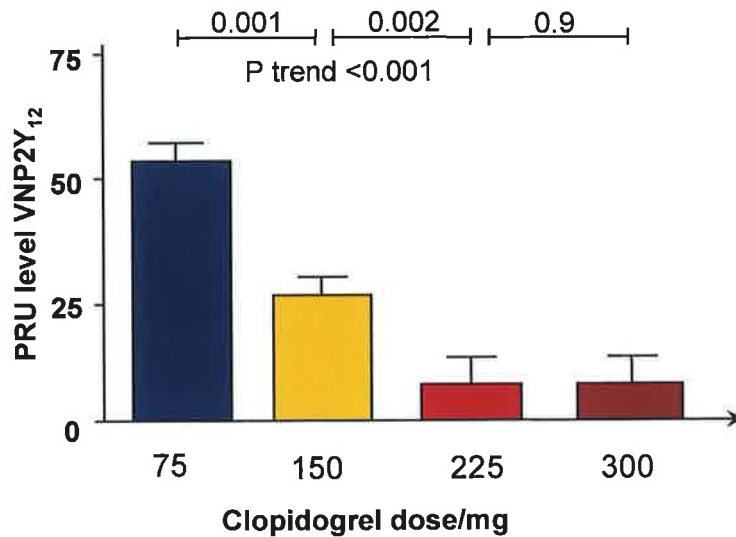
Pharmacodynamic studies have evaluated whether an increased loading dose of clopidogrel can overcome inadequate antiplatelet response in *CYP2C19**2 allele carriers¹³³. In a study by our group, tripling the loading dose (900mg) was effective in overcoming clopidogrel resistance in *1/*2 heterozygotes but had no effect in *2/*2 homozygotes⁷⁹ (**Figure 1.4**).



Collet J-P, et al. *JACC Interv.* 2011;4:392-402

Figure 1.4 The CLOVIS-2 study demonstrating the effect of increasing loading and maintenance clopidogrel dose on platelet reactivity in CYP2C19*2 carriers.

Conversely, studies examining the efficacy of escalation of the maintenance dose from 75mg/day to 150mg/day in carriers of the *CYP2C19**2 genotype have been disappointing with some showing increased platelet inhibition in heterozygotes^{133, 134, 139} and others showing very minimal change¹⁴⁰⁻¹⁴⁴. It is likely that the 150mg maintenance dose is not high enough, as shown in the ELEVATE-TIMI 56 trial where increasing the maintenance dose of clopidogrel to 225mg in *CYP2C19* *1/*2 heterozygotes was needed to achieve similar platelet reactivity to that seen with the standard 75mg dose in non-carriers¹⁴⁵ (**Figure 1.5**). Each 75mg increase in clopidogrel dose led to an 8-9% absolute reduction in platelet reactivity indicating that with the correct dosing, resistance to clopidogrel in heterozygotes can be treated effectively.



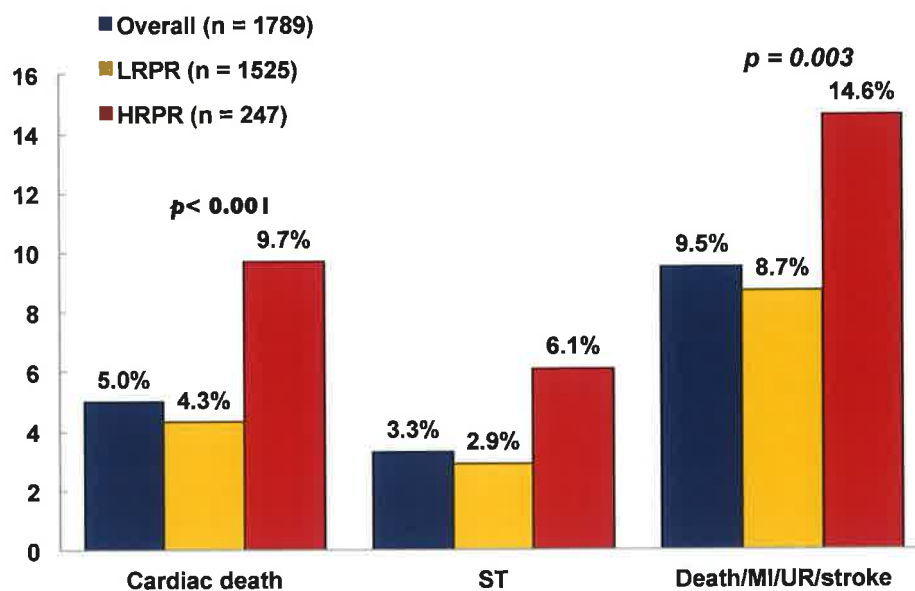
Mega et al. JAMA. 2011;306(20):2221-2228

Figure 1.5 Findings from the ELEVATE-TIMI 56 trial.

Increasing the maintenance dose of clopidogrel to 225mg in CYP2C19 *1/*2 heterozygotes was sufficient to achieve similar platelet reactivity to that seen with the standard 75mg dose in non-carriers. PRU, platelet reaction units; VNP2Y₁₂, VerifyNow point-of-care platelet function test.

There are, however, very little clinical outcome data to support the dose escalation strategy. In the only published randomized trial to date, the Gauging Responsiveness with A VerifyNow assay—Impact on Thrombosis And Safety [GRAVITAS] study, a 150mg dose of clopidogrel did not reduce the composite end-point of cardiovascular death/MI/stent thrombosis at six month follow up when compared with a 75mg dose in patient who had high on treatment platelet reactivity¹⁴⁴. However, the population selected was at very low risk, with 80% having stable CAD resulting in an overall low event rate and, as mentioned, the 150mg dose resulted in only modest antiplatelet effects. In the genetic substudy, Genotype Information and functional

Testing (GIFT)¹⁴⁶, the use of 150mg of clopidogrel was not sufficient at overcoming high on-treatment platelet activity in both homozygote and heterozygote *CYP2C19*2* carriers. Although platelet function analysis and not genotype was used to guide therapy, Parodi *et al.* recently demonstrated in the RECLOSE 2 trial that high on-treatment platelet reactivity was associated with poor long term cardiovascular outcome including a higher mortality but that dose escalation to 150-300mg of clopidogrel or switching to ticlopidine did not have an impact on the event rate¹⁴⁷ (Figure 1.6).



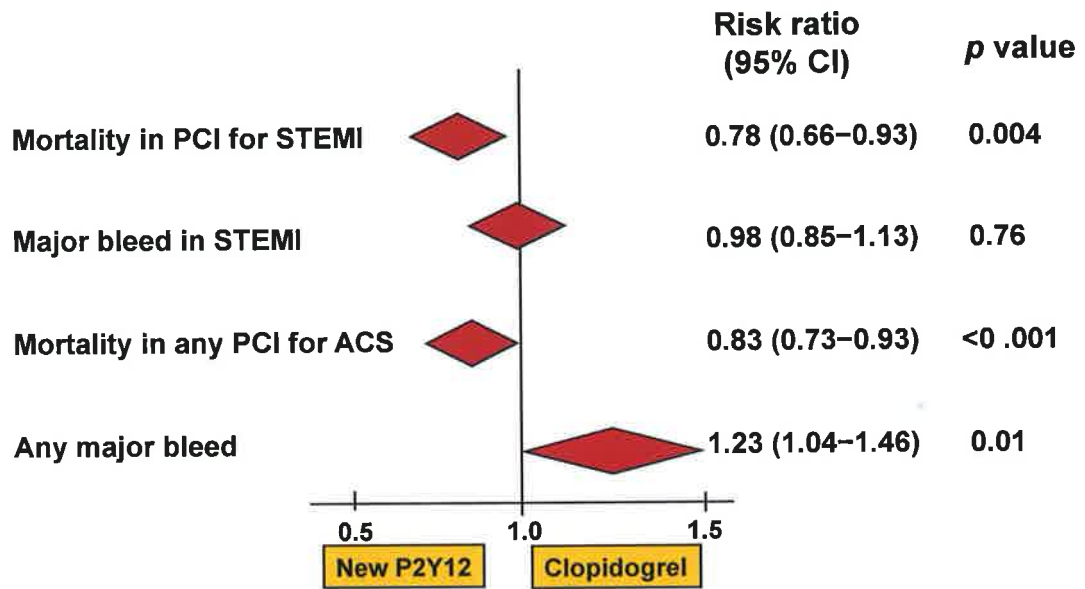
Parodi G, et al. *JAMA*. 2011;306:1215-1223

Figure 1.6 The RECLOSE-2 study.

Impact on mortality of persistent high residual platelet reactivity (HRPR) despite increasing clopidogrel dose. Non-responders defined as >70% aggregation by LTA 12 hours after 600 mg plavix load LRPR, low residual platelet reactivity; UR, Urgent Revascularisation.

Switching to newer antagonists P2Y₁₂

Prasugrel and ticagrelor are newer P2Y₁₂ platelet receptor inhibitors that induce more rapid, potent and predictable platelet inhibition than clopidogrel and whose antiplatelet activity does not depend on *CYP2C19*. Prasugrel is a third generation thienopyridine prodrug that is hydrolyzed by esterases and then transformed into its active metabolite in a single CYP-dependent oxidative step. The CYP enzymes involved are *CYP3A4* and *CYP2B6* with only minor contribution of the *CYP2C9* and *CYP2C19*^{78, 148}. In the TRITON-TIMI 38 study, the common functional CYP genetic variants including *CYP2C19**2 had no effect on platelet inhibition or clinical outcome in patients taking prasugrel¹²⁸. This was also shown in a stable CAD population with *CYP2C19* variants¹⁴⁹. Ticagrelor is a direct acting, reversible P2Y₁₂ inhibitor and also seems to be unaffected by genetic variation in the CYP isoenzymes and *ABCB1*¹¹². In a pooled analysis of these newly developed P2Y₁₂ receptor antagonists, they were shown to decrease mortality after PCI compared with clopidogrel¹⁵⁰. The risk/benefit ratio is particularly favorable in PCI for patients with STEMI (**Figure 1.7**). The use of prasugrel is associated with increased bleeding and is contraindicated in some patients and is more expensive than clopidogrel. Ticagrelor is becoming more widely available with no apparent excess in major bleeding in comparison to clopidogrel¹⁵¹. Due to the demonstrated overall clinical benefit of these drugs, prasugrel and ticagrelor are being used as first line agents in patients presenting with STEMI and moderate to high risk non STE ACS. Furthermore, the 2011 European Society of Cardiology Guidelines recommend either of the two drugs in preference to clopidogrel in patients presenting with NSTEMI unless there were contraindications to their use¹⁵².

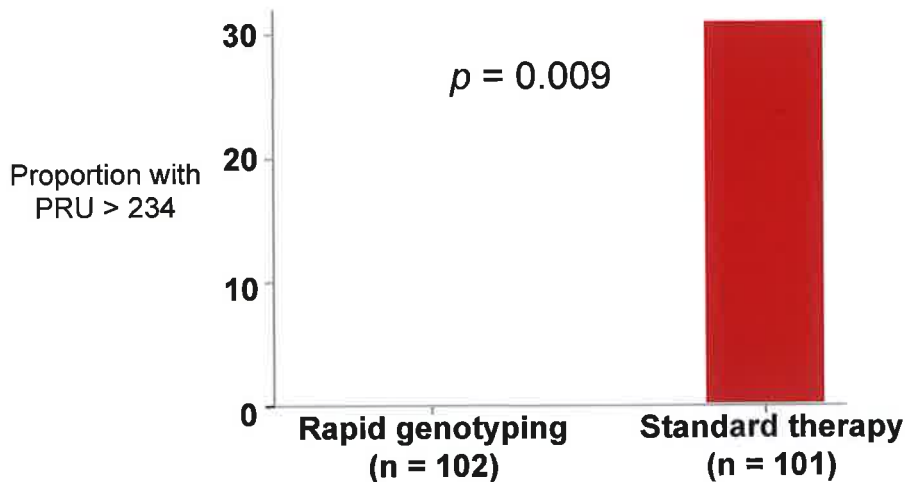


Bellemain-Appaix A, et al. *J Am Coll Cardiol* 2010;56(19):1542-51

Figure 1.7 Class effect of new P2Y₁₂ antagonists of ne P2Y₁₂ receptor antagonists as compared with clopidogrel

The pharmacodynamic efficacy and clinical safety of switching therapy is demonstrated in two studies of CHD patients without genetic information. In the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation–Thrombolysis in Myocardial Infarction 44 Trial (PRINCIPLE-TIMI 44) trial, stable patients post PCI were switched from clopidogrel to prasugrel resulting in higher inhibition of platelet aggregation response to 20 $\mu\text{mol/L}$ ADP¹⁵³. A similar increased platelet inhibition was demonstrated by Angiolillo *et al.* in a group of patients switched from clopidogrel to prasugrel in the maintenance phase after an acute coronary syndrome¹⁵⁴.

However, there are limited data regarding a switching strategy based on genotype. In a pharmacodynamic study with a prospective randomized single-blind crossover design, Alexopoulos *et al.* examined the antiplatelet effects of prasugrel versus a maintenance dose of 150mg clopidogrel in patients with HPR after PCI. It was observed that prasugrel was more effective than high doses of clopidogrel in reducing platelet reactivity in *CYP2C19*2* carriers¹⁵⁵. In the Reassessment of Antiplatelet Therapy Using an Individualized Strategy Based on Genetic Evaluation (RAPID GENE) study an ACS and stable angina population undergoing PCI were randomized to rapid genotyping (<1hour) or standard care¹⁵⁶. *CYP2C19*2* carriers received prasugrel and non-carriers received clopidogrel compared to just clopidogrel in the standard management arm. The tailored treatment arm was shown to have decreased likelihood of high platelet reactivity than the standard arm (Figure 1.8).



Roberts et al: Lancet 2012; DOI:10.1016/S0140-6736(12)60161-5

Figure 1.8 : Findings from the ReAssessment of Anti-Platelet Therapy Using an InDividualized Strategy Based on GENetic Evaluation (RAPID GENE) study. High-on treatment platelet reactivity as measured by VerifyNow was almost eliminated by point-of-care rapid genotyping.

Overall, the current literature would support the use of a novel thienopyridines in *CYP2C19* *2/*2 homozygotes. For *CYP2C19* *1/*2 heterozygotes, it is less clear whether increasing clopidogrel dose or switching to a novel thienopyridine might be the best option. At the other end of the spectrum, patients with the *CYP2C19* extensive or rapid metaboliser phenotypes (i.e. *1/*1, *1/*17, *17/*17) might be best suited to a standard clopidogrel dose on the basis of reduced ischemic events in this group (Figure 1.9).

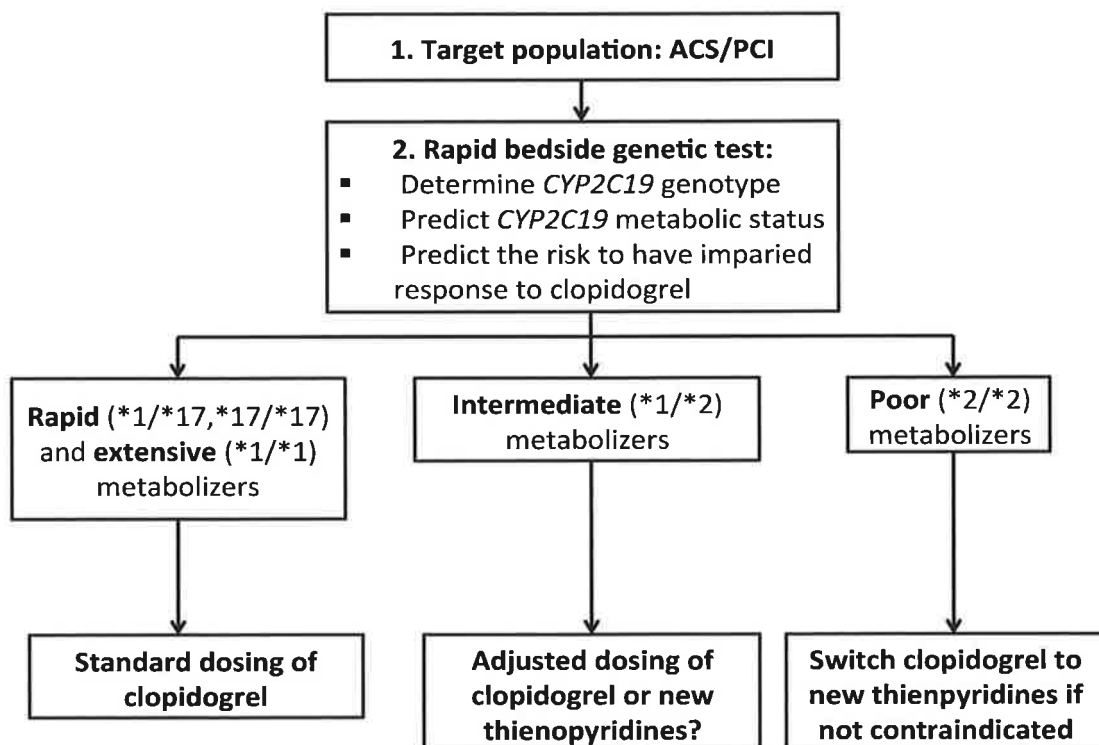


Figure 1.9 An algorithm for the target population of ACS/PCI patients for optimizing clopidogrel therapy and dosage with respect to *CYP2C19* genotype.

The presence of *17 allele could potentially assist in an overall bleeding risk evaluation of patients and thus help guide P2Y₁₂ therapy to minimize bleeding events. For example, *17 carriers that are already on prasugrel, switching to clopidogrel therapy in the maintenance

phase post MI could be considered. For *17 carriers on clopidogrel, it is unknown whether new P2Y12 receptor antagonists are a better option due to a more attractive net clinical benefit. Further studies to examine the clinical efficacy of these strategies are needed.

There are at least 2 ongoing trials that will attempt assess clinical outcome with genotype guided therapy. GIANT is an observational study in ST-segment elevation myocardial infarction treated with PCI where *CYP2C19* testing will be performed after PCI and treatment modified according to the decision of the treating physician.¹⁵⁷ The effect on clinical outcomes will be determined after 1 year of treatment. Researchers in the GeCCO observational study will assess the effectiveness of clopidogrel compared to prasugrel, in patients with ACS undergoing PCI who are *CYP2C19* extensive metabolisers¹⁵⁸. The only other randomized study on the subject of guided therapy, the Thrombocyte Activity Reassessment and GENoTyping for PCI (TARGET PCI) study has been suspended¹⁵⁹. It will be a significant undertaking to complete an adequately powered randomized study on this issue. However, clinical data from the above mentioned studies should help further clarify the issues of concern regarding genotype guided antiplatelet therapy.

P2Y12 receptor antagonists and Bleeding

The clinical benefit of antiplatelet therapy is balanced by the significant morbidity and mortality associated with bleeding events. During treatment for ACS and PCI there is an incidence of major non-CABG related bleeding between 1-5% and minor bleeding at 2-10%^{160, 161 162}. There is wide variability of the incidence due to differences in definition, timing of reporting and concomitant therapies¹⁶³. However, there is a strong, consistent and dose related association between bleeding and morbidity and mortality. This was demonstrated by

Eikelboom et al. in an analysis of patient data from >30,000 patients presenting with acute coronary syndrome from 3 large studies: the Organization to Assess Ischemic Syndromes (OASIS) Registry, OASIS-2, and the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) randomized trial¹⁶⁴. Major bleeding was associated with a 5-fold increase in the risk of death with approximately 1 in 10 patients who developed major bleeding dead at 30 days.(Figure1.10)

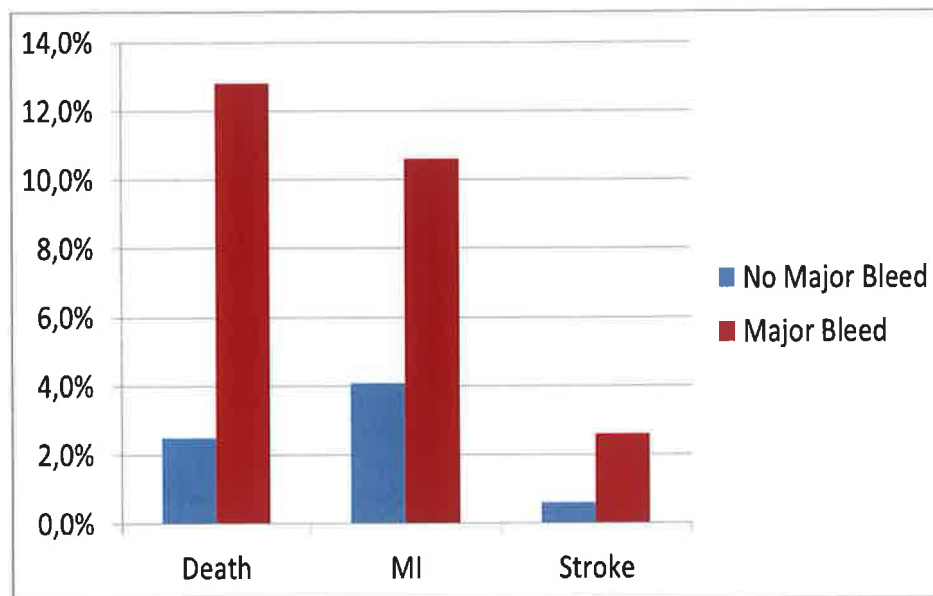


Figure 1.10 Increased morbidity and mortality associated with bleeding events

Furthermore, there appeared to be a dose relationship between severity of bleeding and death. The impact of bleeding appears to be worse in certain subgroups such as the elderly¹⁶⁵. Whether this is direct cause and effect or a reflection that the propensity to bleed is an overall indicator of risk of mortality is unknown.

The more consistent effects of the new P2Y12 have resulted in improved ischaemic benefit but this is counterbalanced by higher rates of bleeding. When compared to clopidogrel, both prasugrel and ticagrelor showed a significant 0.6% absolute excess of TIMI major bleeding not related to CABG surgery. Converging data have shown a relationship between high response to antiplatelet agents or high on treatment platelet inhibition (HPI) and increased risk of bleeding events^{166,167}. The TRITON TIMI 38 pharmacokinetics substudy has provided important information regarding the reasons for the increase in bleeding with the finding of a clear relationship between exposure to the prasugrel active metabolite and TIMI major/minor bleeding¹⁶⁸.

CABG related bleeding

There is also an augmented risk of CABG related bleeding on DAPT with increased blood product requirement and again an association with morbidity and mortality¹⁶⁹. While current practice guidelines advocate the DAPT after an ACS, there is an emphasis on caution in individuals for planned CABG¹⁷⁰. The advocated treatment is to discontinue P2Y12 therapy with adequate washout before surgery as clopidogrel and prasugrel antagonize the ADP receptor irreversibly. In a recent study examining the issue, >75% of the patients returned to baseline reactivity by washout day 7 for prasugrel and day 5 for clopidogrel. Ticagrelor and the newer agents cangrelor and elinogrel have the benefit of being reversible and have shorter offset of action¹⁷¹. Indeed ticagrelor was associated with a significantly lower risk of any major bleeding (OR 1.43; 95% CI 1.10-1.85; p=0.007) and major bleeding associated with CABG (OR 4.30; 95% CI 1.73-10.6; p=0.002) compared with prasugrel¹⁷². These differences have been assigned to the difference in the pharmacokinetic profile and platelet recovery after interruption of ticagrelor¹⁷³.

Due to the recommended caution many patients have their surgery delayed and/or are undertreated to avoid bleeding events^{174, 175}. Many studies have reported improved overall outcome and graft patency rate with continuation of P2Y12 therapy.

Recommendations for treatment of bleeding

For patients who remain on therapy or who have emergency surgery, treatment of can be is a challenge due to the aforementioned irreversibility of effect. Consequently, in the acute setting, significant blood loss can occur leading to haemodynamic instability, prolonged time on CPB, increased blood product transfusion, increased rate of re-exploration, and overall poorer outcome. It is essential therefore that strategies exist for the rapid and effective treatment of bleeding.

The guidelines for intraoperative bleeding advocate several therapeutic goals including: the maintenance of tissue perfusion and oxygenation by restoration of blood volume and hemoglobin; the arrest of bleeding by treating the source; and judicious use of blood component therapy to correct coagulopathy¹⁷⁶. Blood component therapy includes red cells, platelets, fresh frozen plasma (FFP) and cryoprecipitate¹⁷⁷. Plasma-derived and synthetic coagulation factor concentrates such as fibrinogen, factor XIII, and recombinant-activated factor VIIa (rFVIIa) have been used in patients with excessive perioperative bleeding. The antifibrinolytic drug, Aprotinin was commonly used in cardiac surgery to treat excessive haemorrhage. However, the Blood Conservation Using Antifibrinolytics in a Randomized Controlled Trial (BART) showed higher mortality in patients receiving aprotinin. This led to withdrawal of aprotinin from the worldwide market in November 2007 by its manufacturer. There were several reports in the aftermath of this showing an increase of blood product use.

Platelet transfusion

Platelet transfusion is a commonly used in cardiac and non-cardiac surgery for patients on P2Y₁₂ receptor inhibitors for intra-operative and post-operative bleeding. Guidelines propose the empiric platelet transfusion is indicated when platelet function is abnormal secondary to anti-platelet therapy at a dose of 0.7×10^{11} per 7kg weight¹⁷⁸. While the rationale for empiric platelet transfusion is based on the possible reversal of anti-platelet effect with the administration of pooled platelets with normal reactivity, the evidence for this is sparse.

There are some studies where platelet transfusion was performed in-vitro at various proportions to examine the effect at platelet reactivity restoration. One study enrolled 11 health volunteers and administered aspirin 325mg and clopidogrel 300mg 600mg loading doses followed by maintenance doses of 81mg and 75mg respectively¹⁷⁹. Platelet reactivity was assessed by light transmittance aggregometry (LTA) at 4 and 72h post loading. Pooled volunteer platelet rich plasma was added ex vivo in increasing proportions to the treated subject's plasma. At both time points, 40% and 50% V-PRP were needed to overcome platelet inhibition in the 300mg and 600mg arms respectively; an additional 10% V-PRP fully normalized aggregation. Recovery of function was linear with each incremental increase of V-PRP. The authors concluded that, extrapolating the results to the pre-operative setting, 10 platelet concentrate units after a 300-mg clopidogrel loading or 12.5 units after a 600 mg loading may adequately reverse clopidogrel-induced inhibition to facilitate postoperative hemostasis. A limitation of this study is that pooled volunteer PRP was used instead of blood-banked platelet concentrate. Consistent with previous studies a decrease in platelet aggregation ability of blood-banked platelet concentrate was observed when it was used at first and so pooled volunteer PRP was used with more predictable effect^{71, 180}.

In a second study, the restoration of platelet function for healthy volunteers on aspirin and clopidogrel was assessed first by the withdrawal of agents and then using donor platelets from untreated controls. The full recovery of platelet reactivity to ADP after withdrawal of clopidogrel was 10 days versus 4 days for reactivity to AA after withdrawal of aspirin. For clopidogrel treated patients, complete reversal of inhibition was achieved with the addition of at least 90% uninhibited platelets whereas only 30% was needed in aspirin treated patients.

There are very few clinical studies correlating platelet transfusion with haemostasis in patients on P2Y12 receptor antagonists. There are two retrospective analyses and one prospective ex vivo analysis, which attempt to address this issue. The retrospective analyses feature patients with intracranial hemorrhage inconclusive results. One study showed a neutral effect of platelet transfusion on hematoma expansion¹⁸¹ while the other showed an increase risk of hospital death in patients treated with platelet transfusion¹⁸².

There is no single evaluation looking at the effect of restoration of platelet reactivity after platelet transfusion in patients who are bleeding, whatever the underlying DAPT is. Therefore, the second goal of this body of work was to evaluate the effect of platelet transfusion on recovery of platelet reactivity in CAD patients on P2Y12 receptor antagonists (i) in the context of CABG-related bleeds and (ii) after loading dose administration for ACS/PCI. This are challenging situations given the lack of recommendations on how to handle new P2Y12 receptor antagonists in patients undergoing CABG surgery and how to treat patients who bleed post ACS/PCI.

Chapter 2

A POINT OF CARE GENETIC PROFILING APPROACH FOR A FAST IDENTIFICATION OF CLOPIDOGREL METABOLISER PHENOTYPE TO OPTIMIZE PRASUGREL SWITCHING DURING THE MAINTENANCE PHASE AFTER AN ACUTE CORONARY SYNDROME: THE GAMMA STUDY

Introduction

The overall efficacy of antiplatelet therapy is based on the balance between the reduction of recurrent thrombosis and the associated increase in bleeding risk. This has formed the basis for the therapeutic window of on-treatment platelet reactivity whereby there is thresholds for both increased thrombotic and bleeding risk between which therapies should be guided. Clopidogrel with aspirin has been the standard of treatment for patients presenting with an acute coronary syndrome and/or for PCI. However, many patients will have recurrent ischemic events after an ACS despite dual anti-platelet therapy (DAPT). High on-treatment platelet reactivity is a marker of risk with a significant body of evidence demonstrating associated increase in short and long term ischaemic events^{183, 184}. This is related to many clinical factors but also genetic variability in metabolism of clopidogrel. Clopidogrel is an oral platelet P2Y₁₂ receptor inhibitor that requires metabolic activation catalyzed by several Cytochromes P450 (CYP) isoforms. The loss-of-function polymorphism 2C19*2, carried by 30% of individuals, is associated with high-on-treatment platelet reactivity (low level of P2Y₁₂ inhibition) thus a higher risk of stent thrombosis in patients exposed to clopidogrel according to two recent meta-analyses^{48, 122}(OR=3.45 (2.14-5.57 and HR 2.81(1.81-4.37)). On the contrary, the gain-of-function polymorphism 2C19*17 is associated with low-on-treatment platelet reactivity (high level of P2Y₁₂ inhibition- HPI) with a higher risk of TIMI major bleeding (OR=1.85 (1.19 –2.86).

Prasugrel is a new thienopyridine drug that is less dependent on the *CYP2C19* allele for metabolism for metabolism and results in more consistent inhibition of the P2Y12 receptor^{78, 148}. It has improved ischemic outcomes compared with clopidogrel in acute coronary syndrome patients undergoing PCI¹⁸⁵ and is now recommended along side another new P2Y12 inhibitor, ticagrelor as first line treatment in NSTEMI-ACS and STEMI^{152, 186}. The improved and stronger effect of this agent is counteracted by more bleeding complications¹⁸⁷. The recent pharmacogenetic literature suggests that individualized treatment based on rapidly obtained genetic information is possible¹⁸⁸. This has the potential to improve the risk/benefit of therapy by identifying common gain- and loss-of function *CYP2C19* alleles that can be combined to identify the predicted metabolic phenotype. Poor or slow metabolisers are at potential risk for stent thrombosis and normal and rapid metabolisers having a lower risk of thrombosis, but a higher risk of bleeding. As genotype is one of the many clinical factors independently associated with on-clopidogrel platelet reactivity, it has been suggested that point-of-care (POC) platelet function testing may add to this strategy by assessing the pharmacodynamic phenotype in the individual patient, allowing further modification of therapy.

Study Hypotheses

We aimed with this study to assess the feasibility of personalised P2Y12 therapy, choosing either clopidogrel or prasugrel based on phenotypic metaboliser status identified by genotype obtained by rapid platform testing after an ACS. To examine this we formulated two hypotheses:

Primary hypothesis ("genetic hypothesis"):

That the proportion of extensive and ultra-rapid metabolisers according to genotype treated with clopidogrel 75mg MD within the optimal range of P2Y12 inhibition after 30

days, (defined as a % inhibition between 30% up to 80% using the VerifyNow™ P2Y12 platform), would be non-inferior to the proportion of slow metabolisers treated with prasugrel 10mg MD.

Secondary hypothesis (“functional hypothesis”):

That PFT, when added to genetic testing at 30 days in patients outside the pre-specified target of P2Y12 inhibition to further guide antiplatelet therapy (prasugrel 10mg and clopidogrel 75mg), would increase the proportion of patients reaching the prespecified optimal target of P2Y12 inhibition.

Objectives

Primary objective

To demonstrate that a strategy of rapid genetic testing performed on patients after an acute coronary syndrome and stenting allows the appropriate selection one of two antiplatelet treatments approved, prasugrel 10mg MD or clopidogrel 75mg MD as confirmed by a second evaluation to assess the levels of platelet inhibition on the chosen therapy.

Secondary Objective

To demonstrate that further adaption of treatment based on the PFT results can help increase the number of on-target patients. The patients with high on-treatment platelet reactivity (<30% inhibition) would be commenced on prasugrel 10mg od and the patients with low on-treatment platelet reactivity (>80% inhibition) would be commenced on clopidogrel with further repeat PFT evaluation to achieve this objective.

Methods

Study population

The protocol was approved by the institutional ethics committee and all of the subjects gave informed consent. Patients aged >18 years with a history of ACS and PCI with stenting within the previous 6 months, clinically stable and treated with dual antiplatelet therapy including aspirin and either clopidogrel or prasugrel were eligible for inclusion in this study. Those with contra-indications to prasugrel therapy including history of hemorrhagic or ischemic stroke, recent major haemorrhage or planned surgery within the following 8 weeks after inclusion were excluded from the study. The recommendations of caution with regard to prasugrel use in the low weight (< 60 kg) and elderly (>75 years) were not exclusion criteria. Patients with haemoglobin < 10 g/dL, hepatic failure defined as coagulation factors less than 50%, a platelet count < 80,000/ μ L, chronic anticoagulation therapy, patients unable to give informed consent and recent stent thrombosis were excluded from the study.

Study Design

This was a multicenter centre, prospective, non-randomised, proof of concept trial (**Figure 2.1**). We recruited patients on dual antiplatelet therapy after stent implantation undertaken in the setting of an ACS. Thienopyridine treatment at enrolment did not affect eligibility. *CYP2C19* genotype was determined using the VERIGENE® *CYP2C19* TEST (Nanosphere technology).

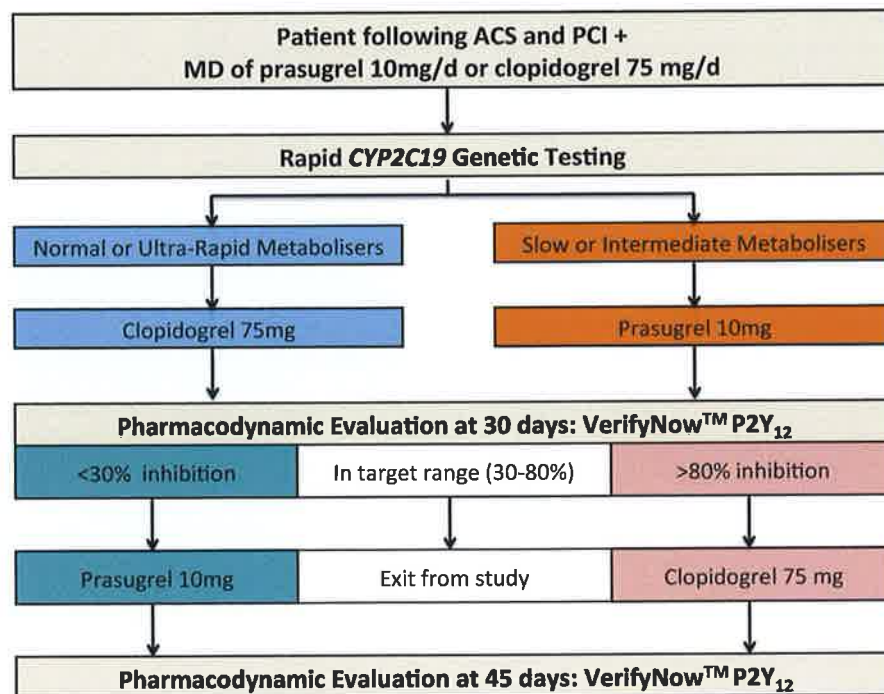


Figure 2.1 Flow design of study

At visit 1, patients were stratified into two groups to represent their predicted phenotypes: normal (*1/*1, *2/*17) or rapid (*1/*17, *17/*17) and intermediate or poor metabolisers (1*/*2, *2/*2) (**Table 2.1**). Normal and rapid metabolisers were treated by 75mg clopidogrel daily while poor/intermediate metabolisers were allocated to prasugrel 10mg daily.

Table 2.1 Categorisation of genotype to predicted phenotype

Metabolic phenotype	CYP2C19 Genotype
Normal Metabolisers +	Wild type carriers (*1/*1) *2 and *17 carriers (*2/*17)
Rapid Metabolisers	*17 allele carriers (*1/*17, *17/*17)
Poor Metabolisers	*2 allele carriers (1/*2, *2/*2) *3 allele carriers (1/*3, *3/*3)

At visit 2, a first evaluation of platelet reactivity was performed 15-30 days after to allow the comparison of the proportion of patients who were within the optimal prespecified window of P2Y12 inhibition (primary hypothesis) between rapid metabolisers and slow metabolisers. Patients within the target range exited the study with no modification of therapy. Patients outside the target with a level of P2Y12 inhibition >80% or <30% were switched to 75mg clopidogrel MD and or to prasugrel 10mg MD, respectively. Visit 1 and 2 could be done the same day if patients were stable, treated with DAPT for at least two weeks and on a thienopyridine that was concordant with their genetic metaboliser profile.

At visit 3, a second evaluation of the level of P2Y12 inhibition was performed at day 45 to allow the comparison of proportions of patients within the prespecified optimal window of P2Y12 inhibition between rapid and slow metaboliser genotypes.

Endpoints

Primary endpoint:

The primary end point was the proportion of patients within the optimal prespecified window of P2Y12 inhibition at visit 2, defined as a % inhibition between 30% up to 80% using the VerifyNow™ P2Y12 platform.

Secondary endpoints:

The secondary endpoint is the proportion of patients within the optimal prespecified window of P2Y12 inhibition at visit 3, defined as as a % inhibition between 30% up to 80% using the VerifyNow™ P2Y12 platform.

The other secondary end points were the proportion of patients within the optimal window of aggregation and activation at 30 and 45 days according to the other platelet function tests performed: Light Transmission aggregometry, Vasodilator phosphorylation reactive index (VASP-PRI) and TEG Platelet mapping. The pre-specified definitions for each test were: Residual platelet aggregation (RPA) in response to 20mmol/L ADP 1-46.2%, VASP-PRI 10-50%, TEG maximum amplitude % in response to ADP (MA ADP%) 30-80%.

Laboratory methods

Genetic Testing

The VERIGENE® *CYP2C19* assay is an automated sample-to-result microarray-based assay in which DNA is extracted from whole blood samples and hybridized to allele-specific probes immobilized on a glass slide. Detection of captured DNA is achieved using nanoparticle-conjugated probes that have been demonstrated to provide excellent sensitivity and that eliminate the need for a target amplification step before array

hybridization^{189, 190}. The CYP technique has been previously validated with conventional methods in an acute coronary syndrome population¹⁹¹.

All participants had genotyping for *CYP2C19**2 (rs4244285) loss-of-function allele as well as *CYP2C19**3 (rs4986893) and *CYP2C19**17 (rs12248560)

To set up the assay, a single-use processing tray containing all necessary reagents to lyse, extract, and purify DNA from whole blood specimens was loaded into the Verigene Processor SP (Nanosphere). A 1.0-mL sample of homogenized blood was transferred to the specimen well in the extraction tray. A single-use CLO test cartridge containing the slide array and hybridization reagents was loaded into the Verigene Processor SP, and the assay was started (run time, 2 hours, 40 minutes). On completion of the assay, the CLO test cartridge was removed from the processor and the hybridization slide was inserted into the Verigene Reader. The reader returned results of *1/*1, *1/*2, *2/*2, *1/*3, *3/*3, *2/*3, *1/*17, *17/*17 within approximately 60 seconds. A message of "no call" was returned if the genotype of the specimen could not be definitively identified owing to specimen deficiency or assay error. If this occurred, testing of these specimens was repeated, provided the original specimen was sufficient for retesting.

Genotyping was validated by conventional laboratory methods using the TaqMan Validated SNP assays with the 7900HT Sequence Detection System (Applied Biosystems).

Light transmission aggregometry

Blood was collected into Becton-Dickinson 3.2% citrate vacuette tubes after having discarded the first 2 to 4 ml of blood to avoid spontaneous platelet activation. Platelet-rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 100g for 10 minutes at 20°C. Platelet poor plasma (PPP) was obtained by further centrifugation at 4500g for 15

minutes. In vitro platelet aggregation in PRP was measured at 37°C by Light Transmission Aggregometry (LTA) (model 490-4D, Chrono-Log Corp, Kordia, the Netherlands) and was induced by the addition of Adenosine Diphosphate (ADP) (Sigma-Aldrich, Saint Quentin Fallavier, France) at final concentrations of 20 µmol/L for prasugrel, clopidogrel testing. The measure was performed twice and the results were expressed in maximal platelet aggregation (MPA) and residual platelet aggregation (RPA), 6 minutes after induction of aggregation by agonist. Prespecified criteria used to define non-evaluable samples were: lack of sufficient signal, hemolysis, and PRP platelet count <150 000/mL and an unstable baseline.

VASP measurement

The phosphorylation of the vasodilator-stimulated phosphoprotein (VASP) was measured with a Beckman Coulter FC500 cytometer (Beckman Coulter, Villepinte, France) using Platelet VASP kits [Platelet VASP®; Diagnostica Stago (Biocytex), Asnières, France] according to the manufacturer's instructions and as previously described¹⁰. Briefly, blood samples were incubated in vitro with ADP and/or prostaglandin E1 (PGE₁) before fixation. The VASP platelet reactivity index (PRI) was calculated from the MFI of each condition according to the formula:
$$\text{VASP PRI} = ((\text{MFI (PGE}_1) - \text{MFI (PGE}_1\text{+ADP)}) / \text{MFI (PGE}_1)) \times 100.$$

VerifyNow™ P2Y₁₂ assay

The VerifyNow(Accumetrics,CA,USA) system is a rapid automated whole blood assay that measures agglutination of fibrinogen-coated beads in response to specific agonists. Measurement of platelet response to clopidogrel and prasugrel with the VerifyNow P2Y₁₂ assay was done according to the package insert (Accumetrics Corporation, San Diego, CA). This is a rapid (less than 5 minutes) platelet function assay designed to measure directly the effects of drugs on the P2Y₁₂receptor. The assay is a turbidimetric-based optical detection system that,

like optical aggregometry, depends on the ability of activated platelets to bind fibrinogen. The assay contains 20 μmol ADP and 22 nmol PGE₁ to reduce the activation contribution from ADP binding to P2Y₁ receptors, thus making the assay specific for the effects of ADP mediated by P2Y₁₂.

Results were expressed in P2Y₁₂ Reaction Units (PRU) in response to iso-Thrombin Receptor Activating Peptide (iso-TRAP) and in response to ADP-PGE₁. Iso-TRAP strongly activates platelets despite of P2Y₁₂ receptor blockage by thienopyridine or aspirin and reflects the platelet reactivity without treatment¹⁹². The device provides an estimated inhibition (in percent) without pre-thienopyridine sample by reporting the ratio of the results of the ADP-PGE₁ and iso-TRAP channels.

Thromboelastograph (TEG)

Analysis was performed with the TEG[®] 5000 Thrombelastograph[®] Hemostasis Analyzer system (Haemonetics Corporation, Braintree, MA). A cuvette was loaded onto the analyser. Immediately before analysis a 1 ml sample of citrated blood was added to a vial containing kaolin (a clotting activator). After good mixing and without delay, 360 μl was added to the cuvette with 20 μl of calcium chloride to reverse the effect of the citrate. The analyser works on the following principle: for a motor to drive a paddle and keep it rotating at a fixed speed in a cuvette containing blood, more current is required as a clot forms and retards paddle movement. Since the paddle rotates alternately in both directions (clockwise and anti-clockwise), two opposing signals are generated. These are combined and graphed against time to form the thromboelastogram.

Platelet Mapping

The degree of suppression of the platelet (adenosine diphosphate) ADP receptor with clopidogrel, prasugrel and the Thromboxane A2 receptor with aspirin can be assessed indirectly with a variation of the standard method. The standard method performed above indicates clot strength with maximum platelet activity independent of the level of aspirin or clopidogrel in the sample. This applies because there is enough thrombin in the blood sample to fully activate the IIb/IIIa receptor in the absence of heparin.

For the platelet mapping analysis three other cuvettes loaded onto the analyser were used. Blood that had been stored in the heparin containing vacuette tubes was used in this analysis. The heparin delays clot formation sufficiently for the action of three activators. Fibrin activator was added to all three cuvettes. This forms a fibrin clot unstrengthened by activated platelets giving a tracing which is consistent with zero platelet activity. The second and third cuvettes had ADP and arachadonic acid added which directly activate the platelets to strengthen the clot irrespective of the blockage of the IIb/IIIa receptor by heparin. This gives traces that are dependent on the degree of suppression of the TXA2 or ADP receptors. If there is no drug suppression of the receptor the tracing settles close to that of the initial citrate sample giving maximum platelet activity (MA ADP and MA AA). If there is total suppression of the receptor then the tracing settles close to the fibrin alone tracing. Intermediate positions of the grey line between the two limits are assessed by the TEG[®] computer software and reported as percentage inhibition of the appropriate receptor.

Statistical analysis

Sample size: We estimated the proportion of prasugrel-treated patients within the prespecified target of P2Y12 inhibition to be 65%. We formulated the hypothesis that 80% of rapid metabolisers on clopidogrel 75mg MD would be in the optimal prespecified

window of P2Y12 inhibition and we wished to demonstrate that this proportion is not inferior to that of prasugrel-treated patients. Considering a power of 80%, an alpha-risk error of 0.05 and a non-inferiority margin of -10%, a sample size of 120 patients per group are required. We have estimated that at least 50% of patients would be normal/rapid metaboliser and that 110 patients per group would be necessary to demonstrate our hypothesis. We planned to enroll 230 patients in total.

Statistical analysis: The non-inferiority was considered as demonstrated (according to the consent risk) if the non-inferiority margin of the 95% confidence interval of the difference in percentages is superior to the non-inferiority margin (ICH Statistical Procedures). The non-inferiority margin was established at 10% according to clinical considerations based on an acceptable maximal loss of efficacy.

Results

Between July 2011 and Sept 2012, 241 patients met the inclusion criteria (**Figure 2.2**). Baseline characteristics of the primary analysis population were well matched in the two genotype groups (**Table 2.2**). The vast majority were men (80.6%) with a high prevalence of cardiovascular risk factors. Almost half of the patients (46.5%) presented with ST-elevation acute coronary syndrome and 96.2% had successful stent implantation (n=231). Patients were included at a mean of 30.2 days (range 1-179) after the ACS. Antiplatelet therapy at inclusion was equally divided between clopidogrel (49.7) and prasugrel (50.3). The concomitant use of PPI was high.

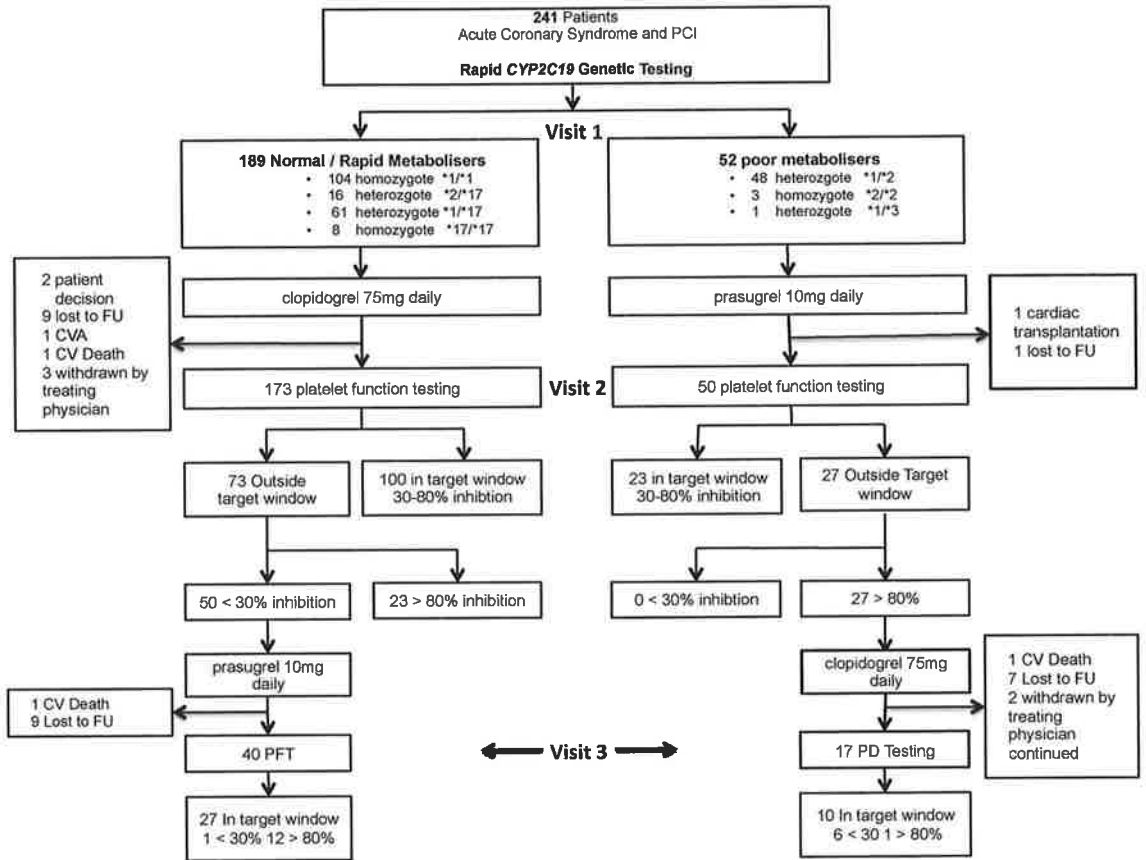


Figure 2.2 Flow diagram of study

Table 2.2 Baseline Characteristics and therapies

	ITT Population (n = 241)	Normal/Rapid metabolisers (n = 189)	Poor Metabolisers (n = 52)	p value
Age, median (Q1);(Q3)	59.65±13.8	59.64±13.9	59.71±13.4	0.9749
Age (> 75)	40(16.6)	32(17)	8(13)	0.7907
Gender (female)	49(20.4)	41(21.8)	8(15.2)	0.3168
BMI (kg/m ²)	27.18±10.99	27.36±12.20	26.53±4.298	0.6301
Diabetes	63(26.1)	51(25.5)	12(28.3)	0.5701
Dyslipidemia	113(46.9)	94(49.7)	19(37)	0.0913
Hypertension	105(43.6)	84(44.8)	21(45.7)	0.6011
Family History	50(20.7)	42(22.4)	8(15.2)	0.2816
Current smoking	97(40.3)	75(39.6)	22(42.3)	0.7325
Prior Myocardial Infarction	48(19.9)	35(18.5)	13(25)	0.3
Prior PCI	55(22.8)	40(21.1)	15(28.8)	0.2424
Prior CABG	72.9(2.9)	7(3.7)	0(0)	0.159
Prior stroke	2(0.8)	2(1)	0(0)	1
CrCl, ml/min	100.1±45.66	102.3±46.13	94.15±41.93	0.2516
CrCl < 60 ml/min	41(17)	33(17.6)	8(15.2)	0.7243
LV Ejection Fraction (%)	52.4±10.1	52.9±10.0	50.5±10.1	0.1346
ACE inhibitors	162(67.2)	130(69.1)	32(60.9)	0.3243
Beta blockers	194(80.6)	150(79.4)	44(84.8)	0.3974
Statins	224(92.9)	175(92.7)	49(89.1)	0.6829
Proton Pump Inhibitors	168(69.7)	135(71.5)	33(63)	0.2682
Calcium channel blockers	38(15.7)	34(18.2)	4(7.7)	0.0712
STEMI	112(46.5)	83(44.2)	29(55.7)	0.1291
NSTEMI/UA	129(53.5)	109(55.8)	23(44.3)	0.0846
One vessel disease	101(42.2)	82(43.6)	19(36.5)	0.3755
Two vessels disease	69(28.9)	56(29.7)	12(23.1)	0.3525
Three vessel disease	67(28)	50(26.7)	17(32.7)	0.374
Radial access	229(95.3)	180(95.2)	49(94.2)	0.7674
Stent Implantation	231(96.2)	182(96.4)	49(94.2)	0.5084
Drug eluting Stent	189(78.7)	147(77.7)	42(80.7)	0.6424
Clopidogrel pre inclusion	120(49.7)	97(51.3)	23(44.2)	0.3650
Prasugrel pre inclusion	121(50.3)	92(48.6)	29(50)	0.3650

***CYP2C19* genotype and predicted phenotype (Inclusion Visit 1)**

The genotype data is illustrated in **figure 2.3A**. At visit 1, the two third of patients who displayed a rapid or normal clopidogrel genetic metabolic profile were left on or switched to clopidogrel, the remaining poor/intermediate clopidogrel metabolisers were left or switched to prasugrel 10 mg maintenance dose (figure 2 and 3A). *CYP2C19**2 and *CYP2C19**17 homozygous carriers were infrequent. Overall, 116 patients had their P2Y12 therapy modified after genetic testing (**Figure 2.3B**). Among the 189 normal and rapid metabolisers, 48.6% had their P2Y12 therapy switched from prasugrel to clopidogrel in accordance with the protocol. Among the 52 poor metabolisers, 44.2% had their P2Y12 therapy switched to prasugrel.

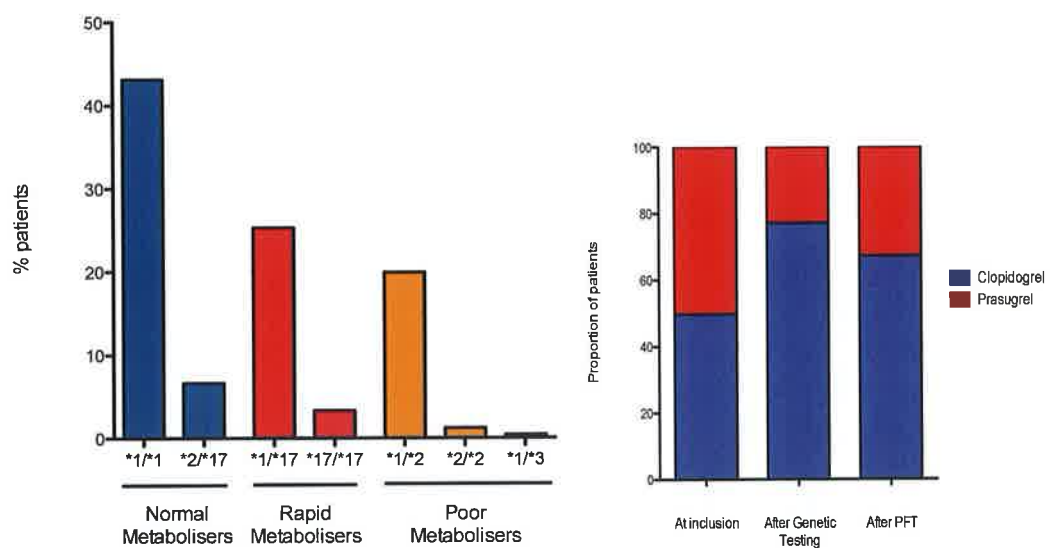


Figure 2.3 A) Proportional representation of identified *CYP2C19* alleles; B) Antiplatelet therapy switching at inclusion, after rapid genetic testing and after two PFT assessments.

Platelet Function Testing (Visit 2)

Of the 241 patients who underwent rapid genetic testing performed using the VERIGENE® *CYP2C19* platform (Nanosphere Inc, Northbrook, IL), 223 (92.5%) patients returned for a pharmacodynamic evaluation (visit 2) (Figure 2.2). Over half the patients (51.3%) were within the prespecified therapeutic window according to the VerifyNow assay. The proportion of the normal and rapid metaboliser patients that were within the therapeutic window (30-80% inhibition) was 52.9% versus 44.2% in the poor metaboliser group ($p=0.27$) with the difference between the two groups of +8.7% (95% CI, -6.7% to 24%). The primary end-point of non-inferiority was achieved because the lower limit of the 95% CI of the difference between the percentages was -6.7%, which is greater than the predefined non-inferiority margin of -10%. (Figure 2.4)

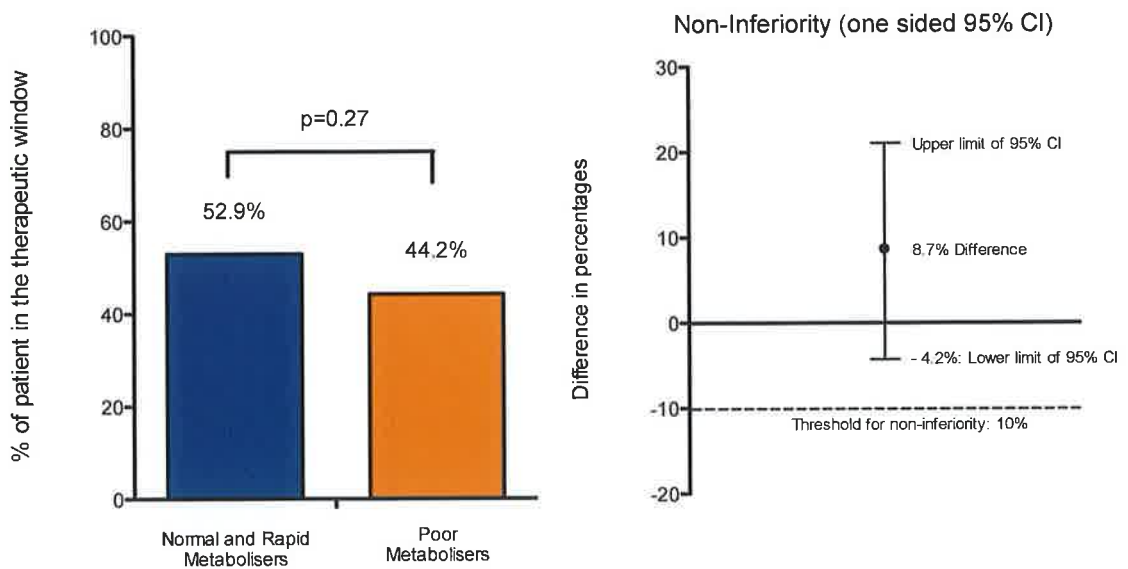


Figure 2.4 Primary end-point of non-inferiority achieved.

The other tests were concordant with these findings showing 67.7%, 50.8% and 67.8% within the pre-specified therapeutic window according to LTA, VASP and TEG platelet mapping respectively (Figure 2.5).

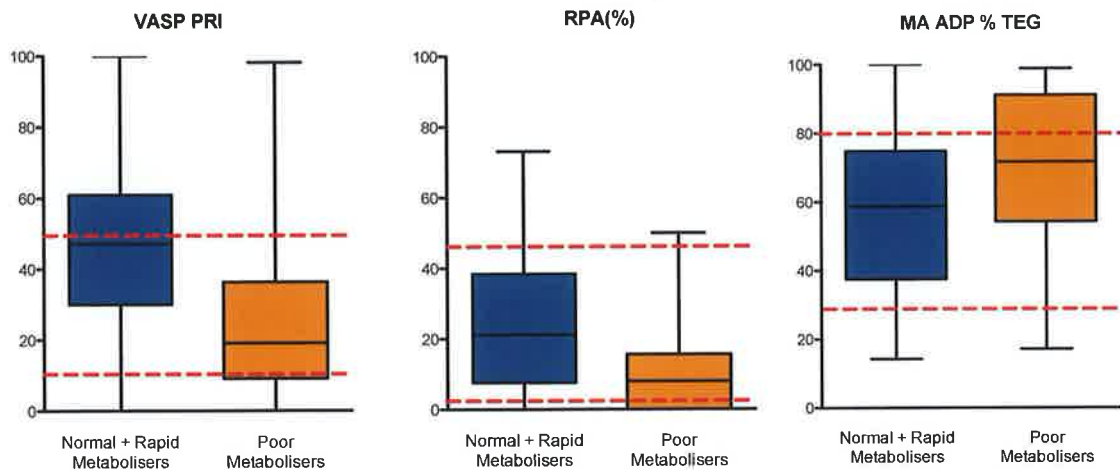


Figure 2.5 Box and whisker plot representation of the vasodilator phosphorylation reactivity index (VASP-PRI%), residual platelet aggregation(RPA%) in response to ADP 20mmol/L , maximum amplitude in response to ADP according to Thromboelastograph (TEG) Platelet Mapping (MA-ADP%). The red scored lines represent the therapeutic window for each test

Among the normal and rapid metabolisers, 73 (38.6%) were outside the pre-specified therapeutic window with 50 patients (26.5%) with VN % inhibition < 30% and 23 patients with high on-treatment platelet inhibition (VN % inhibition > 80%). Among the poor metabolisers, there were no patients with inadequate response to prasugrel (VN % inhibition < 30%) and thus all patients outside the therapeutic window (n=27) were hyper-responders to prasugrel (VN % inhibition > 80%).

Platelet function Testing (Visit 3)

Based on the PFT at Visit 2, 77 patients had their treatment further modified with 27 patients switched to clopidogrel and 50 patients to prasugrel. The 23 patients with hyper-response to clopidogrel were either continued on 75mg daily or switched to 75mg every second day without PFT follow up.

Among the 77 patients that had their therapy switched, 57(74%) returned for follow up PFT. This demonstrated that 37(64.9%) were in the therapeutic window (**Figure 2.6**). Therefore, the proportion of patients in the therapeutic window in the overall cohort increased from 51.5% at the first visit to 66.4% at the second visit ($p=0.0006$). In the normal and rapid metaboliser group, the proportion of on target patients increased as did for the poor metaboliser group with no difference between the two groups (67.2% vs 63.5%, $p=0.613$) (**Figure 2.7**). Only 7 patients (2.9%) had HTPR and 13 had HTPI (5.3%). Of the HTPR, one was on prasugrel and the remaining 6 were poor metabolisers that had

been initially treated with prasugrel that were switched to clopidogrel due to HTPI.

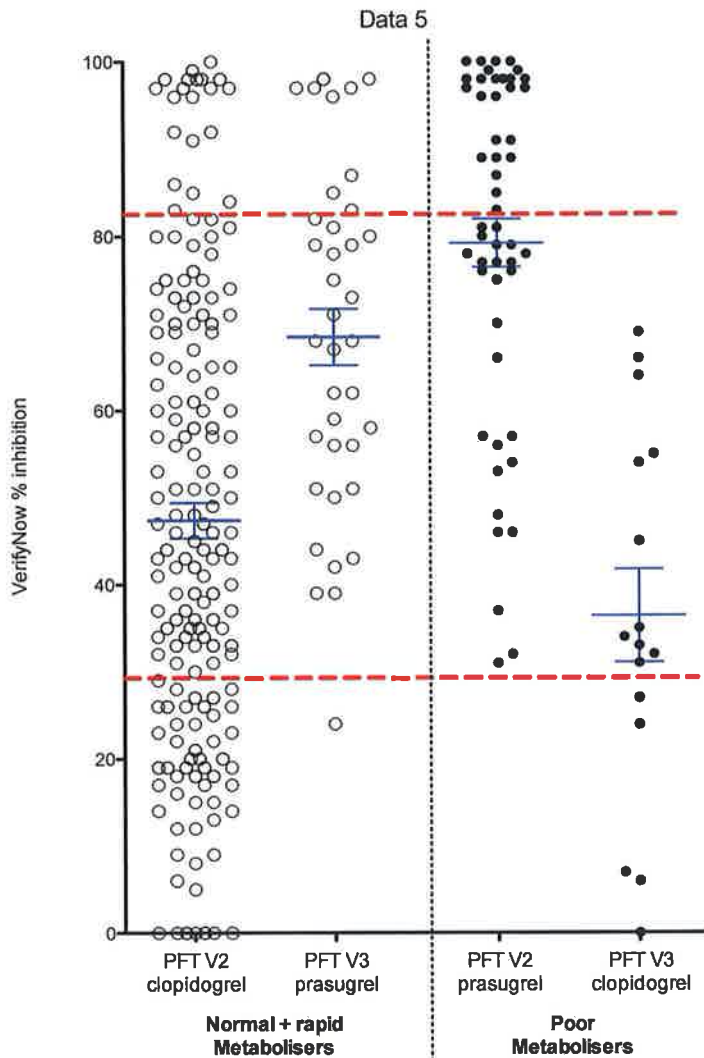


Figure 2.6 Scatter plot of VerifyNow assay results for the two metaboliser groups at PFT visits 2 and 3.

Therapeutic window is represented by scored red lines. Patients with hyporesponse to clopidogrel were switched to prasugrel and retested 15 days later and patients with hyper response to prasugrel were switched to prasugrel and retested 15 days later. Both switching strategies resulted in an increase in on-target patients.

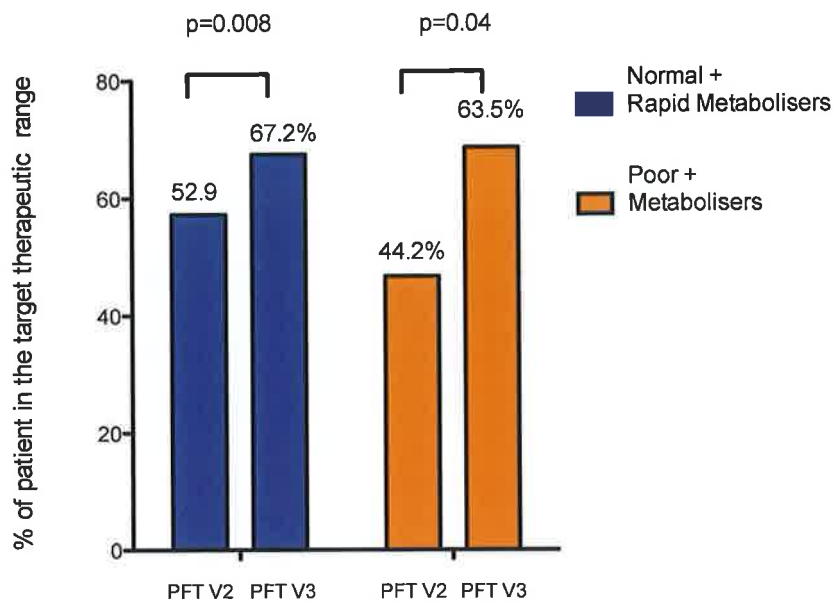


Figure 2.7 Proportion of patients within the therapeutic range of platelet reactivity according to VerifyNow assay with increased proportions after switching of therapy at first PFT visit (V2).

Discussion

The key findings of this study may be summarized as follows 1) Individualized P2Y₁₂ receptor inhibitor therapy using rapidly obtained genetic testing and platelet function testing is feasible in real-world high-risk post ACS patients that have undergone stenting; 2) the primary end-point of the study was achieved, whereby the proportion of patients with a normal or rapid predicted metaboliser phenotype treated with clopidogrel that were within the pre-defined therapeutic window of platelet reactivity, was non-inferior to the poor metaboliser patients treated with prasugrel; 3) with the use of genotype alone, 50% of patients still remained outside the optimal window of platelet reactivity thus demonstrating the important additive benefit of POC PFT to further modify treatment, resulting in increased proportion of on-target patients and reduced the rate of HTPR, which was only 2.9% at the end of the study; and 4) the combined genetic and pharmacodynamic strategy allowed the more selective use of prasugrel therapy thereby avoiding potential future bleeding events without loss of antiplatelet efficacy.

The RAPID gene trial was the first to demonstrate feasibility of rapid genotyping in CAD patients undergoing stenting¹⁸⁸. Using the Spartan RX point-of-care platform, the researchers identified *CYP2C19**2 allele carriers and eliminated HTPR with prasugrel therapy. Although this was not the primary end-point of our trial, there was similarly no HTPR among the 52 carriers of the *2 allele at follow up. There exist, however, several differences between our study and the RAPID gene trial. First, the population assessed in the RAPID gene study mostly presented with stable CAD and only one third presented with ACS. All patients in this cohort presented with ACS and underwent PCI. This design is supported by data that suggests the strongest association between *CYP2C19* genotype and adverse cardiovascular outcome is in ACS patients undergoing stenting^{121, 122}. Second, we used the Verigene *CYP2C19* platform which unlike the Spartan POC platform, is a fully

automated laboratory based test that assessed for the presence of not just *2 but also *3 loss-of-function allele and the gain-of-function *17 allele. Therefore using the genotype, we switched therapy both from clopidogrel to prasugrel and from prasugrel to clopidogrel depending on the predicted metaboliser phenotype. Third, in the second phase of this study we readjusted P2Y12 therapy based on PFT and so our trial allowed for non-genetic clinical factors that have been shown to influence platelet reactivity. Finally, we used cut off values for both HTPR and HTPI, focusing also on the prevention of bleeding events.

The cut-off values chosen 30 and 80% inhibition according to the VerifyNow P2Y12 assay, that correlate to a PRU of 214 and 46 respectively (analysis of GRAVITAS) ref. This therapeutic window was chosen as it was within the margins whereby an increased event rate is seen. The definition of HTPR according to consensus in 2010 is a PRU > 235. Recent studies including a post-hoc analysis of the GRAVITAS study appear to suggest that this is too high and that achievement of a PRU < 208 after PCI and during follow-up was associated with a lower cardiovascular event rate¹⁹³(ref ADAPT DES TCT 2012). This is further supported by the ADAPT DES study, which demonstrated a higher event rate greater sensitivity off the cut off of 208. Our cut-off of 30% is representative of this trend to a lower recommended PRU level.

Debate continues as to whether personalised antiplatelet therapy based on genotype or platelet function testing can impact clinical outcome. The randomized data from the above-mentioned GRAVITAS study and the TRIGGER PCI study have failed to show benefit of this strategy. The RECLOSE 2 study showed high residual platelet reactivity to be an independent prognostic marker of short-term and long-term ischaemic events in an ACS population. Similar to the GRAVITAS study, treatment adjustment with increased clopidogrel dosing or switching to ticlopidine did not impact the event rate. The soon to

be published ARTIC study may shed further light on the issue. A key issue with these trial designs is lack of use of stronger P2Y12 inhibition with newer agents prasugrel and ticagrelor. In our study we adopted a hybrid approach to identify poor metabolisers with genotyping and then follow up platelet function testing. At follow up testing, the rate of HTPR in clopidogrel treated patients with no loss-of-function allele was 26.5% and this was eliminated to one patient (0.5%) with prasugrel treatment.

At the other end of the spectrum, using our predefined cut off, 12 % of clopidogrel and half of prasugrel treated patients had HTPI, representing a significant proportion of total patients outside the therapeutic window. There exists no consensus as to a value of platelet reactivity where bleeding risk is clinically important, but this remains a significant safety issue especially with the new P2Y12 agents. There is more recent data to suggest that a 5mg dose of prasugrel maybe an option in these patients as the consequence of switching therapy to clopidogrel results in 30% rate of HTPR as is demonstrated by our data.

The main limitation of this study is that a surrogate end-point of for clinical efficacy was used. While the presence of HTPR is a well-recognized marker of risk for recurrent cardiovascular events and even though the pharmacodynamic efficacy of the strategy employed in this study is well elicited, we cannot draw definite conclusions on the clinical impact. Even so, we believe that this study demonstrates the feasibility of the combined use of genetic and platelet function testing to appropriately select P2Y12 inhibitor therapy, adding significantly to the field of pharmacogenetics and personalised medicine.

Chapter 3

COMPARISON OF THE EFFICACY OF *EX-VIVO* PLATELET TRANSFUSION IN REVERSING P2Y12 INHIBITION BY CLOPIDOGREL AND PRASUGREL: APTITUDE ACS/PCI STUDY

Introduction

Antiplatelet therapy is the cornerstone of treatment in coronary heart disease patients. It is well established that the addition of P2Y12 receptor inhibiting drugs to aspirin therapy has resulted in improved clinical outcome secondary to reduced recurrent ischaemic events. The benefit of this therapy however, is balanced by bleeding and the significant associated morbidity and mortality. The risk factors for bleeding such as age, female sex and renal insufficiency are in a paradoxical manner the same factors that are associated with thrombotic risk, making the management of many patients somewhat of a challenge. When a major bleeding event occurs the strategies for treatment are dictated by the severity of the bleed and the associated hemodynamic compromise. Many of the therapies have irreversible effect with no antidote and varying lengths of offset of action making the acute treatment difficult. The mainstays of treatment are mechanical haemostasis, intravascular volume expansion and blood product infusions. Platelet transfusion is proposed with two triggers for clinical use; thrombocytopenia of less than 75,000 and impairment of platelet function secondary to antiplatelet drugs. The hypothesis is that replacing the dysfunctional platelet population with functional donor platelets results in overall improved haemostasis. The evidence for the efficacy of such a strategy is sparse with only ex vivo studies in clopidogrel treated healthy volunteers and there are no studies with either newer P2Y12 receptor antagonists or coronary heart disease patients.

The **Antagonize P2Y₁₂ Treatment Inhibitors by Transfusion of platelets in an Urgent or DElayed Timing after ACS or PCI presentation (The APTITUDE study)** was designed to demonstrate the effect of in vitro platelet transfusion in a coronary population receiving loading doses of P2Y₁₂ receptor antagonists.

Study hypothesis

The proportion of untreated platelet rich plasma (PRP) to normalize platelet reactivity is higher in prasugrel versus clopidogrel-treated patients.

Primary Objective

To compare the effects of ex vivo PRP addition to normalize PR between ACS/PCI patients loaded with clopidogrel and prasugrel.

Methods

Study Population

The protocol was approved by the institutional ethics committees and all of the subjects gave written informed consent. Men and women age 18 years and older were recruited after admission to the Heart Institute at Pitié-Salpêtrière University Hospital between January and August 2012. Patients were suitable for inclusion in the study if they were for planned coronary angiography and/or PCI with a loading dose of either clopidogrel 600/900mg, prasugrel 60mg or ticagrelor 180mg having presented either electively or with an acute coronary syndrome. Those with a personal or family history of bleeding disorders, a platelet count outside the normal range (<150,000/ μ l or >450,000/ μ l), haemoglobin < 8 g/dl, or current treatment (within 15 days) with another P2Y₁₂ inhibitor, glycoprotein IIb/IIIa inhibitor NSAID were excluded. 35 patients met the inclusion criteria and consented to take part in the study.

Study Design

The study design is outlined in figure 3.1. Blood was drawn from participants at two different time points; just before administration of the P2Y12 inhibitor loading dose (H0) and at 4 hours (H4). Light transmission aggregometry was performed at H0 and treatment naïve platelets in the form of platelet rich plasma (PRP-H0) was stored for mixing *ex vivo* with PRP at H4 (PRP-H4). Increasing proportions of PRP H0 were added as shown in figure 4.1 to normalize platelet function.

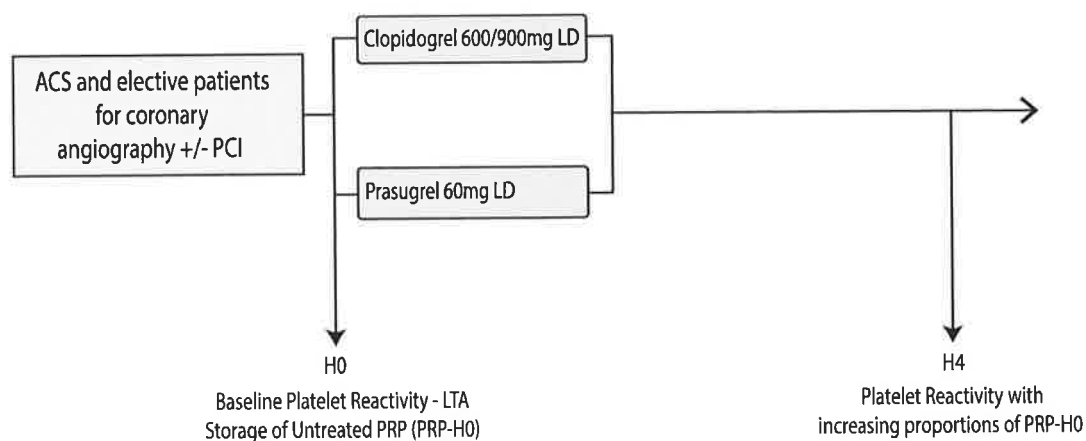


Figure 3.1 Scheme design of APTITUDE ACS/PCI study

Table 3.1 Platelet mixing *ex-vivo* with increasing concentrations of treatment naïve platelets.

Treated platelets PRP Post LD (H4)	Untreated platelets PRP H0
100%	0%
70%	30%
50%	50%
20%	80%
0%	100%

Light transmission aggregometry

Blood was collected into Becton-Dickinson 3.2% citrate vacuette tubes after having discarded the first 2 to 4 ml of blood to avoid spontaneous platelet activation. Platelet-rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 100g for 10 minutes at 20°C. Platelet poor plasma (PPP) was obtained by further centrifugation at 4500g for 15 minutes. In vitro platelet aggregation in PRP was measured at 37°C by Light Transmission Aggregometry (LTA) (model 490-4D, Chrono-Log Corp, Kordia, the Netherlands) and was induced by the addition of Adenosine Diphosphate (ADP) (Sigma-Aldrich, Saint Quentin Fallavier, France) at final concentrations of 20 µmol/L for prasugrel, clopidogrel testing. The measure was performed twice and the results were expressed in maximal platelet aggregation (MPA) and residual platelet aggregation (RPA), 6 minutes after induction of aggregation by agonist. Prespecified criteria used to define non-evaluable samples were: lack of sufficient signal, hemolysis, and PRP platelet count <150 000/mL and an unstable baseline.

Study Endpoint

The primary study endpoint was the difference in % restoration of platelet function with addition of 80% proportion of PRP H0 (treatment naïve platelets) between clopidogrel 600, 900 and prasugrel 60 measured by the residual platelet aggregation(RPA) in response to 20 µM ADP. The % restoration was calculated as $(RPA_{80\% \text{ mix}}/RPA_{\text{baseline}}) \times 100$. The secondary endpoint was the relative increase in RPA(RI-RPA, %). The RI-RPA% was defined as $((\% RPA_{80\% \text{ mix}}) - (\% RPA_{H4})) / (\% RPA_{H4}) \times 100$

Statistical Analysis

Continuous variables were expressed as mean ± SD unless otherwise stated, and categorical variables as frequencies and percentages. Baseline characteristics of patients by thienopyridine were compared using the chi-square test for categorical variables, and

Student t test or 1-way analysis of variance for continuous variables as appropriate. For RPA measures, data were included for subjects with evaluable RPA measurements at baseline and at all % PRP-H0 proportions. The PD response to PRP-H0 addition was primarily defined as RI- RPA (%) to adjust for post LD platelet reactivity. After checking for the absence of carryover effect, the comparison between clopidogrel response between the clopidogrel and prasugrel groups and the 2 LDs of clopidogrel tested (600-mg vs. 900-mg LD vs 60mg LD) was evaluated by the Kruskal-Wallis test. Two-sided unpaired Wilcoxon test was then used to compare endpoints between the two groups. All statistical analysis was performed using the GraphPad Prism (Dan Diego, California). All tests were two-sided with a statistical significance of 0.05. All p values are 2 sided.

Results

Study population

A total of 35 patients (clopidogrel, n= 30 prasugrel, n=5) were included and given loading doses of clopidogrel 600/900mg and prasugrel 60mg pre-angiography for acute coronary syndrome or stable angina. Baseline characteristics were typical of a coronary heart disease population (Table 3.2). All prasugrel patients presented with ACS compared with clopidogrel treated patients that presented both acutely and electively.

Table 3.2 Baseline Characteristics and in hospital treatment of patients

Baseline Characteristics	n=35
Age (years)	66,66 ± 8,9
Male sex	28(80%)
Body mass index (kg/m ²)	26,72 ± 3,41
Creatinine (mmol/L)	74±15
Creatinine Clearance	92±20
Cardiovascular Risks	
Hypertension	34%
Dyslipidaemia	64%
Family History	12%
Smoking	54%
Diabetes	15%
Peripheral Vascular Disease	1%
Cardiovascular History	
Percutaneous Coronary Intervention	25%
Drug Eluting Stent	15%
MI	18%
CABG	2%
Presentation	
STEMI	0%
NSTEMI/UA	60%
Elective Angiography + PCI	40%
Left Ventricular Ejection Fraction	55±12
In-Hospital Treatment	
PCI	55%
DES	40%
Medical Treatment	45%

Baseline RPA did not differ significantly between groups (p=0.546) (**Table 3.3**). Predictably the response to LD was more pronounced in prasugrel patients versus clopidogrel 900mg LD versus clopidogrel 600mg (% inhibition of platelet aggregation (IPA) 96.5±12, 70.2±23, 65±15 respectively). Patients with poor pharmacodynamic response (RPA>46.6) or with uninterpretable curves were excluded from the final

calculations (n=10). There was a stepwise increase in RPA and MPA with increasing concentrations of PRP H0 in all groups (**Figure 3.2**)

Table 3.3 Reversal of P2Y12 inhibition by addition of treatment naïve PRP in increasing proportions as measured by RPA and MPA induced by 20 µM ADP. % restoration of platelet aggregation in response to ADP represented by % RPA four hours after LD and after 30, 50 and 80% proportion of PRP from baseline.

	Baseline	4 hrs Post LD	30%	50%	80%
RPA (%) ADP 20 µM					
Clopidogrel 600 mg (n = 9)	66.4 ± 8.2	22.9 ± 13.9	38.3 ± 12.6	50.8 ± 10.4	57.8 ± 9
% (RPA/Baseline)		34.0	58.0	77.0	87
Clopidogrel 900 mg (n = 11)	52 ± 18.2	15.4 ± 13.8	23.4 ± 16.1	28 ± 10.9	38.1 ± 15.8
% (RPA/Baseline)		29.0	45.0	53.0	71
Prasugrel 60mg (n=5)	60 ± 5.1	2 ± 2.2	14 ± 9.4	25.3 ± 11.6	42.3 ± 6.6
% (RPA/Baseline)		3.0	23.0	42.0	67
MPA (%) ADP 20 µM					
Clopidogrel 600 mg (n = 9)	67.6 ± 8.1	40.8 ± 8.1	50.1 ± 6.6	57.3 ± 6.5	60.5 ± 9.4
% (MPA/Baseline)		60.0	73.0	84.0	89
Clopidogrel 900 mg (n = 11)	59.2 ± 14.8	35 ± 10.3	39.7 ± 12.3	40.5 ± 7.4	46.7 ± 9.3
% (MPA/Baseline)		59.0	66.0	68.0	79
Prasugrel (n=5)	62.8 ± 4.4	22.5 ± 17.6	29.3 ± 15.7	39.5 ± 14.5	47.5 ± 7.6
% (MPA/Baseline)		36.0	47.0	63.0	75

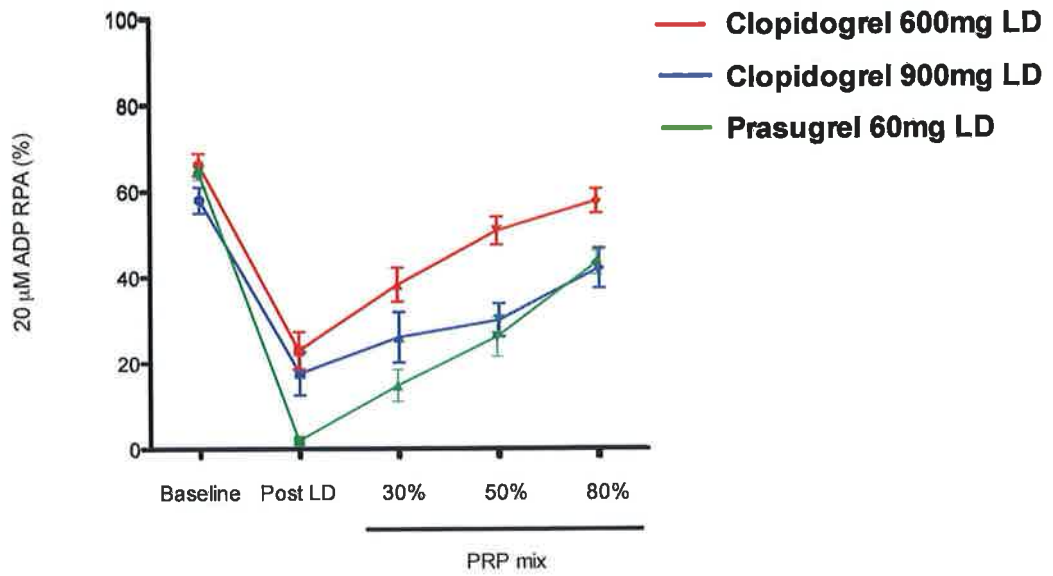


Figure 3.2 Platelet aggregation in response to 20μM ADP at baseline before loading and after loading and then with increasing concentrations of donor PRP from preloading.

The % restoration of platelet aggregation after 80% mixing was significantly higher in clopidogrel 600mg loaded patients than clopidogrel 900 loaded patients (87.6 ± 13.9 versus 71.8 ± 15.1 ; $p=0.04$) and prasugrel 60mg loaded patients (87.6 ± 13.9 versus 67.6 ± 10.5 ; $p=0.02$ and p for trend 0.036). However, the RI-RPA% was significantly higher in prasugrel 60 group than both the clopidogrel 600 and 900 groups ($p=0.0193$, $p=0.04$).

Discussion

The key findings of this study are as follows: ex-vivo platelet transfusion in increasing proportions resulted in a stepwise increase in platelet aggregation according to RPA and MPA in response to 20 μ M ADP in patients presenting for ACS and PCI loaded with clopidogrel and prasugrel; platelet transfusion had significantly more effect at restoration of platelet function in clopidogrel 600mg treated patients than clopidogrel 900mg and prasugrel 60mg treated patients; the relative increase of RPA (RI-RPA%) was significantly higher in the prasugrel treated group compared to clopidogrel suggesting that the overall effect of dose effect of platelet transfusion is substantial with this new agent.

However due to the mean lower level of platelet aggregation seen in prasugrel compared to clopidogrel loaded patients, the overall effect of platelet transfusion in prasugrel at restoration of platelet function is less than clopidogrel. Therefore, a greater dose of platelets are likely needed to completely normalise platelet function.

This is the first study to examine the biological effect of ex-vivo platelet transfusion on platelet function in clopidogrel and prasugrel treated patients presenting after an ACS and/or for PCI. There have been other studies, which have demonstrated the effect of in-vitro transfusion in healthy volunteers on aspirin and clopidogrel. Vilahur et al and Li et al both demonstrated that a greater proportion of untreated PRP was required to restore platelet reactivity in clopidogrel than aspirin (50 vs 20% and 90 vs 20% respectively^{179, 194}. To follow on from these studies, the aim of this study was firstly, to compare two doses of clopidogrel and the newer P2Y12 receptor antagonist, prasugrel and secondly, to assess the effect in a CAD population. We similarly demonstrated that least 80% of donor platelets are needed to restore platelet function after clopidogrel 600mg loading with more than this needed for patients loaded with more the more potent 900mg dose and prasugrel

60mg. This has important implications for the use of platelet transfusion in-vivo in this group of patient in that, more units of donor platelet could be needed to achieve haemostasis.

Our study has several limitations. This was an exploratory study and so sample size could not be estimated with precision. We used the patient own untreated platelets as the donor platelets, which differs somewhat from real-life whereby multiple donors may contribute to a pool of blood bank platelets. However, other studies have shown that blood bank platelets have variable platelet function and so this would have introduced inconsistent effect into this comparison study.

Conclusion

Ex-vivo platelet transfusion in increasing concentrations resulted in restoration of platelet reactivity in response to ADP in CAD patient loaded with P2Y₁₂ receptor antagonists. The degree of restoration was less after in clopidogrel 900mg and prasugrel 60mg in comparison with clopidogrel 600mg loading doses. It there maybe concluded that the stronger the platelet inhibition, the higher dose of platelet transfusion needed to fully reverse the antiplatelet effect.

Chapter 4

THE EFFICACY OF *IN-VIVO* PLATELET TRANSFUSION IN THE REVERSAL OF P2Y12 INHIBITION BY CLOPIDOGREL AND PRASUGREL: THE APTITUDE BLEED STUDY

Introduction

The management of patients with coronary heart disease on antiplatelet therapy undergoing cardiac and non-cardiac surgery is a challenge. Prevention of recurrent ischemic events including stent thrombosis is paramount especially in patients in the acute setting post ACS and PCI. For patients undergoing CABG, maintaining graft patency is also important¹⁹⁵. Cardiac and non-cardiac surgery is therefore performed not infrequently on patients treated by DAPT with an associated higher rate of perioperative bleeding resulting in increased morbidity and mortality¹⁷⁷. Many high risk patients are either continued on treatment or need to be operated before the time of offset of antiplatelet effect of P2Y12 receptor antagonists, namely a period of 7-10 days for adequate replacement of the inhibited platelet population with uninhibited ones. If these patients bleed during cardiac surgery, hemostasis can be challenging. The current recommended treatment strategy is similar to the general strategy adopted for any bleeding patient and includes mechanical diathesis, volume replacement and blood product transfusion. The inhibition of the P2Y12 receptor by the thienopyridines, clopidogrel and prasugrel is irreversible and thus there is no antidote to their effects. Platelet transfusion has been a proposed treatment strategy to restore platelet reactivity on the basis that the transfused, uninhibited platelets would act to augment overall platelet function, thus assisting haemostasis. However, there are no studies demonstrating the in-vivo biological efficacy of platelet transfusion used in this

setting. Furthermore, it is unknown whether platelet transfusion could have a detrimental effect especially in the recently stented patient.

. For the patient at high risk for recurrent thrombotic event or in the setting of urgent or emergent surgery where discontinuation of P2Y₁₂ inhibitor therapy is not possible, platelet transfusion is recommended acutely as a therapy and is commonly used in CABG. However, there are no studies that demonstrate the biological or clinical efficacy of this strategy. Furthermore it is uncertain whether platelet transfusion poses increased thrombotic risk especially in recently stented patients.

The Antagonize P2Y₁₂ Treatment Inhibitors by Transfusion of platelets in an Urgent or DElayed Timing after ACS or PCI presentation (The *APTITUDE* study) was designed to assess the effect of platelet transfusion on the restoration of platelet reactivity in patients who bleed during coronary artery bypass surgery undertaken on dual antiplatelet therapy.

Hypothesis

That platelet transfusion is a biologically efficacious strategy to treat to restore platelet reactivity in perioperative bleeding patients on P2Y₁₂ therapy

Methods

Study Population

Between January and August 2012, 33 patients were enrolled in this single centre study at The Heart Institute, Pitié-Salpêtrière University Hospital, a busy cardiovascular centre that performs 1500 procedures per year. The protocol was approved by the institutional ethics committees and all surviving subjects gave written retrospective consent. Patients were suitable for enrolment if they were aged 18 years and over, were on either clopidogrel or prasugrel therapy for at least 48 hours and with a last dose taken

within the previous 24 hours, and were to be transfused at least one pool of platelets intra or perioperatively. Exclusion criteria included treatment with IV glycoprotein IIb/IIIa inhibitor therapy within the previous 15 days and any participation in another clinical research study.

The number of standard concentrate units transfused were at the discretion of the surgeon and anesthetist and guided by the recommendations of the French Agency for the Safety of Health Products (AFSAPPS) and the British Committee for Standards in Haematology Guidelines^{178, 196}. Transfused platelets concentrates were collected and prepared according to the Council of Europe (CoE) and the and one of two types of products were used: apheresis platelet concentrate or pooled buffy-coat derived platelet concentrate.

Blood loss in the intensive care unit was measured by calculating the volume of mediastinal and pleural drainage for the first 24 h. The number of units of red blood cells (RBC), platelets (PLTS), fresh frozen plasma (FFP), and cryoprecipitate (CRYO) transfused in the operating theatre and intensive care unit were recorded. Baseline demographics for each patient were recorded.

Study design

Blood sampling was performed immediately before (T0) and after each pool of platelets transfused was finished infusing (T1, T2 etc.). Samples for analysis were drawn from an arterial or central venous catheter connected to a flush system containing 0.9% sodium chloride solution. The first 10 ml drawn was discarded to eliminate the dilution effect of the saline. Blood was collected into Becton-Dickinson 3.2% citrate, 60 USP units of Sodium Heparin and K₂EDTA vacuette tubes.

Study Definitions

Type of surgery was classified as one of three categories: coronary artery bypass grafting, valve surgery (repair or replacement of valve), or complex surgery (multiple valve surgery or coronary artery bypass grafting with valve surgery or surgery involving the aortic arch). Urgency of surgery was classified as one of three categories: elective (the procedure could be deferred without risk), urgent (surgery indicated within 72hr of angiography or of unplanned admission), and emergency (surgery required same day). Redo surgery was defined by the patient having undergone cardiac surgery on a previous admission.

The operative and anaesthetic management was similar in all patients. Extracorporeal circulation was maintained by a roller peristaltic pump with a heparin-coated oxygenator and arterial filter. After the initial anticoagulation, additional doses of heparin were given to maintain activated clotting time above 480 s. After CPB, heparin was neutralized with protamine sulfate, 1mg protamine/ 100U of the total heparin dose. Blood products transfusion was performed according to the local protocol. The volume of chest tube drainage in the first 24h on the ICU was documented. The results of the platelet function analysis were not provided to the surgeons, the anaesthetists and the intensivists in charge of the patients.

Flow cytometry

VASP measurement

The phosphorylation of the vasodilator-stimulated phosphoprotein (VASP) was measured with a Beckman Coulter FC500 cytometer (Beckman Coulter, Villepinte, France) using Platelet VASP kits [Platelet VASP®; Diagnostica Stago (Biocytex), Asnières, France] according to the

manufacturer's instructions and as previously described¹⁰. Briefly, blood samples were incubated in vitro with ADP and/or prostaglandin E1 (PGE₁) before fixation. The VASP platelet reactivity index (PRI) was calculated from the MFI of each condition according to the formula: $VASP\ PRI = ((MFI\ (PGE_1) - MFI\ (PGE_1+ADP)) / MFI\ (PGE_1)) \times 100$.

P-Selectin measurement

Flow cytometry was performed in <2 h after venipuncture for P-selectin measurement. To determine platelet P-selectin expression, blood samples previously activated by a dose of 20 mM thrombin receptor activating peptide (TRAP), 20 mM and 10mM ADP were mixed with saturated concentrations of anti-CD62p-PE (Beckman Coulter, Villepinte, France) monoclonal antibody and anti-CD41a-FITC monoclonal anti- body (Beckman Coulter, Villepinte, France). After staining with antibodies, samples were incubated for 30 min in the dark and diluted with 1 mL of FACS solution. Samples were immediately processed for flow cytometric analysis. To determine platelet CD62P expression, individual platelets were identified by size (forward and scatter) and anti-CD41a-FITC immunofluorescence using a logarithmic scaled dot plot. P-selectin expression on the surface of platelets was defined as positive for anti-CD62P-PE. Variation in activation corresponded to percent of gated platelets after activation by agonist minus percent of gated platelets at rest.

Light transmission Aggregometry

Platelet-rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 100g for 10 minutes at 20°C. Platelet poor plasma (PPP) was obtained by further centrifugation at 4500g for 15 minutes. In vitro platelet aggregation in PRP was measured at 37°C by Light Transmission Aggregometry (LTA) (model 490-4D, Chrono-Log Corp, Kordia, the Netherlands) and was induced by the addition of Adenosine Diphosphate (ADP) (Sigma-Aldrich, Saint Quentin Fallavier, France) at final concentrations of 20 µmol/L for prasugrel, clopidogrel

testing. The measure was performed twice and the results were expressed in maximal platelet aggregation (MPA) and residual platelet aggregation (RPA), 6 minutes after induction of aggregation by agonist. Prespecified criteria used to define non-evaluable samples were: lack of sufficient signal, hemolysis, and PRP platelet count <150 000/mL and an unstable baseline.

VerifyNow

The VerifyNow(Accumetrics,CA,USA) system is a rapid automated whole blood assay that measures agglutination of fibrinogen-coated beads in response to specific agonists. Measurement of platelet response to clopidogrel and prasugrel with the VerifyNow P2Y₁₂ assay was done according to the package insert (Accumetrics Corporation, San Diego, CA). This is a rapid (less than 5 minutes) platelet function assay designed to measure directly the effects of drugs on the P2Y₁₂receptor. The assay is a turbidimetric-based optical detection system that, like optical aggregometry, depends on the ability of activated platelets to bind fibrinogen. The assay contains 20 µmol ADP and 22 nmol PG E1 to reduce the activation contribution from ADP binding to P2Y₁receptors, thus making the assay specific for the effects of ADP mediated by P2Y₁₂.

Results were expressed in P2Y₁₂ Reaction Units (PRU) in response to iso-Thrombin Receptor Activating Peptide (iso-TRAP) and in response to ADP-PGE₁. Iso-TRAP strongly activates platelets despite of P2Y₁₂ receptor blockage by thienopyridine or aspirin and reflects the platelet reactivity without treatment¹⁹². The device provides an estimated inhibition (in percent) without pre-thienopyridine sample by reporting the ratio of the results of the ADP-PGE₁ and iso-TRAP channels.

Thromboelastograph (TEG)

Analysis was performed with the TEG[®] 5000 Thrombelastograph[®] Hemostasis Analyzer system (Haemonetics Corporation, Braintree, MA). A cuvette with a surface coating of heparinase to deactivate any heparin in the sample was loaded onto the analyser. Immediately before analysis a 1 ml sample of citrated blood was added to a vial containing kaolin (a clotting activator). After good mixing and without delay, 360 μ l was added to the cuvette with 20 μ l of calcium chloride to reverse the effect of the citrate. The analyser works on the following principle: for a motor to drive a paddle and keep it rotating at a fixed speed in a cuvette containing blood, more current is required as a clot forms and retards paddle movement. Since the paddle rotates alternately in both directions (clockwise and anti-clockwise), two opposing signals are generated (**Figure 3.1**).

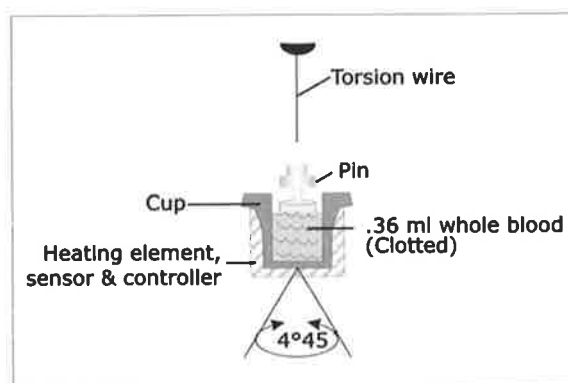


Figure 4.1 These are combined and graphed against time to form the thromboelastogram.

The thromboelastogram (**Figure 3.2**) was displayed on a computer running TEG[®] analytic software which is connected to the analyser via an A/D interface box. The values of normal controls were displayed enabling comparison. The time taken to get a result was of the order of 15 to 30 mins.

TEG[®] variables included reaction time (time to initiate the coagulation cascade), k (time to reach an amplitude of 20 mm), maximal amplitude (MA, measuring clot strength), angle (speed of clot formation), and the fibrinolytic percentage 30 minutes after MA (LY30).

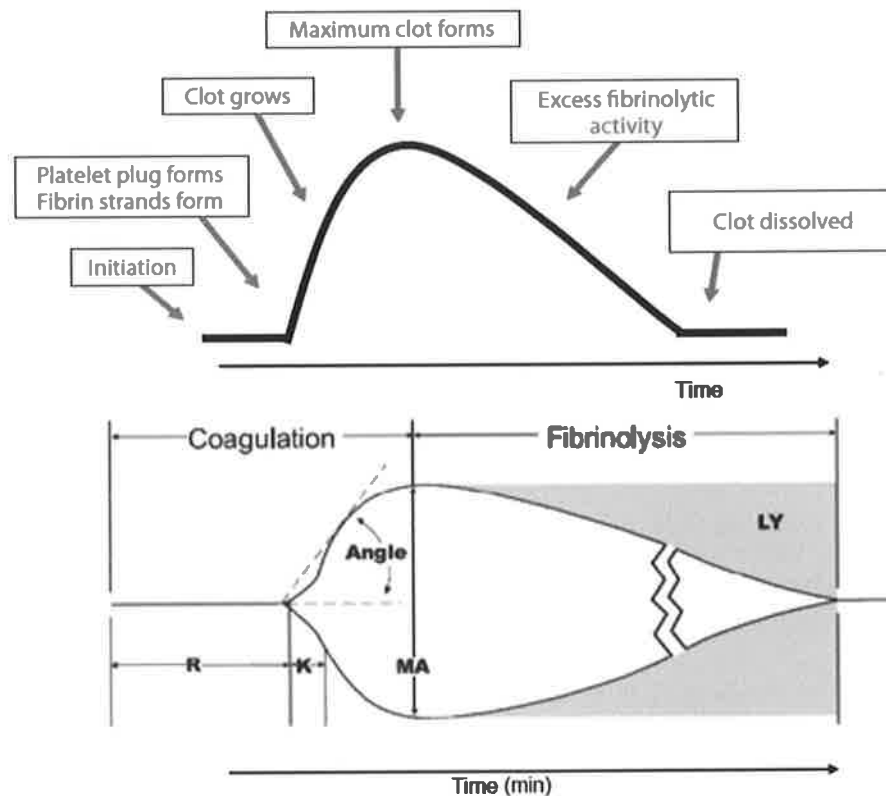


Figure 4.2 Thromboelastograph (TEG) tracings

The R time, angle (A), maximum amplitude (MA) and the lysis 30 which represents the amplitude after 30 min. This final value is equivalent to the percentage lysis after 30 min; in other words, the A_{30} value divided by the MA value multiplied by 100 gives the percentage lysis which is a function of fibrinolysis.

Platelet Mapping

The degree of suppression of the platelet (adenosine diphosphate) ADP receptor with clopidogrel, prasugrel and the Thromboxane A2 receptor with aspirin can be assessed indirectly with a variation of the standard method. The standard method performed above

indicates clot strength with maximum platelet activity independent of the level of aspirin or clopidogrel in the sample. This applies because there is enough thrombin in the blood sample to fully activate the IIb/IIIa receptor in the absence of heparin.

For the platelet mapping analysis, three other cuvettes loaded onto the analyser were used. Blood that had been stored in the heparin containing vacuette tubes was used in this analysis. The heparin delays clot formation sufficiently for the action of three activators. Fibrin activator was added to all three cuvettes. This forms a fibrin clot unstrengthened by activated platelets giving a tracing which is consistent with zero platelet activity. The second and third cuvettes had ADP and arachadonic acid added which directly activate the platelets to strengthen the clot irrespective of the blockage of the IIb/IIIa receptor by heparin. This gives traces that are dependent on the degree of suppression of the TXA2 or ADP receptors. If there is no drug suppression of the receptor the tracing settles close to that of the initial citrate sample giving maximum platelet activity (MA ADP and MA AA). If there is total suppression of the receptor then the tracing settles close to the fibrin alone tracing. Intermediate positions of the grey line between the two limits are assessed by the TEG[®] computer software and reported as percentage inhibition of the appropriate receptor.

Statistical Analysis

Continuous variables are expressed as mean \pm SD and categorical variables as frequency and percentage. After testing the normal distribution and equal variances using the Levene F-test, unpaired t tests were used to compare continuous variables between groups. Baseline characteristics of patients were compared by chi2 tests for categorical values and continuous variables by Student's test. Two groups were compared by Student's test, in case of unequal variances the Welch correction was made. Paired t-tests were used to compare continuous variables before and after loading dose in the same group.

The primary endpoint of APTITUDE BLEED was the difference in relative increase of VASP PRI (platelet reactivity index) obtained by flow cytometry.

All statistical analysis was performed using the GraphPad Prism (Dan Diego, California). All tests were two-sided with a statistical significance of 0.05.

Results

A total of 33 patients (67% male) were included in the study. The baseline characteristics are outlined in **Table 1**. The prevalence of cardiovascular risk factors was high, including diabetes mellitus (48.5%), hypertension (81.8%) and peripheral vascular disease (24.2%). Almost half had a recent history of ACS (< 6 weeks) and 73% had PCI within the previous year. All patients were on DAPT comprising aspirin and either clopidogrel (n=28) or prasugrel (n=5). The majority of patients included underwent CABG (n=23), 15.2% had single valve surgery and the remaining patients either had complex surgery and one patient underwent cardiac transplantation. All operations were performed on cardiopulmonary bypass (CPB). Post-CPB cell salvage and processing was used in 24 (73%) patients.

Table 4.1 Baseline characteristics and operative details.

	Total (n=33)
Age (years)	64.4±10.5
Male sex	22(66.7)
Body mass index (kg/m ²)	26.1±5.4
Creatinine Clearance	60.8±44.6
Cardiovascular Risks	
Hypertension	27(81.8)
Dyslipidaemia	23(69.7)
Family History	5(15.2)
Current Smoking	13(39.4)
Diabetes	16(48.5)
Cardiovascular History	
Percutaneous Coronary Intervention	24(72.7)
Drug Eluting Stents	12(36.4)
History of Myocardial Infarction	18(54.5)
Recent ACS within 30 days	15(45.5)
STEMI	8(24.2)
NSTEMI/UA	7(21.2)
Left Ventricular Ejection Fraction	47.69±14.8)
Antiplatelet therapy pre op	
Aspirin	33(100)
Clopidogrel	28(84.8)
Prasugrel	5(15.2)
Surgical urgency	
Elective	20(60.6)
Urgent	7(21.2)
Emergency Surgery	6(18.2)
Type of surgery	
Coronary artery bypass grafting	23(69.7)
Single valve	5(15.2)
Cardiac Transplant	1(3)
Complex	4(12.1)
Aortic valve	6(18.2)
Mitral valve	2(6.1)
Tricuspid valve	0(0)
Redo	1(3)
Grafts	
Arterial only	19(82.6)
Total number of grafts	3.045(1)
Arterial	1.957(0.4)
Vein	0.1379(0.4)

Platelet transfusion

The decision to transfuse was at the discretion of the cardiothoracic surgeon and anesthetist. Most patients were transfused due to excessive intraoperative bleeding and the transfusion was commenced either just after discontinuation of CPB and administration of protamine sulphate (n=25) or in the immediate post-operative period (<12 hours)(n=8).

Patients were transfused according to the weight-based guidelines, a mean of 8.7 concentrate units of platelets (1 unit = 0.7×10^{11} plts) with the majority transfused with pooled buffy-coat platelets (n=25). No platelet transfusion reactions occurred. Other products including units of red blood cells, fresh frozen plasma and fibrinogen were administered (**Table 3.2**).

Table 4.2 Details of blood loss and blood product transfusion

Blood Product Transfusion	
Platelet Concentrate Units (T0-T1)	8.7±1
Apheresis platelet concentrate	25(75.8)
Pooled buffy-coat platelet concentrate	8(24.2)
RBC within 48hours post operatively	26(78.8)
RBC units	3.3±2.6
Fresh Frozen Plasma(FFP)	10(30.3)
FFP units	3.9±3.5
Fibrinogen	5(15.2)
Blood loss	
Cell-saver volume(ml)	495.3±337.8
Chest tube drainage (mL)/24hours	575.8±439.7
Chest tube drainage (mL) total	965.1±794.9

Bleeding

Laboratory values for hemoglobin, hematocrit and platelet count pre-operatively, at the time of transfusion, the nadir and the ICU values are shown in **table 3.3**. Blood volumes from post-CPB cell salvage (Cell saver®) and chest drain volumes are also shown.

Table 4.3 Haematological laboratory values perioperatively.

	Pre-operatively	At time of transfusion	Nadir	ICU Post-operative
Hematocrit	12.2±1.9	9.5±1.5	8.3±1.4	10.8±1.8
Hemoglobin	36.1±5.2	28.3±4.2	24.9±3.8	31.9±5.0
Platelets	238.2±106.9	190.5±79.8	158±60.9	210.1±81.5

Effect of platelet transfusion on platelet activation

Successful cytometric analysis was achieved in 30/33 patients and the mean baseline (T0) VASP PRI was $40.5 \pm 22.9\%$. When compared to baseline values, transfusion resulted in a 30.1% increase in VASP-PRI % ($p=0.0014$) (**Table 3.4**). When comparing the effect seen in clopidogrel versus prasugrel patients, transfusion resulted in a significant increase in clopidogrel patients (42.0 ± 22.8 vs $56.1 \pm 15.74\%$ + $p=0.0009$) and although numerically higher, the increase was not significant in prasugrel patients ($20,86 \pm 14,20$ vs $34,58 \pm 15,40\%$; $P = 0.296$)(**Figure 3.3**).

Table 4.4 Results of platelet function tests performed before and after transfusion

	Pre- Transfusion T0	Post- Transfusion T1	P value
Flow Cytometry			
VASP PRI (%)	40.56 ± 22.91	52.88 ± 17.02	0.0014*
P-selectine TRAP	79.3 ± 9.7	85.5 ± 4.5	0.0186*
P-selectine ADP 20	28.2 ± 18.3	23.5 ± 9.1	0.2052
P-selectine ADP 10	21.8 ± 12.6	18.9 ± 11.3	0.5872
Light Transmission Aggregometry			
MPA (%) ADP 20 µM	27.5 ± 24.9	33.4 ± 17.9	0.1189
RPA (%) ADP 20 µM	23 ± 24.2	24 ± 21.5	0.7609
MPA (%) AA 250 mM	0.5±1.2	0.75±1.4	0.243
RPA (%) AA 250 mM	0.5±0.75	0.6±1.1	0.567
VerifyNow			
PRU	260.6 ± 132.3	258.1 ± 99.2	0.6682
% inhibition	28.1 ± 31.5	27.6 ± 22	0.9405
Thromboelastograph (TEG)			
MA (CK-Hep)	60 ± 6.5	61.6 ± 6.3	0.0419*
R	6 ± 1.9	5.5 ± 1.7	0.3179
K	1.7 ± 0.6	1.5 ± 0.5	0.0459*
α Angle	63.4 ± 16.2	69.1 ± 5.2	0.0178*
Platelet Mapping			
MA ADP	24.6 ± 17.7	25.8 ± 16.1	0.4221
% inhibition ADP	72.4 ± 27.3	69.8 ± 25.6	0.4326
G (ADP)	2.1 ± 2.2	2.1 ± 1.7	0.5328
MA (AA)	19.7 ± 16	40 ± 18.4	0.0011*
% inhibition AA	89.9 ± 12.9	46.6 ± 29.7	0.0004*
G (AA)	1.2 ± 1	3.7 ± 2.6	0.0012*

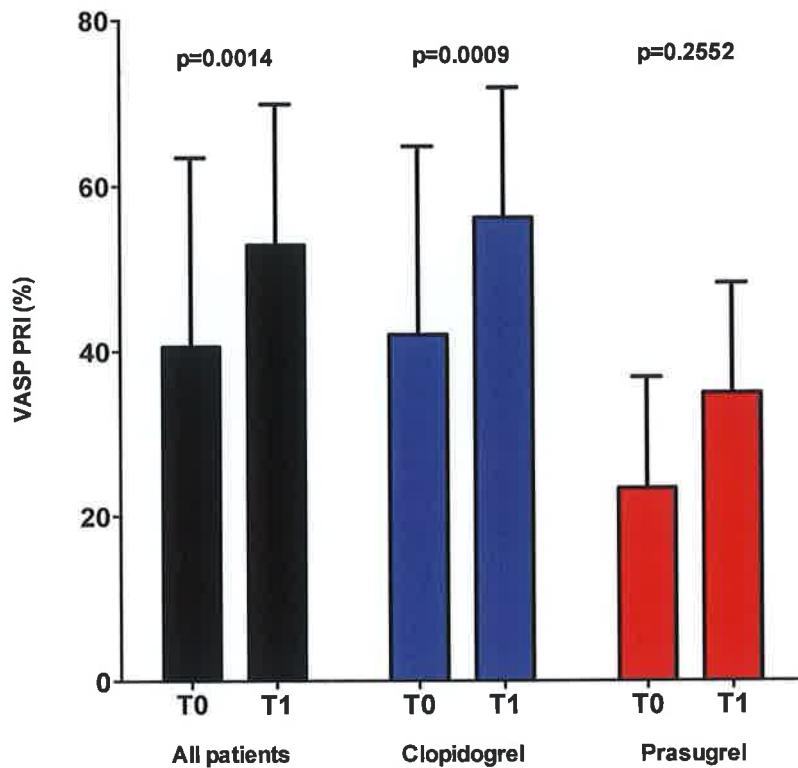


Figure 4.3 Comparison of vasodilator phosphoprotein (VASP) platelet reactivity index PRI% before and after platelet transfusion for all patients and according to P2Y12 receptor inhibitor therapy. Columned data with whiskers representative of SD.

The change in platelet aggregation measured by Δ VASP (T2-T1) did not correlate with clinical parameters known to affect platelet function including age ($r=0.07$; $p=0.713$), BMI ($r=0.108$; $p=0.579$) and creatinine clearance ($r=0.109$; $p=0.1$).

Other factors including diabetes ($p=0.666$) and recent MI ($p=0.62$) did not appear to affect the Δ VASP. However there was a trend to lower Δ VASP in active smokers (8.58 ± 14.9 vs. 17.6 ± 19 ; $p=0.181$) and higher Δ VASP in patients with hypertension (15.5 ± 18.2 vs. 5.8 ± 16.8 ; $p=0.240$).

The level of P-selectin expression at rest was subtracted from the raw value of P-selectin expression after activation by ADP in order to compare the variation in delta of P- selection expression at baseline and after platelet transfusion. There was a 7.8% increase in the variation of P-selectin expression after activation with 20µM TRAP ($P = 0.0186$). However there was non-statically significant decrease after transfusion after activation with 20µM ADP and 10µM ADP (**Table 3.4**).

Effect of platelet transfusion on platelet aggregation

There were many samples (n=11) that were deemed unsuitable for analysis due to hemolysis, platelet counts less than 150,000 per mm³ or unstable baseline signal. When compared with baseline values, transfusion did not cause a significant change in MPA or RPA after activation by 20 µM ADP and 1.25 mM arachidonic acid.

Effect of platelet transfusion on TEG[®] and platelet mapping[®]

Results of global hemostatic parameters by TEG and platelet mapping before and after the transfusion are detailed in Table X. There were increases in three of the four TEG curve parameters measured: a 2.6% increase in the MA, an 8.9% increase in the α Angle and an 11.7% decrease in the K time. With respect to the Platelet mapping, there was no significant difference between the parameters with ADP as agonist. However, platelet transfusion resulted in a two-fold increase in MA and % inhibition in response to arachidonic acid (AA) (**Figure 3.4**).

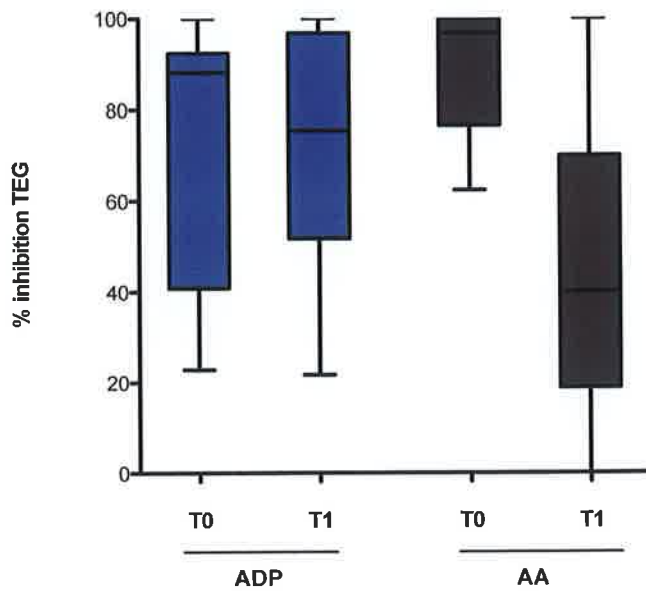


Figure 4.4 TEG Platelet mapping with estimated % inhibition in response to ADP and to AA.

Discussion

The main finding of this study is that platelet transfusion in patients treated by DAPT undergoing cardiac surgery with CPB appears restore platelet activity. VASP PRI is a very specific marker for P2Y12 receptor activation and was significantly increased directly after platelet transfusion. The effects of platelet transfusion not only affect P2Y12 receptor activation; we demonstrated in this study that P-selectin expression in response to TRAP was modestly increased after transfusion. The Thromboelastograph (TEG) is point-of-care technique that is frequently used test in the specific setting of cardiac surgery^{197, 198}. It gives an indication of the global haemostatic picture, measuring viscoelastic clot properties. Transfusion appeared to increase the rate and the extent of clot formation. Several ex-vivo studies have demonstrated the biological effect of platelet transfusion in clopidogrel and prasugrel treated healthy volunteers^{179, 194}. This is the first study to show in vivo biological

evidence supporting this commonly used therapy to reverse the effects of P2Y12 therapy to aid haemostasis.

These findings were not supported by other techniques commonly used in clinical research including other forms of testing using ADP as an agonist; LTA, VerifyNow, and platelet mapping. Although the MPA in response to 20 mM ADP showed a trend to increase, this was not statistically significant. This modest effect was also reflected with the data from VerifyNow and ADP platelet mapping. This may have several explanations. Firstly, many of patients underwent blood sampling at the nadir of values for hemoglobin, hematocrit and platelets. This was due to blood loss and the well described hemodilutional effects of cardiac surgery secondary to infused fluids and blood products and the CPD extra-corporeal circuit. Therefore, many of the samples were strictly not suitable for these techniques, which may have affected the accuracy of the findings. By contrast, the technique/assay used to measure VASP is less dependent on the quality of the sample with range for platelet count from 50,000 to 300,000 platelets/ μ L and testing can be performed on samples kept at room temperature for up to 48 hours after drawing with no loss of accuracy/validity. Secondly, it well described the degree of platelet dysfunction that occurs during CPD^{186, 199}. Platelet transfusion may not have been insufficient to reverse this dysfunction and increase platelet aggregation measured by these methods. This may explain why the effect is only seen with VASP which measures platelet activation and not aggregation.

Finally, it may be more difficult to reverse the effect of P2Y12 inhibitors. This is supported by our findings with TEG platelet mapping. Platelet transfusion was associated a two-fold increase in maximum amplitude (MA) in response to AA compared to no increase in response when using ADP as the agonist. This suggests that the effects of aspirin are

easier to overcome than P2Y12 receptor inhibitors. Ex-vivo studies have also demonstrated this with one study showing that clopidogrel treated healthy volunteers required replacement of 90% or more of their platelets with donor platelets to reach normal platelet reactivity as assessed by ADP-induced LTA versus 30% in aspirin treated patients as assessed by AA-induced LTA¹⁹⁴. It is uncertain as to the exact mechanism explaining this phenomenon.

The increase in VASP levels is modest but may be clinically significant. This study was not powered or designed to correlate this change with clinical values such as blood loss and mortality. What is evident is that, similar to the general population, one third of clopidogrel patients were resistant to clopidogrel therapy using the cut-off by consensus of 50% VASP PRI. By contrast, of the prasugrel patients included, there were no hypo responders and the mean VASP PRI was lower than that of clopidogrel before and after platelet transfusion. This would suggest that a higher dose of platelets are required in prasugrel patients to have the same final biological impact.

Limitations

There are several limitations to this study. Due to the exploratory nature of the study, a sample size calculation could not be conducted. There was no control group of either patients on P2Y12 receptor inhibitor or naïve of treatment undergoing cardiac surgery with CPB. An effect of improvement in platelet function after discontinuation of CPB regardless of platelet transfusion cannot be out ruled. However, each patient was its own control and blood sampling was performed before and directly after the treatment.

No blood sampling was performed a second time point and so the possible delayed enhancement of platelet function was not assessed. The design of the study was as such for

two reasons: Firstly, our objective was to examine the acute effect of this treatment owing to several reports by surgeons and anesthetists of the immediate intra-operative observed effect of transfusion. Secondly, there are very significant hemodynamic, hemostatic and platelet function changes that occur in the first hours after discontinuation of CPB and transfer to the intensive care unit. These changes coupled with concurrent potential blood components that would likely be transfused in a bleeding patient which are shown to impact platelet function were seen as significant confounders and would make it difficult to examine the precise impact of platelet transfusion. To limit these confounders, we performed blood sampling before and within 30 mins of the transfusion.

Finally, it has been demonstrated the platelet function of different pools of platelets is variable depending on issues such as preparation techniques and storage time. It therefore possible that the effect on the patients studied was variable but, at the same time, this is reflective of real-life treatment and thus reflects this variability.

Conclusion

This is the first study to examine the biological efficacy of platelet transfusion during cardiac surgery in patients treated by P2Y₁₂ receptor inhibitors. Our data indicates that platelet transfusion has an immediate impact on the augmentation of platelet function. However, the overall effect appears to be modest on thienopyridines and increased quantity of platelets may be required to have sufficient impact on hemostasis. Further larger scale studies are warranted especially with newer P2Y₁₂ receptor antagonists to correlate the biologic with clinical efficacy to further guide management of this treatment group.

Chapter 5

CONCLUSION AND FUTURE DIRECTIONS

This body of research showed that patients at either end of the spectrum of platelet response to P2Y₁₂ receptor antagonists can be accurately identified using genotype and platelet function testing. With adjustment of therapy we demonstrated that these patients can be correctly orientated to the optimal therapeutic window of platelet reactivity. This has the very significant implications for the field of personalised therapy. Whether modification of therapy based on this information this will result in improved clinical outcome remains to be answered in adequately powered, larger scale studies.

The concept of a therapeutic window for platelet reactivity in response to ADP has been proposed in recent years. Variability of response to clopidogrel was first described in 2003.

Since then, there is a significant body of evidence suggesting that high on-treatment platelet reactivity (HPR) is an independent risk factor for recurrent ischaemic events in clopidogrel.^{183, 184} The definition of HPR remains an issue of some debate and is a key issue in the design of trials on this issue. There are a number of factors that illustrate the complexity of this debate. Firstly, platelet reactivity can be assessed using multiple laboratory and point-of-care assays that have variable correlation between tests^{200, 201} and so the incidence of HTPR is test dependent. Secondly, there is a temporal variation in platelet reactivity after an acute coronary syndrome, leading to the question of when patients should be assessed and whether multiple assessments are indicated¹⁹³. Finally, the cut-off for each test is derived from correlation studies which all vary in design¹⁸⁴ and more recent data have challenged the cut-off sensitivity of in particular, the VerifyNow P2Y12 and the VASP-PRI% assays favouring more sensitive cut-off values (VN 208 vs 235 and VASP-PRI 60% vs 50%)^{193, 202}. We chose a cut-off of 30% inhibition with VerifyNow, which correlates with a PRU of 214 and thus correlates well with the newer threshold. VerifyNow is the most validated of the POC test and is FDA approved for this purpose.

At the opposite end of the spectrum, there exists no consensus on a threshold for increased bleeding risk associated with low on-treatment platelet reactivity or high platelet inhibition. The data would suggest however that these patients are at higher risk^{166, 167}. However, this has become more of an issue with newer P2Y12 receptor antagonists with more potent effect.

Clinical implications of genetic testing

From the evidence to date, there is no clear indication that routine platelet function testing in daily clinical practice improves outcome. Similarly, there evidence does not support the use of genetic testing in the general population. Our study is the first proof of concept study

to examine the use of a hybrid approach combining platelet function analysis with easily performed rapid genetic testing to appropriately select P2Y12 antagonist therapy. By way of contrast to platelet function testing, genetic testing gives a fixed result that is not variable. The *CYP2C19**2 loss-of-function allele has the potential to be a powerful risk factor for ischemic cardiovascular events. Looking into the selected papers of the most recent meta-analysis, there were 14 per thousand patients in whom stent thrombosis could have been avoided using genotyping, which is actually more than many drugs that have been tested in mega trials¹²⁵. Genotyping and alternative treatment strategies could therefore potentially prevent stent thrombosis and its associated high mortality and morbidity. Genotyping has somewhat been hampered by high cost and laboratory testing taking 3-5 days for a result. Point-of-care genotyping has the potential to provide more rapid and inexpensive testing. Therefore, genotyping should not appear as an overly exuberant approach and the current lack of randomized trials should not be an argument against following best practice. The future of genetic testing in this field may lie in improved risk stratification in certain coronary populations such as the elderly who have both bleeding and thrombotic risk concurrently and in whom P2Y12 therapy selection is a complex issue. As we demonstrated in the GAMMA study, individualizing the treatment regimen based on clopidogrel responsiveness can be performed with bedside platelet-function testing, providing risk stratification for ischemic events²⁰³ and avoiding the overtreatment that is associated with an excess of bleeding events. In practical terms, rapid genotyping and platelet function analysis could be performed together in high risk patients with further platelet function analysis on follow up to further refine therapy. Toward the future, refinements in genotype markers which could predict more common events such as peri-PCI myocardial infarction and bleeding in vulnerable populations such as the elderly are the next challenges²⁰⁴.

In this body of work, we focused on how to minimize bleeding events and also on how to effectively treat bleeding that occurs thereby minimizing the associated significant morbidity and mortality. We showed how, faced with the irreversible action of these agents, platelet transfusion appears to be a valid option for the rapid restoration of P2Y12 receptor activity in cases of bleeding after ACS or in the setting of surgery.

Up to 15% of patients with ACS will require coronary artery bypass surgery (CABG) and bleeding complications in patients have been associated with adverse outcomes. This risk appears to be higher with newer P2Y12 receptor antagonists. However, in an analysis of the TRITON-TIMI 38 data, the use of prasugrel was associated with greater 12-hour chest tube drainage volumes and platelet transfusion rates but without any significant differences in red blood cell transfusions total hemostatic components transfused, or total blood donor exposure²⁰⁵. It maybe hypothesized that platelet transfusion had a clinical impact thus reducing the overall red cell transfusion.

Our findings give some biological evidence for this possible clinical benefit. Just as with personalised therapy, further larger scale studies correlating biological efficacy with the reduction in adverse outcomes with these agents, especially with newer more potent agents are warranted.

PUBLICATIONS ARISING FROM THIS THESIS

1. O'Connor SA, Hulot JS, Silvain J, Cayla G, Montalescot G, Collet JP.
Pharmacogenetics of clopidogrel.
Curr Pharm Des. 2012 Jun 19. [Epub ahead of print] PubMed PMID: 22724417.
2. O'Connor S, Montalescot G, Collet JP.
The P2Y12 receptor as a target of antithrombotic drugs
Purinergic Signal. 2011 Sep;7(3):325-32. Epub 2011 Jun 28. PMID: 21710143
3. Collet JP, O'Connor S,
Clinical effects and outcomes with new P2Y12 receptor antagonists in ACS
Fundamental and Clinical Pharmacology 2011 Sep 5. doi: 10.1111/j.1472-8206.2011.00984.x.[Epub ahead of print] PubMed PMID: 21895760
4. Cayla G, Hulot JS, O'Connor SA, Pathak A, Scott SA, Gruel Y, Silvain J, Vignalou JB, Huerre Y, de la Briolle A, Allanic F, Beygui F, Barthélémy O, Montalescot G, Collet JP.
Clinical, angiographic, and genetic factors associated with early coronary stent thrombosis.
JAMA. 2011 Oct 26;306(16):1765-74. PMID: 22028352.

SPECIFIC WORK CARRIED OUT BY CANDIDATE

Chapter 2: The GAMMA study

I worked on a fulltime basis in the research department for this entire study and I intrinsically involved in all aspects of the conduction of this study from beginning to end. This was prospective study that had an intense recruitment of 241 patients over a 14 month period. The study was carried out by a team of three: clinical research assistant, masters student and I as team leader. I performed over 60% (130 patients) of the work including patient screening, recruitment, consent, blood sampling, rapid genetic testing, eCRF completion and patient follow up.

With regard to the laboratory techniques, I trained and became proficient in all tests before study commencement including light transmission aggregometry, VerifyNow, TEG analysis and platelet mapping and flow cytometric analysis for VASP measurement. I also completed training in rapid genetic analysis with the VERIGENE® *CYP2C19* assay which is a fully automated test.

The platelet function laboratory is a high volume laboratory performing all of the above tests on a regular basis for several ongoing studies. There is a full-time laboratory technician who performed some of the testing, however I performed the majority of testing for this study.

I performed the data extraction from the database and independently performed all the statistical analysis.

Chapter 3: APTITUDE ACS/PCI

In contrast to the GAMMA study, which was somewhat a team effort, the APTITUDE ACS/PCI and APTITUDE BLEED studies were down to almost total individual effort on my part with some assistance from a masters student for a two-month period.

The concept for the study was Jean-Philippe Collet's and I designed the studies in particular the platelet mixing technique. I performed the screening and recruitment of almost all the patients for APTITUDE ACS/PCI. I performed over 80% of the platelet mixing and platelet function analysis studies.

Chapter 4: APTITUDE BLEED

Again I designed the study and I performed the majority of platelet function analysis including light transmission aggregometry, VerifyNow, TEG and platelet mapping and flow cytometric analysis for P-selectin expression and VASP measurement

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STUDY ABBREVIATIONS

ADP	Adenosine Diphosphate
AE	Adverse Event
AFFSAPS	Agence française de sécurité sanitaire des produits de santé
AHA	American heart Association
APTITUDE	To Antagonize P2Y12 Treatment Inhibitors by ex-vivo Transfusion of platelets in an Urgent or DELayed Timing after ACS presentation
ASA	Acetylsalicylic Acid
ARC	Academic Research Consortium
AUC	Area Under the Curve
BARC	Bleeding Academic Research Consortium
BMS	Bare Metal Stent
CABG	Coronary Artery Bypass Graft Surgery
CPB	Cardiopulmonary bypass
CV	Cardiovascular
DES	Drug Eluting Stent

EACTS	European Association for Cardio-Thoracic Surgery
EMA	European Medicines European Agency
ESC	European Society of Cardiology
GRAVITAS	Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis and Safety
GUSTO	Global Strategies for Opening Occluded Coronary Arteries;
IRB/IEC	Institutional Review Board/Independent Ethics Committee
ICH GCP	International Conference on Harmonization Good Clinical Practice
ISTH	International Society on Thrombosis and Haemostasis
ITT	Intention To Treat
IVRS	Interactive Voice response System
JAMA	Journal of Medical Association
LOE	Level Of Evidence
MD	Maintenance Dose
MACE	Major Adverse Cardiovascular Event
NEJM	New England Journal of Medicine
NSTE-ACS	Non-ST-Segment Elevation Acute Coronary Syndrome
PCI	Percutaneous Coronary Intervention

PEP	Primary End Point
PP	Per Protocol
PR	Platelet Reactivity
PT	Platelet Transfusion
SAE	Serious Adverse Event
SC	Steering Committee
STEMI	ST-segment elevation myocardial infarction
STEEPLE	Safety and Efficacy of Enoxaparin in Percutaneous Coronary Intervention Patients, an International Randomized Evaluation
TIMI	The TIMI Study Group. Named for a series of national clinical studies known as the TIMI (Thrombolysis in Myocardial Infarction) studies launched in 1984 by Brigham and Women's Hospital.
PRU	P2Y ₁₂ Reaction Unit
ROC	Receiver operating characteristic
ST	Stent Thrombosis

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