In Vivo Studies of Coronary Stent Thrombosis in Model Systems and in Man

Doctor of Medicine (Research)

2019

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Abstract

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Introduction

Coronary stent thrombosis (ST) is a significant complication of percutaneous coronary intervention (PCI) associated with poor clinical outcomes. The aim of this thesis was to investigate the underlying causes and mechanisms of ST and to study the potential use of novel coronary stents.

Methods

ST patients and controls were recruited to the PRESTIGE study and underwent detailed clinical assessment, optical coherence tomography (OCT), platelet function testing (PFT) and thrombin generation studies. A rabbit iliac model was developed to investigate the bio-neutrality of RGD-peptide stents and the thrombogenicity of Absorb bioabsorbable vascular scaffolds (BVS).

Results

ST patients in the PRESTIGE study were younger, had more diabetes mellitus, thromboembolic antecedents, recent non-cardiac surgery, first generation drug eluting stents (DES), less aspirin use and less circumflex arteries involved. The OCT studies showed predominantly malapposition and under-expansion in early ST and uncovered struts and neoatherosclerosis in very late ST. Clopidogrel 'high on treatment platelet reactivity' (HPR) rates were high. The in vivo studies demonstrated more platelet adherence to the BVS than standard DES. The recovery model showed good healing and endothelialisation of RGD stents, with no ST at 28-days. However, this positive outcome was similar in the control bare metal stents (BMS).

Conclusion

ST patients have risk factors that can potentially be used to predict risk in patients undergoing PCI. The rate of malapposition and under-expansion seen on OCT in early ST highlights the importance of adequate stent sizing and post dilation. The rate of uncovered struts and neoatherosclerosis in very late ST highlights the need for a more bio-neutral stent and aggressive secondary prevention. The in vivo results may partly explain some of the disappointing outcomes reported in the ABSORB II and III studies. Finally, the HPR rate on clopidogrel warrants further consideration when deciding on DAPT strategy in high risk patients.

Acknowledgements

The investigations in this thesis would not have been possible without the assistance of many individuals, but I would specifically like to thank the following key people:

- My supervisors, Professor Anthony Gershlick and Professor Alison Goodall for their support and guidance, without which this thesis would not have been possible.
- Dr Chirag Patel and Dr Katherine Simpson in the Cardiovascular Sciences department at the University of Leicester for their help with the in vitro and in vivo studies as well as the administration and follow-up of PRESTIGE patients.
- Debbie Bursnall in the Division of Biomedical Sciences at the Central Research Facility (CRF), University of Leicester for her help with the in vivo studies.
- Florence Lai for her help with the statistical analyses.
- The cardiac catheter lab staff at the Glenfield Hospital in Leicester and all the staff at the CRF.
- All my PRESTIGE study co-investigators and research nurses.

Finally, but certainly not least, I would like to acknowledge the continued patience and support of my wife, Mandeep, over the last several years. I am deeply grateful for her unwavering encouragement, understanding and love throughout my career and during my time in research. I would like to dedicate this thesis to both her and our son, Kabir.

Table of contents

List of tables

Table of Figures

Chapter 1: Introduction

1.1. Thesis introduction

Coronary artery disease is commonly treated with coronary angioplasty and stenting. Despite major advances in device technology and pharmacotherapy, coronary stent thrombosis continues to be the most serious complication of percutaneous coronary intervention and is associated with a high rate of acute myocardial infarction and death. Although relatively rare, the widespread use of coronary stents worldwide has resulted in a substantial increase in the absolute number potentially at risk of this complication.

This thesis describes translational research into the pathophysiology of stent thrombosis, including in vitro studies, an in vivo animal model to test stent thrombosis and a clinical study. The in vitro studies were designed to compare the potential for thrombin generation measured in the stored plasma of stent thrombosis patients and 'healthy' controls, with the aim of potentially providing an additional therapeutic target to reduce the risk of stent thrombosis. An in vivo rabbit iliac model was developed to investigate the acute thrombogenicity of bio-absorbable vascular scaffolds and the long-term healing of novel prototype coronary stents designed to be more biocompatible and thereby reduce the ongoing risk of stent thrombosis. The clinical study is the UK arm of a European multi-centre observational study, PRESTIGE, funded through the European Union Seventh Framework programme (FP7), which aimed to determine the characteristics of patients presenting with stent thrombosis, using detailed demographic and clinical data, as well as platelet function studies and intra-coronary imaging.

The overall aim of these studies was to understand the underlying risk factors contributing to the development of stent thrombosis and focus in on mechanisms involved, through in vitro and clinical studies, and to develop strategies to reduce its risk, including the potential use of more potent antithrombotic agents and novel intracoronary stents.

1.2. Coronary artery disease

Coronary artery disease (CAD) remains the leading causing of death worldwide, resulting in 8.14 million deaths (16.8%) in 2013, an increase from the 5.74 million deaths (12%) in 1990 (1). In addition to its high mortality, CAD carries with it a significant burden of morbidity in terms of health and economic costs. In the UK, there were 500,000 NHS hospital admissions related to CAD in 2012/13, with over 175,000 inpatient episodes of acute myocardial infarction (MI) (2). According to prevalence data from the Clinical Practice Research Datalink (CPRD) database (2013), more than 915,000 people living in the UK have a history of MI and over 1.3 million people are living with angina. More than £6.8 billion (0.4% of the UK total GDP) was spent on treating cardiovascular disease as a whole within the NHS in England in 2012/13 (2).

CAD is thought to be initiated by changes to the vascular endothelium, which can start at an early age, but is exacerbated by various risk factors including smoking, hypertension, hyperlipidaemia and diabetes mellitus. This leads to progressive thickening of the smooth elastic inner lining of the coronary arteries and development of atherosclerotic plaques containing varying degrees of lipid, calcium, old red blood cells and inflammatory cells. In the presence of significant atheromatous luminal narrowing, myocardial ischaemia occurs, which usually manifests clinically as angina. With plaque erosion or rupture, a thrombotic response leads to partial or complete coronary occlusion, causing an acute coronary syndrome (ACS).

Arterial thrombi typically form under conditions of high blood flow and are predominantly made up of platelet aggregates, giving them the appearance of ‘white thrombus’. Arterial thrombi principally originate at the site of atherosclerotic plaque erosion or rupture and their formation involves the release of prothrombotic material, including tissue factor, platelet aggregation and platelet adhesion to the vascular wall. The initially labile plaque is then stabilised by insoluble fibrin produced on activation of the coagulation cascade (figure 1.1 and 1.2 below).

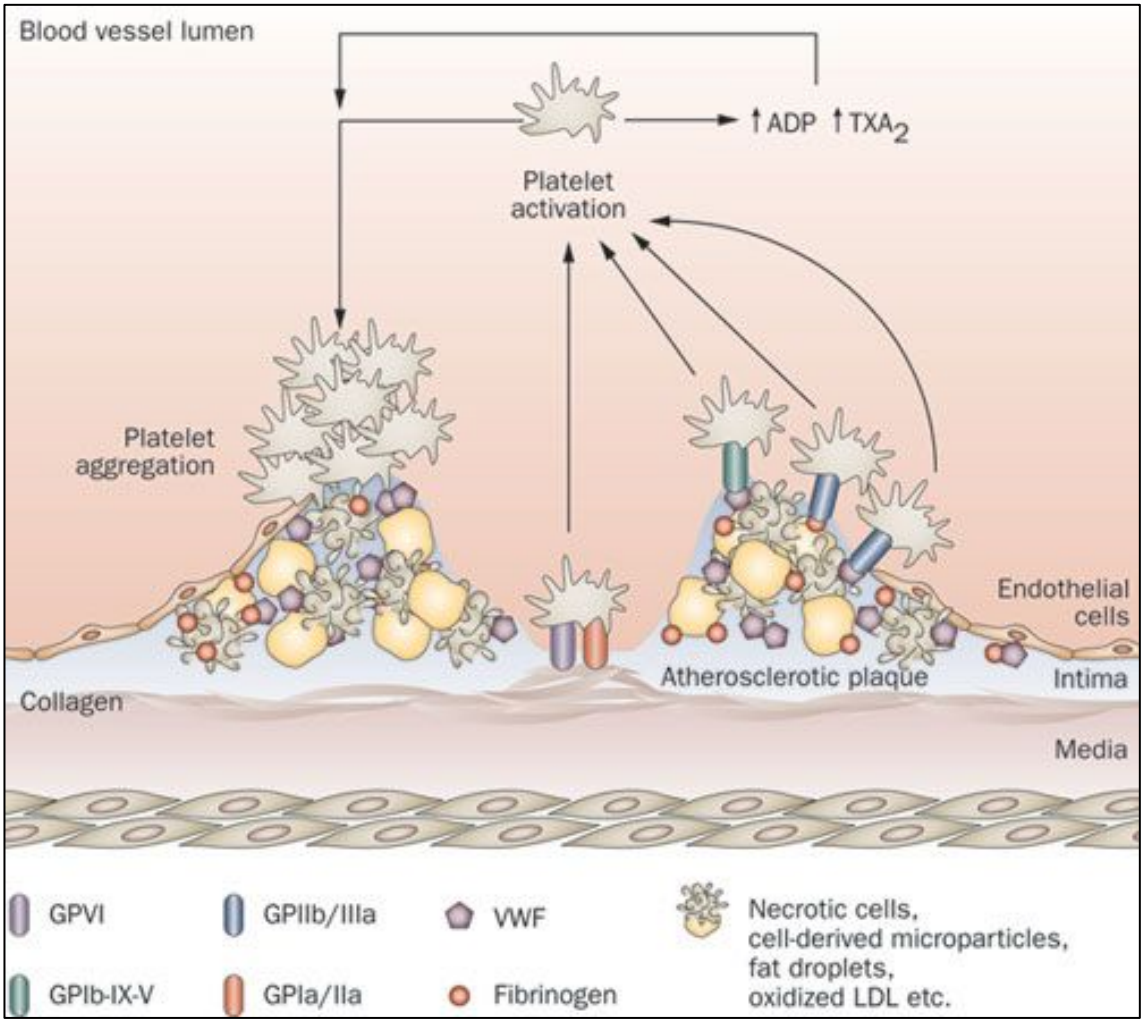


Figure 1.1. Platelet activation and aggregation in arterial thrombosis formation; reproduced from Lipp et al. (3)

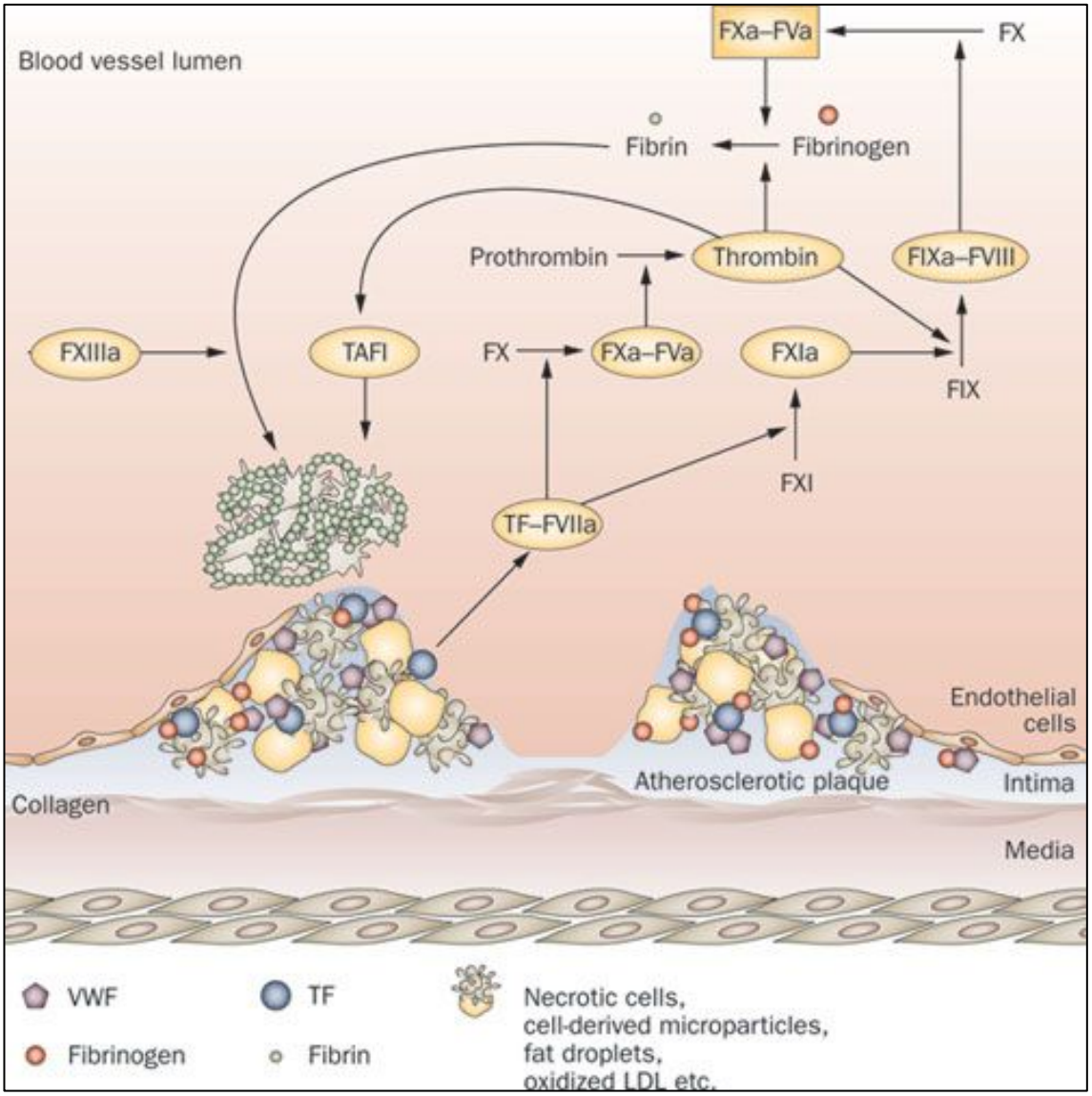


Figure 1.2. The activation of coagulation in arterial thrombus formation; reproduced from Lippi et al. (3)

Management of CAD involves the combination of lifestyle changes, antiplatelet medication, statins, anti-anginals and secondary prevention of modifiable risk factors. In addition, coronary revascularisation may be needed in the presence of ongoing ischaemic symptoms or a significant burden of ischaemia despite optimal medical therapy. This can be either with coronary artery bypass grafting (CABG) or more commonly with percutaneous coronary intervention (PCI). According to the latest BCIS national audit data, there were just over 97,000 PCIs and just under 17,000 CABGs undertaken in the UK for the year 2015.

1.3. Intracoronary stents

1.3.1. Historical background

Andreas Gruentzig performed the first percutaneous transluminal coronary angioplasty (PTCA) on the 16th September 1977 in Zurich, Switzerland (4). Up until this point, the only option for coronary revascularisation had been the more invasive CABG. He used a primitive balloon angioplasty catheter fashioned on his kitchen table to treat a significant stenosis in the proximal left anterior descending (LAD) coronary artery of a 38-year old man, with very acceptable acute and long term results (5). However, PTCA in general became known to be associated with a significant risk of acute vessel closure and a high rate of restenosis, due to plaque prolapse, elastic recoil, negative remodelling and smooth muscle cell intimal hyperplasia. These limitations, which led to a need in 35% of patients for a repeat procedure, were partly addressed by the introduction of metallic stents, with the first human coronary implant in March 1986 by Ulrich Sigwart in Lausanne, Switzerland (6). Such bare metal stents (BMS) scaffolded the vessel, sealing angioplasty-induced dissections and disrupted atherosclerotic plaques, thereby reducing the risk of acute vessel closure. In addition, they reduced late negative remodelling and therefore significantly reduced the rate of subsequent restenosis (7). They had no impact however on intimal hyperplasia (8).

As stent implantation became a standard treatment for CAD, two important limitations of early bare metal stents became apparent. The first was early stent thrombosis (ST) (up to 25% at that time), with the majority occurring in the first 14 days after implantation (9). The underlying mechanism was thought to be a combination of endothelial denudation and medial wall injury caused by PCI, leading to platelet adherence and aggregation on both the stent struts and exposed sub-endothelial surface. The introduction of dual antiplatelet therapy and antithrombotic agents used at the time of PCI reduced the risk of ST to a degree, as described later in this chapter (section 1.4). In addition, routine high-pressure stent deployment was considered important to abolish stent-wall pro-thrombotic spaces. The second important limitation was in-stent restenosis (8),

which occurred in 15-20% of cases, a higher incidence particularly seen in diabetic patients, in diffuse lesions, in vessels <3mm and in bifurcation lesions (10). Restenosis frequently resulted in recurrent ischaemic episodes requiring further coronary intervention. Over the next two decades, this led to the third major revolution in interventional cardiology, the development of drug eluting stents (DES) which reduced restenosis rates from 15% with BMS to ~5 % with DES.

1.3.2. Drug eluting stents

By the late 1990s, there was improved understanding of the underlying mechanisms responsible for in stent restenosis, and was due to an exaggerated healing response to injury following PCI, combined with the presence of a metallic foreign body in the vessel, stimulating the growth of neointimal (8). This was thought to be caused by the recruitment of inflammatory cells to the site of injury, the migration and proliferation of vascular smooth muscle cells from the media to intima and late remodelling of the vessel. To address this issue, stents, coated with polymers to deliver anti-proliferative drugs were developed. Initially, sirolimus (rapamycin), a macrolide antibiotic with immunosuppressant, anti-proliferative and anti-migratory properties, along with paclitaxel (taxol), a microtubule-stabilising agent with potent anti-proliferative activity, were extensively investigated. The RAVEL study (11) and later the SIRIUS trial (12), using a sirolimus-eluting stent, demonstrated dramatic reductions in restenosis rates in simple (type A) lesions, with reductions from 26.6% to 0% at 1 year and from 35.4% to 3.2% respectively, translating to a significant reduction in major adverse cardiac events when compared to BMS. This led to the approval of the first DES, the sirolimus-eluting Cypher stent (Cordis), in April 2002 for use in Europe and in April 2003 for use in the USA.

Numerous clinical trials also evaluated paclitaxel-eluting stents, initially TAXUS I (13), TAXUS II (14) and TAXUS III (15). Again, there was a substantial reduction in rates of restenosis as compared to BMS and the Taxus stent (Boston Scientific) was approved for use in Europe in February 2003 and shortly after for use in the USA.

Although DES were shown to significantly reduce the rates of restenosis and target lesion revascularisation (TLR) compared to BMS, some concerns were raised regarding the long-term safety of these devices, specifically the development of ST occurring both early and late after DES implantation. Following the presentation of meta-analyses by Camenzind (16), Nordmann (17) and the BASKET-LATE (Basel Stent Kosten Effeckivitats Trial-Late Thrombotic Events) study and registry (18) at the European Society of Cardiology (ESC) meeting in 2006 (commonly known as the “ESC firestorm”), an FDA panel was convened because of the suggested significantly increased mortality associated with DES. Multiple analyses were subsequently published and reassuringly did not support an increase in long-term mortality from the use of DES over BMS (19-21). However, although the use of DES was associated with a substantial decrease in the incidence of restenosis and need for repeat revascularisation, there was evidence for an increased rate of ST with first generation DES beyond 1 year.

Despite these initial concerns, DES have enabled the expansion of PCI to allow treatment of even very high-risk patients and complex lesions, including left main stem disease, ostial lesions, bifurcation lesions, long diffuse disease, calcified vessels and chronic total occlusions with longer term predictable robust outcomes. Iterative developments have led to thinner stent struts, a variety of anti-proliferative drugs (mostly limus derivatives), more biocompatible polymers (e.g. phosphorylcholine and hexafluoropropylene/vinylidene fluoride) and more recently, biodegradable polymers (e.g. poly-l-lactic acid, PLLA and poly-lactic-co-glycolic acid, PGLA), polymer-free DES (e.g. BioFreedom, Biosensors International) and most recently, fully bioresorbable DES (e.g. Absorb BVS, Abbott Vascular). There are now at least 60 different DES approved for use in Europe. The key characteristics of the most widely used current generation DES, which have published large-scale randomised controlled trial data, are summarised in figure 1.3.

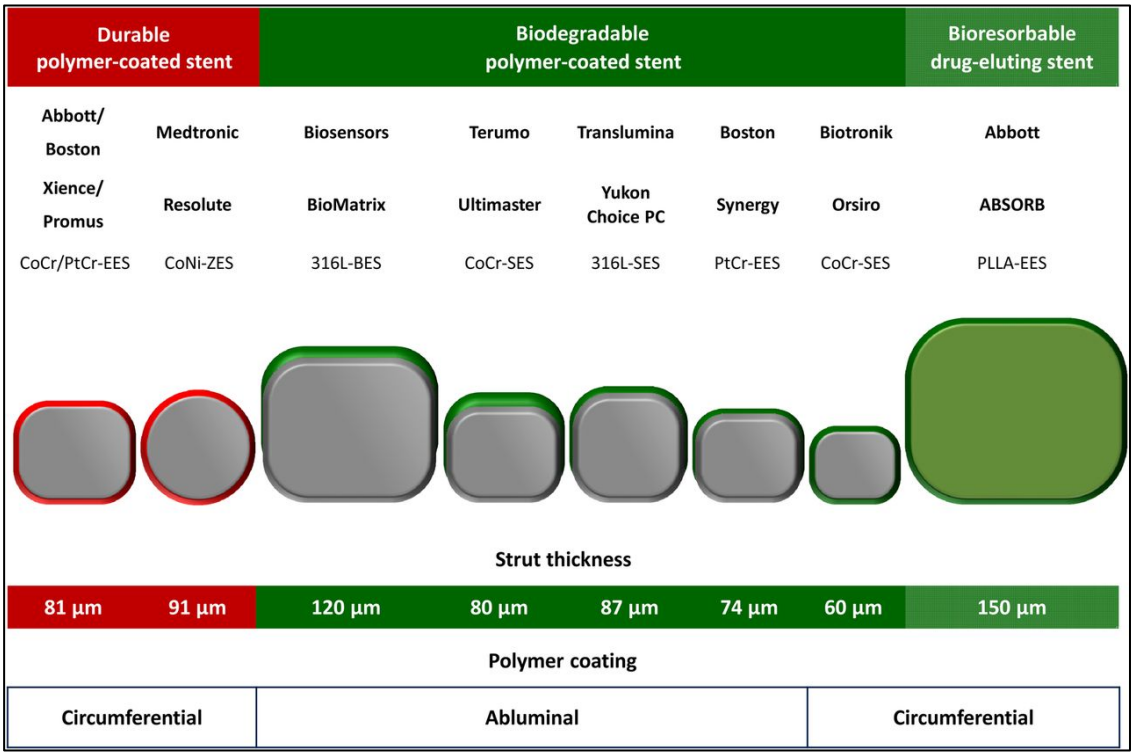


Figure 1.3. Key characteristics of most widely used and studied current generation DES. BES, biolimus-eluting stent; CoCr, cobalt chromium; CoNi, cobalt nickel; EES, evorolimus-eluting stent; PtCr, platinum chromium; SES, sirolimus-eluting stent; ZES, zotarolimus-eluting stent; reproduced from Byrne et al. (22)

Several recent meta-analyses (23, 24) of randomised controlled trials using second generation everolimus eluting DES have shown a significant reduction in rates of ST compared to first generation sirolimus-eluting, paclitaxel-eluting and zotarolimus-eluting DES and also compared to BMS. Overall, these studies support the early and long-term safety and efficacy of current generation DES in all patient groups, including when used for ACS and diabetic patients (25, 26).

1.4. Coronary stent thrombosis

With modern day stent technology, together with the use of optimal techniques and major advances in antiplatelet and antithrombotic therapy, ST has become a relatively rare complication of PCI. However, with the widespread use of coronary stenting worldwide (up to 4.5 million predicted in 2016: source JP Morgan), the absolute number of patients at risk of this complication remains substantial (45,000 events even with an incidence as low as 1%), and since there is an

associated high morbidity and mortality rate, this continues to be an important clinical issue.

1.4.1. Definitions

Following the ESC congress in 2006, the Academic Research Consortium (ARC) developed universal definitions of ST in order to standardise reporting across clinical trials (27). This categorises the occurrence of ST as *definite*, *probable* or *possible* (table 1.1) and the timing following PCI as *early* (within 30 days), *late* (30 days to 1 year) and *very late* (more than 1 year). The early time-point is further subdivided in to *acute* (within 24 hours) and *subacute* (24 hours to 30 days).

Table 1.1. ARC definitions of diagnostic criteria of ST (27)

Definite ST	Angiographic confirmation of ST (thrombus within the stent or in the segment 5mm proximal or distal to the stent) and the presence within 48hours of at least one of the following criteria: • Acute ischaemic symptoms at rest • New ischaemic ECG changes • Raised cardiac enzymes
	Pathological confirmation of ST (evidence of recent thrombus within the stent determined at autopsy or by examination of tissue retrieved from thrombectomy)
Probable ST	Any unexplained death occurring within the first 30 days after coronary stenting
	Any MI, regardless of time from stenting, with documented ischaemia in the territory of the implanted stent, without angiographic confirmation of ST and in the absence of any other obvious cause
Possible ST	Any unexplained death occurring after 30 days from coronary stenting

1.4.2. Incidence

The estimated incidence of ST varies considerably in studies, reported between 1% and 5% (18, 28-33). Most of the early studies were limited by small sample sizes, differing risk populations, an inconsistent definition of ST and limited data on very late ST. With contemporary stent technology and antiplatelet therapy, the incidence of ST is currently estimated at 1% at 12 months, independent of stent type (34). A recent systematic review of randomised clinical trials involving DES showed a median incidence of definite ST of 0.61% at 1 year (35). Overall rates of cumulative ST rates up to 3 years have halved with newer generation DES from about 3% to 1.5% (figure 1.2) (36, 37). Despite its relatively low incidence, ST remains a very significant complication of PCI, causing acute myocardial infarction in over 90% of cases with mortality rates as high as 20-40% (38-40).

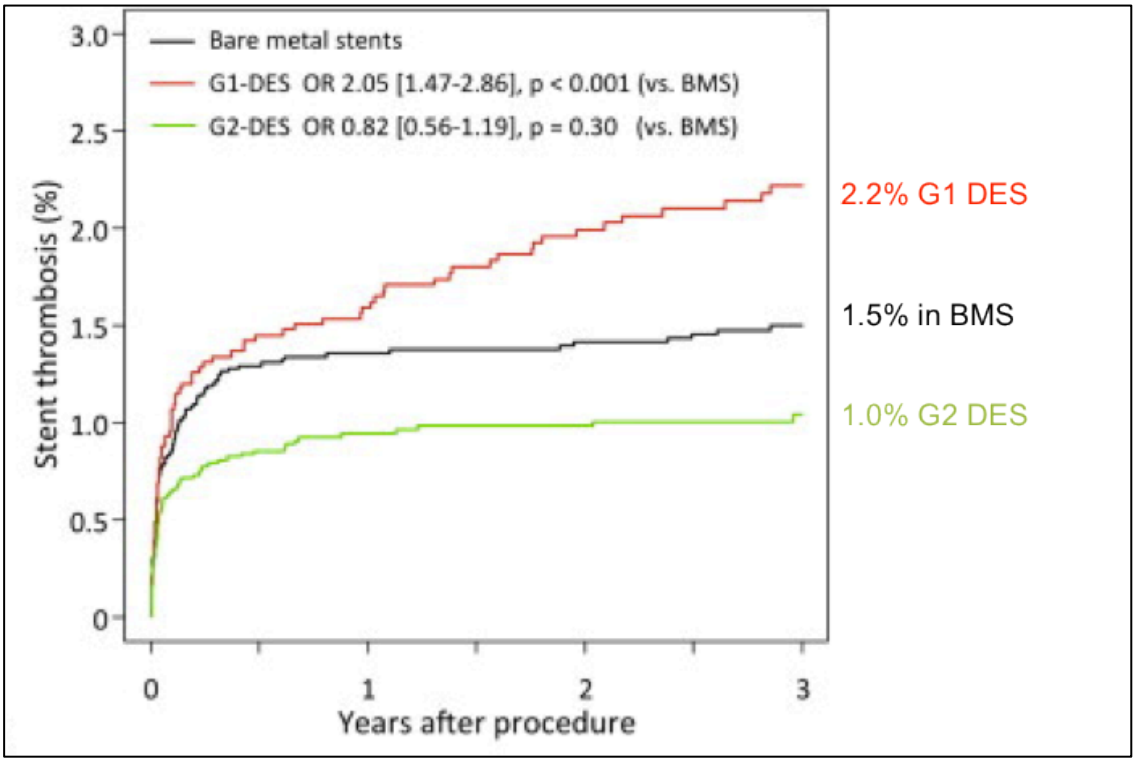


Figure 1.4. Kaplan-Meier curves of ST through to 3 years in different stent groups (G1 DES, first-generation DES; G2 DES, second-generation DES) and risk estimates compared to BMS; adapted from Tada et al. (36)

1.4.3. Mechanisms and risk factors

The underlying mechanisms leading to ST are heterogeneous and vary according to incident time point (i.e. early, late and very late ST). As ST is a relatively uncommon complication, most prospective randomised trials are underpowered to study the underlying risk factors for, and outcomes from ST. Therefore, much of the data regarding mechanisms, incidence and outcomes of ST are derived from observational studies, meta-analyses and post-mortem studies, all of which have inherent limitations.

The pathophysiology of ST reflects the underlying fundamental mechanisms of thrombosis that make up Virchow's triad (41); vessel wall injury after stent placement, hypercoagulability affected by coagulation and platelet reactivity, and blood stasis caused by mechanical factors.

1.4.3.1. Vessel wall injury

Coronary balloon angioplasty and stenting cause significant injury to the coronary vascular endothelium, exposing the underlying strongly pro-thrombotic collagen, von Willebrand factor and tissue factor to the bloodstream. This leads to the interdependent activation of platelets and coagulation (primarily through the tissue factor-initiated extrinsic pathway), thereby potentially leading to a risk of ST (42). During an ACS, the underlying vascular injury and inflammatory response from atherosclerotic plaque erosion or rupture are more marked and thought to be the reason for a higher risk of future ST in PCI undertaken for ACS (43).

Although DES were developed to suppress the vascular inflammatory response to stenting and so reduce the rate of in stent restenosis, first generation DES (sirolimus- and paclitaxel-eluting stents) were associated with a higher rate of late and very late ST (as described in section 1.3.2), perhaps through the polymer (sirolimus-eluting) or the drug (paclitaxel-eluting) being less bio-neutral than planned or hoped for, but most likely also through delayed endothelial healing. Moreover, there appears to be an ongoing risk of ST long-term with no evidence

of a reduction in risk over time (30). The underlying substrate for this ongoing risk of ST has been identified from post-mortem studies to be likely due to delayed arterial healing caused by incomplete re-endothelialisation, persistent fibrin deposition and ongoing vessel wall inflammation (44). Whilst both sirolimus and paclitaxel reduce in stent restenosis by interrupting smooth muscle cell proliferation, they also both inhibit endothelial cell proliferation. The therapeutic window for the beneficial effect on the smooth muscle cells and adverse effect on endothelial inhibition is indeed narrow for such drugs. Furthermore, as with the vast majority of DES, the drug was delivered by being loaded onto the stent through polymer-binding and, amongst other factors, there has also been an association with local hypersensitivity reactions to the polymer coatings used in first generation DES (45).

Newer generation DES, with thinner cobalt chromium and platinum chromium metallic struts, more biocompatible and biodegradable polymer coatings, and lower doses of sirolimus-analogue drugs (see figure 1.1), appear to have made significant progress in addressing this problem of delayed healing (36, 37, 46), translating, as already described, to a marked reduction in the incidence of ST. In fact, recent studies have suggested that the rates of early ST maybe slightly higher with BMS compared to the newer generation DES, possibly due to a reduced acute thrombogenicity provided by polymer coatings on the DES (47). This has been supported by randomised clinical trial data providing some evidence for a reduction in early ST in the treatment of acute MI with polymer-coated stents compared to BMS (48). The EXAMINATION trial compared the performance of the newer generation everolimus-eluting stents (EES) versus BMS in nearly 1500 STEMI patients. The trial confirmed the safety and efficacy of the EES compared with BMS in the setting of STEMI and in addition showed a reduction of target lesion revascularisation (2.9% vs 5.6%; p=0.009) and ST (0.8% vs 2.1%; p=0.03) with the newer generation DES up to 2-years follow-up (49).

However, despite these encouraging results from newer generation DES, it has become increasingly evident over recent years, from intracoronary imaging and

autopsy studies, that a previously unrecognised process of neoatherosclerosis is responsible for a moderate proportion of ongoing late stent failures.

Neoatherosclerosis

Neoatherosclerosis refers to the development of atherosclerotic plaque disease within existing coronary stents and is characterised firstly by early foamy macrophage infiltration, then atherosclerotic plaque development, and finally the formation of necrotic core plaque with or without a thin fibrous cap (figure 1.3).

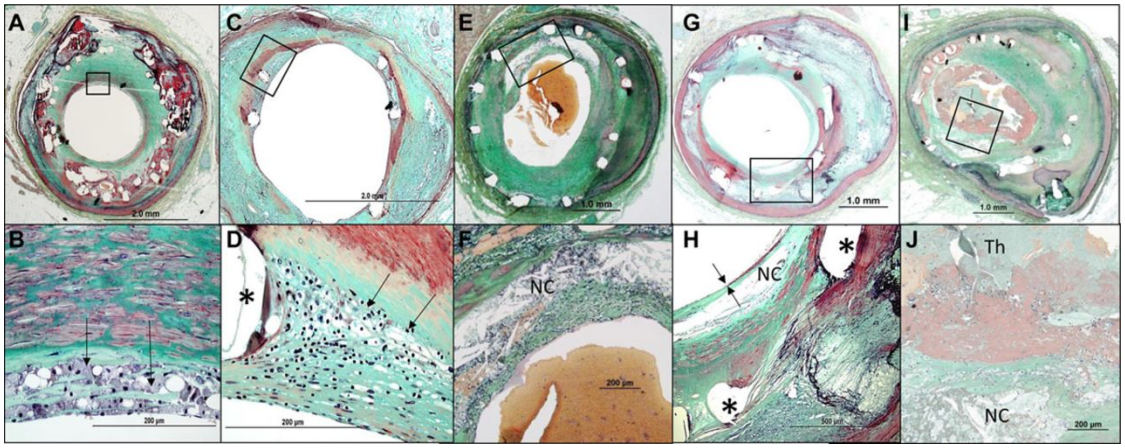


Figure 1.5. Pathological progression of neoatherosclerosis occurring within DES. A and B, Low- and high-power images of early atherosclerotic change characterised by luminal foam cells (arrows) with underlying fibrointimal thickening. C and D, Low- and high-power images of foamy macrophage clusters (arrows) in the peristrut region adjacent to luminal surface. E and F, Low- and high-power images of fibroatheroma with necrotic core (NC) within neointima. G and H, Low- and high-power images of thin cap fibroatheroma (arrows) with NC. I and J, Low- and high-power images of plaque rupture resulting in continuity between the overlying thrombus (Th) and the NC. * represents stent strut; reproduced from Romero et al. (50)

Neoatherosclerosis has been observed with both BMS and DES, but it appears to occur earlier and more frequently with DES (51). Moreover, it is seen as frequently with latest generation DES, as with first generation DES (52). The reason for this is as yet unclear, but may be due to endothelial dysfunction (both physical and functional) induced by DES implantation, allowing infiltration of

circulating lipid particles in to the stented arterial wall, leading more often to accelerated neoatherosclerosis (22). Other predictors of neoatherosclerosis, other than DES implantation, include time since stent deployment (>48 months), current smoking and chronic renal impairment (53).

The prevalence of neoatherosclerosis can only be detected by intracoronary imaging or on post-mortem studies. Intravascular ultrasound, even virtual histology imaging, which only has a spatial resolution of 100-200μm, will only detect necrotic core. However, optical coherence tomography (see chapter 2.1.3) has an axial resolution of 12-15μm and improves the likelihood of distinguishing neoatherosclerosis, with the ability to identify even foamy macrophages.

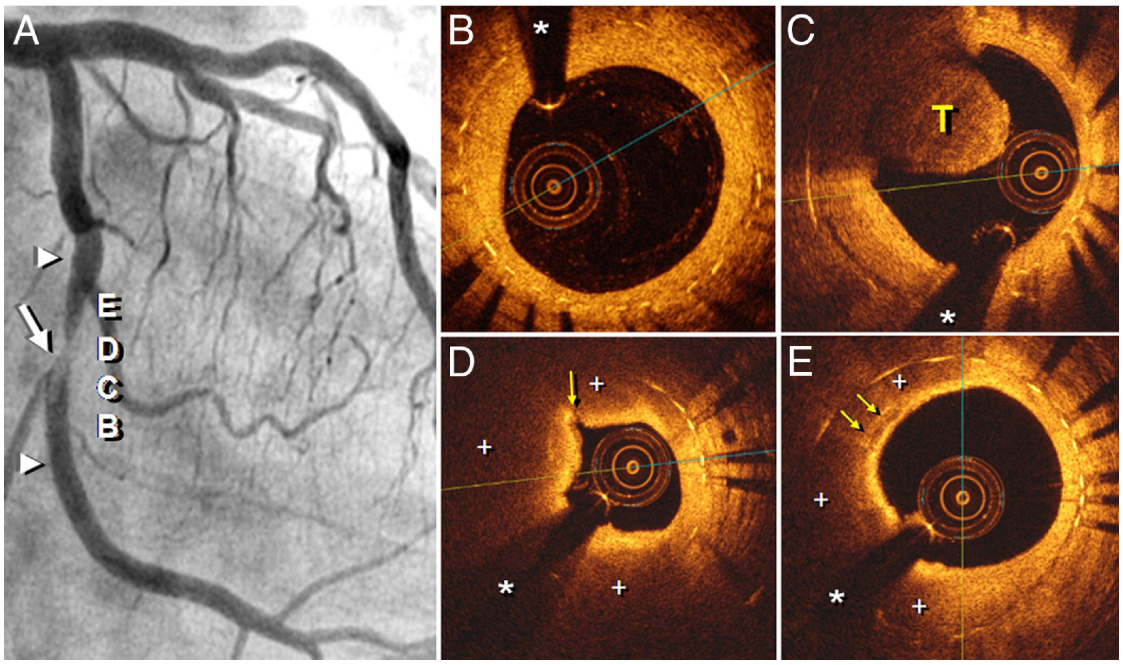


Figure 1.6. Coronary angiography image (A) showing focal in-stent restenosis in the left circumflex artery, together with a large thrombus (white arrow) within the stent (arrowheads). OCT shows mild neointimal hyperplasia with a bright homogenous pattern in the distal stent segment (B, asterisk denotes wire artefact). Proximally, there is a large protruding red thrombus (T) (C). There are features of neoatherosclerosis at the proximal stent edge (D, E), including heterogeneous neointima with attenuation (plus signs), a ruptured thin-capped fibroatheroma (yellow arrow in D) and bright linear images (yellow double arrow in E), suggestive of macrophage accumulation.

1.4.3.2. Coagulation and platelet reactivity

In addition to stent technology, developments in antiplatelet and antithrombotic therapy over the last two decades have markedly reduced the rate of early and late thrombotic complications of PCI. Indeed, one of the most important developments in the growth of PCI was the demonstration that dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ ADP platelet receptor antagonist reduced the rate of ST and bleeding complications by comparison with anticoagulant therapy used in earlier strategies (54, 55). As described later in section 1.4.5.1, DAPT is recommended for a duration post PCI dependent on the clinical setting and type of stent implanted. Although the optimal duration of DAPT post PCI is still the subject of much debate, we know that premature discontinuation of this therapy before the prescribed duration, especially in the first 30 days post PCI, is one of the most important predictors of subsequent ST, with up to a six-fold increase in the risk of ST in those patients who are non-compliant or who disrupt there DAPT (28, 56). The PARIS registry (56) prospectively observed over 5000 patients, undergoing PCI in 15 clinical sites in the USA and Europe and examined over 2 years the effect of DAPT cessation, on MACE (composite of cardiac death, definite or probable ST, myocardial infarction, or target-lesion revascularisation). Compared to those on DAPT, disruption due to non-compliance or bleeding was associated with an overall hazard ratio of 1.50 (1.14-1.97; p=0.004). The earlier the disruption of DAPT, the higher was the risk; within 7 days being 7.04 (3.31-14.95), 8-30 days being 2.17 (0.97-4.88) and after 30 days being 1.3 (0.97-1.76).

Response to antiplatelet therapy

Although the risk of ST is high when DAPT is discontinued prematurely (before the prescribed duration), ST events may occur whilst patients are still taking DAPT. While this may be due to various mechanical factors, as described in the next section, there has also been some association between individual response to antiplatelet therapy and risk of ST. Many studies have shown that so called *high on treatment platelet reactivity* (indicating a poor response to antiplatelet therapy), that is detected with platelet function testing, is associated with a higher

risk of ST (57, 58). The response to both aspirin and $P2Y_{12}$ receptor antagonists such as clopidogrel, is known to vary widely between individuals (59) with hypo-responsiveness rates of up to 28% to aspirin (60, 61) and about 30% to clopidogrel (62). Certain co-morbidities, such as diabetes mellitus, renal failure and obesity are known to predispose to this phenomenon (63). Moreover, clopidogrel is a pro-drug and requires intestinal absorption and hepatic metabolism by several cytochrome P450 enzymes to its active metabolite before being able to irreversibly inhibit the platelet $P2Y_{12}$ receptor. In addition to factors such as drug interactions (with conflicting data implicating drugs such as proton pump inhibitors that use the same metabolic pathways (64-66)), polymorphisms in genes modulating absorption (e.g. ABCB1) and metabolism (e.g. CYP2C19) of clopidogrel have been associated with poor individual response to the drug. Cytochrome P450 2C19 (CYP2C19) is a drug metabolism enzyme that is involved in converting clopidogrel to its active metabolite. It is encoded by the *CYP2C19* gene on chromosome 10. A common single nucleotide polymorphism (SNP) of *CYP2C19* (designated *2) encodes for a cryptic slice variant that results in a protein product with no enzyme activity. The loss-of-function *CYP2C19*2* allele has been shown in several studies to be associated with a decreased clopidogrel responsiveness, as measured by ex-vivo platelets function assays, and an increased risk of adverse outcomes after PCI (67-69).

However, whilst both platelet function and pharmacogenetic testing are useful to identify poor or non-responders to prescribed antiplatelet therapy and thereby inform risk, no study to date has shown any significant reduction in adverse outcome if antiplatelet treatment is modified on the basis of these test results (i.e. by increasing dose or switching to more potent agents) (70-72). This may be due to the inclusion in these studies of low risk patients with mostly stable coronary artery disease who have very low event rates. In order to show a benefit of ‘tailored’ antiplatelet therapy, one would have to design a study to include only those patients with the highest risk profile for adverse events post stenting, predominantly ACS patients, and this would have to be adequately powered.

1.4.3.3. Mechanical factors

Various mechanical factors related to the underlying coronary lesion and stented area can increase blood turbulence and stasis, increasing the risk of ST. This includes complex lesions, such as long diffuse disease, tortuous vessels, calcified lesions and complex bifurcation lesions (73, 74). Stent inflow and outflow obstruction, including residual disease and proximal or distal stent edge dissections can predispose to ST. Within the stent, mechanical factors such as mal-apposition, whether due to stent under-sizing, under-expansion or late acquired mal-apposition from positive remodelling of the vessel, as well as stent fractures are all thought to increase the risk of ST (33, 74-77). In addition, stenting in the context of ACS, especially with cardiogenic shock or cardiac arrest, predisposes to subsequent ST due to the frequent presence of thrombus within the vessel along with slow blood flow, stasis and distal embolisation of material causing *no reflow* during PCI (78).

1.4.3.4. Risk factors for ST

The risk factors associated with the development of ST have been categorised into *patient-related*, *PCI procedure-related* and underlying *coronary lesion-related*, as summarised in figure 1.5. In general, procedural and lesion-related factors increase the risk of ST more during the early phase post-stenting and patient-related factors increase the ongoing long-term risk. However, procedural issues, such as mal-apposition can cause a problem at a later time point, for example when DAPT is discontinued, even beyond a year or more. In addition, patient-related risk factors, such as compliance to and responsiveness to prescribed DAPT therapy can cause a problem at any time point. The Dutch Stent Thrombosis Registry (33), which enrolled patients with predominantly acute and subacute ST, identified various risk factors, including stent under-sizing, TIMI flow grade less than 3, malignancy, residual coronary disease, dissection, lack of aspirin, lack of clopidogrel, bifurcation lesions, LVEF<30% and younger age.

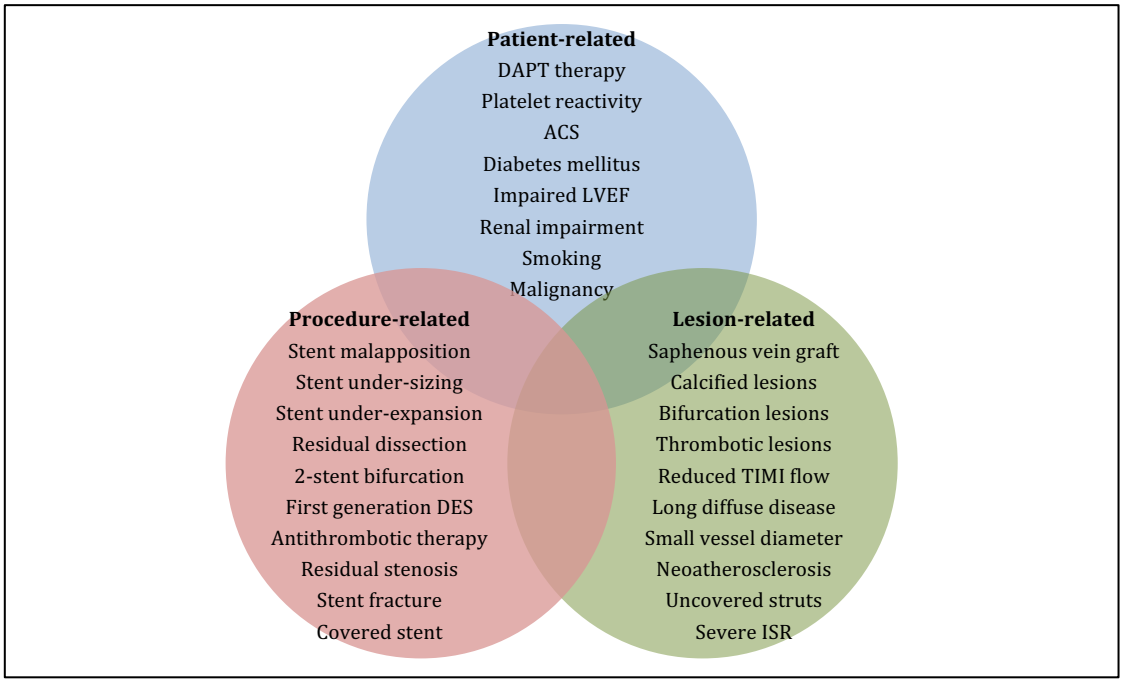


Figure 1.7. Mechanisms and risk factors for the development of ST; DAPT, dual antiplatelet therapy; LVEF, left ventricular ejection fraction; ACS, acute coronary syndrome; DES, drug eluting stent; TIMI, thrombolysis in myocardial infarction

1.4.4. Treatment of ST

ST most commonly presents as acute vessel closure with ST elevation myocardial infarction (STEMI) and treatment should be focused on urgent reperfusion. Outcomes are poorer and success rates lower compared to primary percutaneous coronary intervention (PPCI) for de novo coronary plaque rupture (79). This can partly be explained by a higher baseline risk profile in patients with ST, including a higher rate of renal failure, prior stroke, prior MI and multi-vessel disease. In addition, however, thrombus burden is usually very high leading to poorer procedural results, with a lower rate of successful reperfusion and more distal embolisation. Mechanical thrombectomy during treatment of ST has been shown to improve epicardial flow and myocardial perfusion, with a trend towards reducing the risk of recurrent ST (52, 80, 81).

Thrombus within this setting is predominantly platelet-rich (82) and in addition to loading with the more potent oral antiplatelet drugs (see section 1.4.5), various intravenous antithrombotic agents are available for use at the time of PCI. The

importance of antithrombotic therapy has been illustrated by trials comparing different combinations. Several large-scale PCI trials have previously established the efficacy of intravenous glycoprotein IIb/IIIa inhibitors (GPI), that block platelet aggregation at the final common pathway, so reducing ischaemic outcomes (83-90). A strategy of heparin combined with GPI has more recently been compared to bivalirudin (a direct thrombin inhibitor) in several trials. In both the HORIZONS-AMI (Harmonising Outcomes with Revascularisation and Stents in Acute Myocardial Infarction) (91) and EUROMAX (European Ambulance Acute Coronary Syndrome Angiography) (92, 93) trials, bivalirudin was associated with a higher risk of acute ST when compared to heparin and a GPI. This was in the context of clopidogrel use. With the newer, more potent ADP-receptor antagonists, prasugrel and ticagrelor, the HEAT-PPCI (How Effective Are Therapies in Primary Percutaneous Coronary Intervention) trial (94) randomised STEMI patients undergoing PPCI to treatment with bivalirudin versus heparin monotherapy (with bailout GPI if needed). Again, bivalirudin was associated with a higher risk of acute ST, with no difference in major bleeding. Given these findings, it may be preferable to use a strategy of heparin with additional GPI if necessary when undertaking PCI for ST. Another option would be to use a higher dose prolonged infusion of bivalirudin, which appeared to mitigate the risk of acute ST in the EUROMAX study (93). However, these strategies are, as yet, unproven.

Further treatment after ST depends on the underlying mechanism(s) and should be guided by intravascular imaging with intravascular ultrasound (IVUS) or optical coherence tomography (OCT) (77, 95). Mal-apposition should be treated with appropriately sized balloons inflated to high pressure. Stent edge dissections, edge restenosis, stent fractures and neoatherosclerosis may need the deployment of additional stents. Neointimal hyperplasia can either be treated with drug-eluting balloons or DES. The use of rotational atherectomy or excimer laser ablation may be necessary for un-dilatable, under-expanded stents (96, 97).

In the absence of obvious mechanical factors causing ST, a history of non-compliance to DAPT should be assessed. If ST occurs whilst on clopidogrel,

platelet function testing can be considered and more potent antiplatelet agents initiated, especially for ‘non-responders’.

1.4.5. Prevention of ST

1.4.5.1. Dual antiplatelet therapy

As already described, compliance with the prescribed duration of DAPT is one of the most important factors in reducing the risk of ST and this should be communicated with patients and assessed at all possible opportunities. In general, 1 month of DAPT is advocated for BMS and 6 months of DAPT for DES treatment in stable angina. DAPT may be used for longer than 6 months in patients at high ischaemic risk and low bleeding risk. A shorter duration of 1 to 3 months DAPT may also be considered after DES implantation in patients at high bleeding risk or with up-coming non-deferrable non-cardiac surgery or concomitant anticoagulant treatment. In the context of ACS, 1 year of DAPT is still recommended for both BMS and DES. This can be shortened in patients deemed at high bleeding risk to 1 month for patients receiving BMS and 3-6 months for those receiving DES (98, 99). When treating ACS, two newer $P2Y_{12}$ receptor antagonist drugs, prasugrel and ticagrelor, have been shown in large multi-centre clinical trials, TRITON-TIMI 38 (100, 101) and PLATO respectively (102, 103), to reduce the risk of ST as compared to clopidogrel when used in addition to aspirin. Their metabolism and mode of action, compared to clopidogrel, are summarised in figure 1.6 and table 1.2 below.

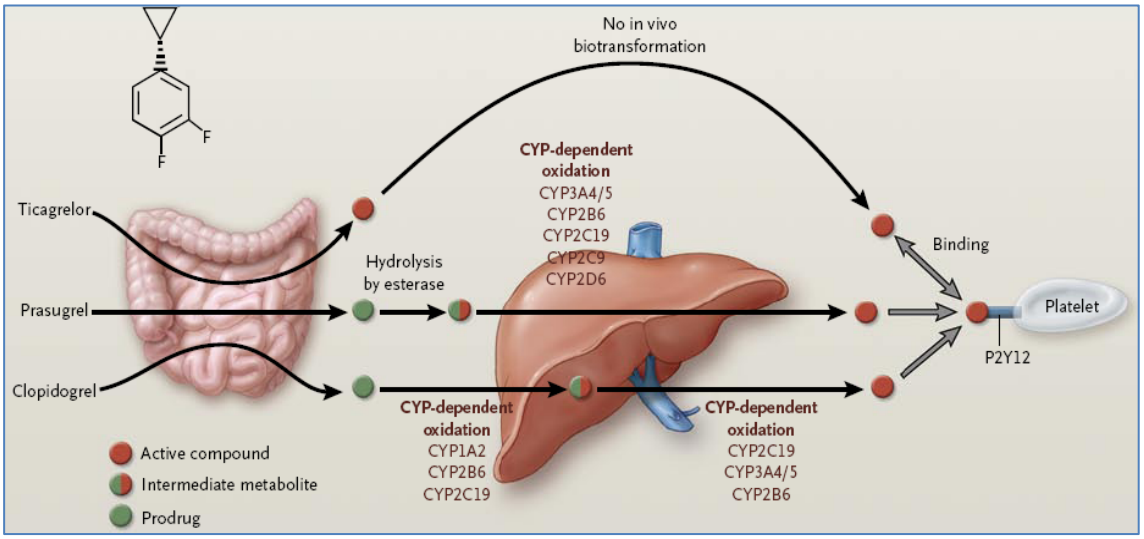


Figure 1.8. Biotransformation and mode of action of currently used oral $P2Y_{12}$ receptor antagonist drugs; CYP, cytochrome P450; reproduced from Schomig (104).

Table 1.2 Drug characteristics of oral $P2Y_{12}$ receptor antagonist drugs. CPTP, cyclopentyltriazolopyrimidine; *50% inhibition of platelet aggregation

	Clopidogrel	Prasugrel	Ticagrelor
Class	Thienopyridine	Thienopyridine	CPTP
Binding	Irreversible	Irreversible	Reversible
Activation	Prodrug, limited by metabolisation	Prodrug, not limited by metabolisation	Active drug
Onset of effect*	2–4 hours	30 minutes	30 minutes
Duration of effect	3–10 days	5–10 days	3–4 days
Withdrawal before major surgery	5 days	7 days	3 days

Clopidogrel is a thienopyridine that irreversibly blocks the ADP receptor $P2Y_{12}$ on platelets. It has a much slower onset of action than the newer agents and as already described, it is associated with a significant variability in antiplatelet response (105). The two-step activation process, involving a series of cytochrome P450 isoenzymes, is susceptible to the effect of genetic polymorphisms and drug interactions (106).

Prasugrel is a newer thienopyridine that also irreversibly binds to the $P2Y_{12}$ receptor, but has a more rapid onset of action (107) with a stronger inhibitory effect than clopidogrel and shows less variability in antiplatelet response (108). However, its irreversibility is thought to be partly responsible for the significant increase in the risk of CABG-related bleeding seen with prasugrel compared to clopidogrel in the TRITON-TIMI 38 study (109).

Ticagrelor is an orally active drug that binds reversibly to the $P2Y_{12}$ receptor and has a stronger and more rapid antiplatelet effect than clopidogrel. In the PLATO study, ticagrelor was associated with a 16% relative risk reduction in the primary end point (a composite of death from cardiovascular causes, MI and stroke), with no significant increase in the overall risk of major bleeding (110).

In recent years, there has been considerable debate as to the optimal duration of DAPT post stenting with DES and studies have investigated durations ranging from 3 months up to 30 months (111-120). The DAPT study, comparing a standard 12 months with a prolonged 30 months of DAPT in 9921 patients treated with DES, reported a significant reduction in ST events and MI with prolonged DAPT, albeit with a significant increase in major bleeding. This was the same for stable angina and ACS patients (121). Other studies investigating the use of 3 or 6 months of DAPT versus a standard 12 months have indicated non-inferiority of efficacy and safety outcomes when implanting second generation DES in patients with stable angina or low risk ACS (112-115). More recently, based on the PEGASUS TIMI-54 trial (122), the National Institute of Health and Care Excellence (NICE) have recommended the extended use of a lower dose of ticagrelor beyond 1 year and up to 3 years, as an option for preventing further

atherothrombotic events in patients with a previous MI who are at high risk of a further event (Technology Appraisal Guidance 420).

These studies highlight the need for individualisation of antiplatelet therapy with regards to potency and duration of DAPT depending on the clinical setting and the risk of ST and bleeding. The recently published DAPT score, developed by the DAPT study investigators, provides a potential decision tool and improves prediction of patient benefit and harm from prolonged DAPT (123, 124).

1.4.5.2. Novel antithrombotic therapy

Recent studies have assessed the addition of oral anticoagulants to DAPT therapy to further reduce the risk of ST. The ATLAS-ACS 2 TIMI 51 study showed a significant reduction in the rate of ST and mortality in patients with ACS treated with rivaroxaban, an oral direct factor Xa inhibitor, in addition to DAPT (125). Another agent, although not an anticoagulant, voraxapar, which is a protease-activated receptor (PAR-1) antagonist that blocks thrombin-mediated platelet activation, has been studied in patients who have previously undergone coronary stenting. The addition of voraxapar to DAPT was associated with a significant reduction in the risk of ST, with however, an increased risk of moderate to severe bleeding (126). With the now widespread use and proven efficacy of the newer, more potent oral antiplatelet agents (ticagrelor and prasugrel), especially in the context of ACS, these additional oral anticoagulant/antithrombotic agents have not taken off for use in coronary artery disease.

1.4.5.3. Procedural considerations

With modern day stent technology, increasingly complex coronary lesions can be treated successfully with PCI. Careful coronary lesion preparation, stent sizing and post dilatation should avoid mechanical issues such as mal-apposition and edge dissections and thereby minimise the risk of ST. Intracoronary imaging with IVUS or OCT should be used to guide procedural strategies where there is any doubt and have been shown to improve overall outcomes, especially in patients with acute coronary syndromes and complex lesions (127).

1.4.6. Future developments

In recent years, there has been a move towards developing DES with absorbable polymers and polymer-free DES, with the aim of improving biocompatibility and reducing late complications. Fully bioresorbable stents are considered by some to be the next big revolution in coronary intervention. They aim to temporarily scaffold the vessel after balloon angioplasty, eluting an anti-proliferative drug to limit restenosis and having allowed the vessel to "heal", fully degrade in to carbon dioxide and water, leaving no residual material. This theoretically removes the ongoing risk of very late stent thrombosis and potentially restores normal vasomotor tone of the stented segment, as well as increasing lumen calibre due to positive remodelling within the vessel (128).

Clinical trials using the first generation of these devices implanted in selected patients with relatively simple coronary lesions initially showed encouraging results (129), although some concern was raised from registry studies regarding the apparent increase in early ST events (130). Initial results from the ABSORB-III trial, a large multi-centre randomised controlled trial, comparing the bioabsorbable vascular scaffold (BVS), Absorb (Abbott Vascular), to a 'best in class' second generation DES, Xience (Abbott Vascular), in non-complex coronary lesions, showed non-inferior results at 1 year with regards to target lesion failure. However there was a higher rate of *subacute* ST ("definite" or "probable") with the BVS, with however no increase in *acute* or *late* ST at 1 year (131). The excess ST was put down to an "operator learning curve". More recently, the 2-year data from this study were presented at the American College of Cardiology Conference (March 18, 2017). These showed a higher rate of target lesion failure (cardiac death, target vessel MI or ischaemia-driven target lesion revascularisation) in the Absorb BVS arm compared to the Xience DES arm (10.9% vs 7.8%, p=0.03). A post hoc analysis of the 2-year data was however also presented at the same session, suggesting that increased event rate was concentrated in the patients with a reference vessel diameter (RVD) <2.25mm by quantitative angiography, who were not intended to be treated in the trial. The intended RVD range for patients to be treated in the study was between 2.5mm and 3.75mm, but 19% of patients had a RVD <2.25mm.

The 3 year results of the ABSORB II study were also recently published and these aimed to demonstrate two mechanistic properties of the BVS: recovered vasomotion of the scaffolded vessel and an increase in luminal dimensions (132). However, at 3 years, the vasomotor reactivity was not statistically different between the Absorb BVS group and Xience metallic DES group and moreover, the late luminal loss was larger in the Absorb cohort. In terms of secondary endpoints, although there was an increase in the rate of device-oriented composite events (mainly driven by target vessel MI), the ABSORB II study with only 501 patients was not powered for clinical endpoints. Whilst these findings are concerning, it remains to be seen from the results of long term clinical outcomes and ongoing studies whether we will eventually see any superiority of this device. It is likely that future iterations will be needed before widespread adoption of bioabsorbable stent technology can be recommended.

1.4.7. Summary

There have been substantial developments in interventional cardiology and we are now at a time when PCI has become the treatment of choice in most patients with ischaemic heart disease. The main risk attributable to bare-metal stenting was restenosis requiring repeat revascularization. The introduction of DES has reduced the occurrence of clinical restenosis to <5%. However, for both types of stent, ST remains a serious clinical adverse event after PCI, resulting in abrupt vessel closure with a high risk of MI and death. Therefore, prevention of ST is paramount and although there is no established risk score for ST, several predictors may help identify those patients at highest risk.

Previous registries looking at potential risk factors have been limited by small numbers, especially in terms of late and very late ST and of those involving DES. For example, in the previously published Dutch Stent Thrombosis Registry, more than 75% of the ST cases were in the early time-point (<30 days) and approximately 65% involved BMS (33). In addition, there has been a relative lack of complete patient profile, including a paucity of data on intracoronary imaging, platelet function testing (PFT), thrombus histopathology and genetic studies.

Such data may ultimately provide important insights in to the underlying mechanisms and risk factors for ST and potentially other conditions involving thrombus formation, providing surrogate markers of high risk and should help individualise patient management and allow the development of strategies and technologies to minimise the ongoing risk of ST with contemporary stent technology.

As such, the PRESTIGE study (Prevention of Stent Thrombosis by an Interdisciplinary Global European effort) was designed to investigate in detail the risk factors and underlying mechanisms in patients presenting with definite ST, including demographic and clinical variables, as well as intracoronary imaging at the time of ST. With the knowledge that antiplatelet and antithrombotic therapy in ST patients is often suboptimal, platelet function and thrombin generation studies were undertaken as described in chapter 3. Finally, an in vivo rabbit iliac model was set up, chapter 4, to investigate the potential clinical use of novel bio-neutral stents and the acute thrombogenicity of the Absorb BVS, the first in a generation of fully bioabsorbable vascular scaffolds, that promise to eventually eliminate the ongoing risk of very late ST.

Chapter 2: The PRESTIGE clinical study (UK cohort)

2.1. Introduction

2.1.1. Background

The PRESTIGE study (PREvention of Stent Thrombosis by an Interdisciplinary Global European effort, grant agreement no. HEALTH-F2-2010-260309) is a European multi-centre study, funded by the European Union's Seventh Framework Programme (FP7/2007-2013), designed to investigate the underlying causes and mechanisms of coronary stent thrombosis (ST). This is part of a much wider European project, involving four work packages, as outlined in figure 2.1 below.

The clinical work package (WP4), involves eight participating European countries (UK, Germany, France, Belgium, Spain, Italy, Netherlands and Poland) with the overall aim to perform a highly detailed analysis of patients with ST, with regard to its predictors and clinical outcomes. This would be achieved through the establishment of a robust and comprehensive database, including details on demographics, clinical presentation, ECG, biochemical and haematological data, as well as angiography details, procedural description and data on any of the following that the patients underwent: pathological, imaging, genetic and follow-up information of patients with definite ST. All data was compared with matched control patients and the target was to recruit a of total 500 patients with definite ST and at least 500 matched controls.

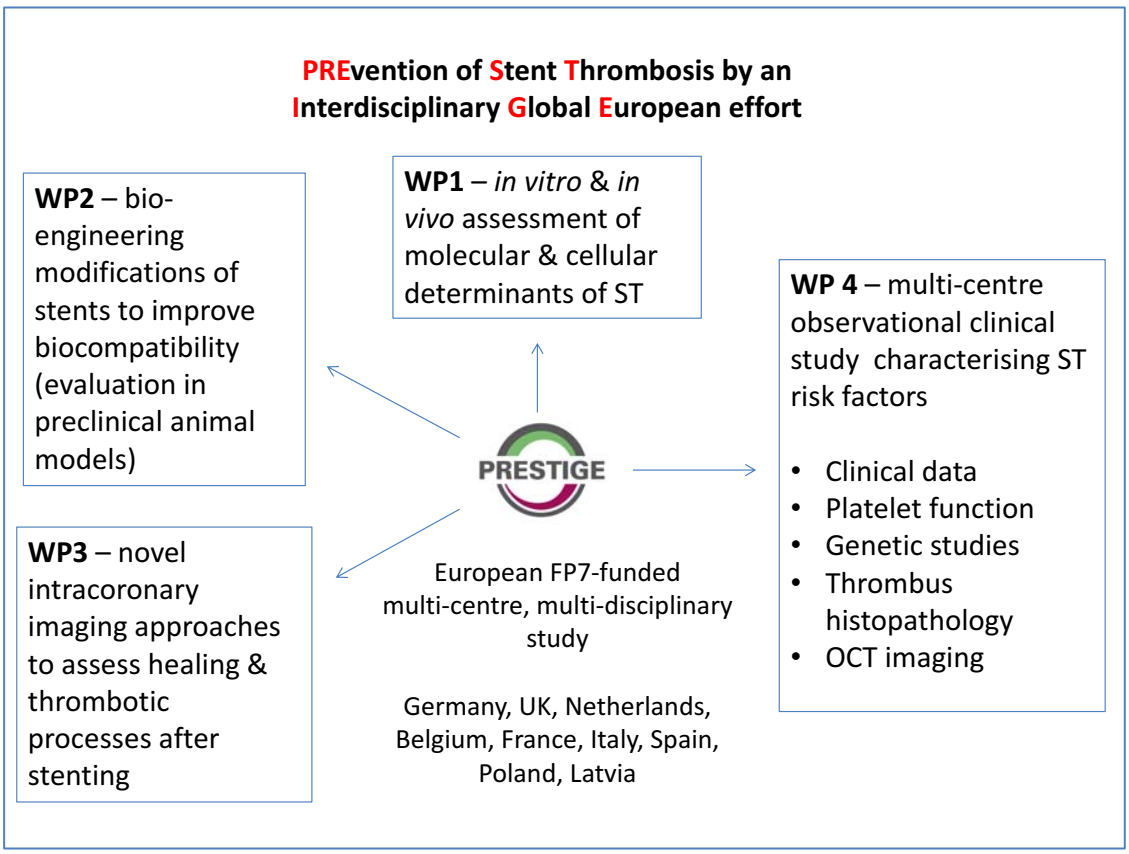


Figure 2.1. Overview of work packages involved in the PRESTIGE study in Europe. WP, work package; ST, stent thrombosis; OCT, optical coherence tomography

Our goal in the UK was to contribute at least 50 ST patients and 250 controls, so matched 5:1, as it was felt that given the large number of potential variables, this would provide a comparable control group. Data from these patients were entered into the Europe-wide PRESTIGE database but was also analysed as a specific cohort for the purpose of this thesis. The following data was collected both in Leicester and in the other UK centres, each of which are described in detail in the following sections:

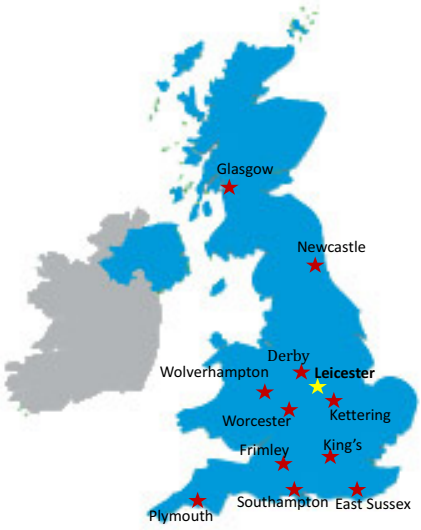
- Platelet function testing (chapter 3)
- Optical coherence tomography (OCT) images
- Thrombus aspiration
- Blood for genetic analysis
- Blood for thrombin generation (chapter 3)

2.1.2. UK satellite sites

Having successfully gained adoption of the study on the National Institute for Health Research (NIHR) Comprehensive Local Research Network (CLRN) portfolio, a further 11 UK cardiac centres were enrolled to take part in the study and these were coordinated through the University Hospitals of Leicester (UHL) NHS Trust. These were:

- The Royal Wolverhampton Hospitals NHS Trust
- University Hospital Southampton NHS Trust
- Newcastle Upon Tyne Hospitals NHS Foundation Trust
- King's College Hospital NHS Foundation Trust
- Kettering General Hospital NHS Foundation Trust
- Derby Hospitals NHS Foundation Trust
- Golden Jubilee National Hospital, Glasgow
- Plymouth Hospitals NHS Trust
- Worcestershire Acute Hospitals NHS Trust
- Frimley Health Foundation Trust
- East Sussex Healthcare NHS Trust

I carried out a site initiation visit to the cardiac research and clinical departments of each satellite centre, in order to present the study protocol in detail and for training with data entry, blood sampling, platelet function testing (PFT), processing of thrombus samples, OCT acquisition and other study-related activities (figure 2.2). Following local research and development (R&D) approval, protocols, equipment and consumables for PFT, thrombus collection and OCT catheters were supplied. Advice and support were provided at all times by myself to each site by telephone, email and further site visits as necessary. All case report forms (CRFs) and other study-related documents, such as serious adverse event (SAE) reports, were regularly sent to UHL for filing and uploading to the online database. At the satellite sites, blood samples for DNA analysis were collected into two EDTA sample bottles and shipped to Glenfield Hospital for centrifugation and storage of the buffy coat at -80°C for future genetic testing.



Recruiting Centre	PFTs	OCT	TA	DNA	TG
Leicester	Y	Y	Y	Y	Y
Wolverhampton	Y	Y	Y	Y	N
Southampton	Y	N	Y	Y	N
Newcastle	Y	Y	Y	Y	N
King's	Y	Y	Y	Y	N
Kettering	Y	N	Y	Y	N
Derby	Y	N	Y	Y	N
Glasgow	N	Y	Y	Y	N
Plymouth	N	N	Y	Y	N
Worcester	Y	N	Y	Y	N
Frimley	Y	N	Y	Y	N
East Sussex	Y	N	Y	Y	N

Figure 2.2. UK cohort of PRESTIGE clinical study with details of investigations carried out at each satellite site. PFTs, platelet function tests; OCT, optical coherence tomography; TA, thrombus aspiration; TG, thrombin generation

2.2. Methods

2.2.1. Ethics and R&D approval

The National Research Ethics Committee (REC reference 12/EM/0132), and the UHL NHS Trust R&D department as the sponsor (reference UHL 84300), approved the study and recruitment was commenced at Glenfield Hospital in May 2012. I wrote the ethics and R&D application, as well as the clinical protocol, standard operating procedures (SOPs), assent and consent forms, patient information leaflets (PILs), patient invite letter, GP letters and all other study-related documents.

2.2.2. Inclusion and exclusion criteria

All patients over the age of 18 years with definite ST (angiographic confirmation of ST with evidence of ACS) presenting to a PRESTIGE study centre and able to provide informed consent were included in the study. The only exclusion was refusal to participate or the absence of informed consent.

2.2.3. Clinical protocol

Patients presenting with suspected ST were verbally assented for inclusion in the PRESTIGE study prior to transfer to the cardiac catheter laboratory. This included patients with ACS, with a history of previous PCI, with or without electrocardiography (ECG) changes in the territory of the stented region. The subsequent sequence of events is summarised in figure 2.3.

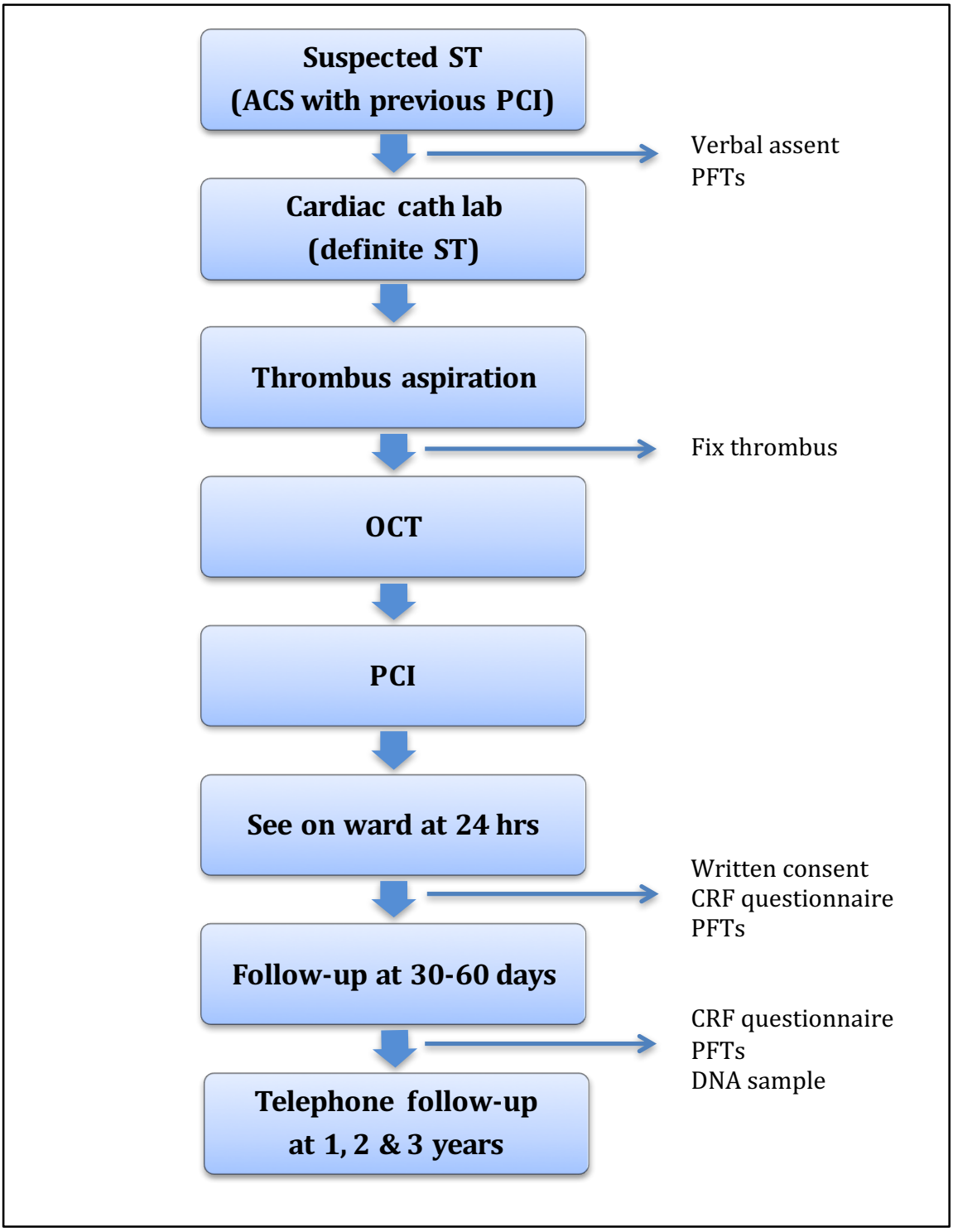


Figure 2.3. Summary of PRESTIGE UK clinical protocol. ST, stent thrombosis; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; PFTs, platelet function tests; OCT, optical coherence tomography; CRF, case report form

2.2.3.1. Blood sampling

On admission, and ideally prior to loading with oral antiplatelet therapy, 30mls of whole blood was withdrawn (after discarding the first 5mls) from the antecubital vein or arterial sheath at the time of coronary angiography. This was transferred to two 2mls vacuette® tubes containing 3.2% sodium citrate (Greiner Bio-One) and/or two 3mls Multiplate® hirudin blood tubes (Roche Diagnostics) for PFTs using the VerifyNow® and/or Multiplate® systems (see chapter 3) and to the appropriate tubes for clinically relevant blood testing. PFTs were repeated at 24 hours and during follow-up between 30 and 60 days. These are described in detail in chapter 3.

2.2.3.2. Coronary angiography and thrombus aspiration

Background of thrombus studies

As described in chapter 1, one of the proposed mechanisms of late and very late ST in general is delayed coronary arterial wall healing post stenting, characterised by persistent fibrin deposition, incomplete re-endothelialisation and chronic inflammation. These findings are mainly derived from pivotal studies from the Virmani group, who performed autopsy studies in patients with very late ST (44, 133, 134). In addition, late hypersensitivity reactions may play a role, especially with first generation DES (135). Cook et al. demonstrated evidence of hypersensitivity reactions in thrombi aspirated from coronary arteries in ST (136). These demonstrated extensive eosinophilic infiltration, typical of type IVb hypersensitivity (sirolimus eluting stents) and macrophage infiltration, typical of type IVc hypersensitivity (paclitaxel-eluting stents). Thus, aspirated thrombus samples were collected from the UK cohort of the PRESTIGE study. For standardisation reasons, these were sent to a core laboratory in Munich, Germany for analysis and so the results are not presented in this thesis. These data have recently been published, with the author of this MD being a co-author on the paper (82).

Thrombus aspiration protocol

If ST was confirmed on coronary angiography (defined as a filling defect suggestive of thrombus within a stent or within 5mm proximal or distal to the stent), intracoronary thrombus was aspirated using a thrombectomy catheter of operator choice. The harvested material was passed through a 40μm Becton Dickinson cell strainer filter and the aspirated material was retained and transferred to a pot containing 10% neutral buffered formalin (NBF) solution. After 48 hours of fixation at room temperature, the material was transferred to 5% sucrose phosphate buffered saline (PBS) and stored at 4°C until shipment to the core laboratory in Munich, Germany for subsequent histopathological evaluation.

2.2.3.3. Optical coherence tomography

Background of intracoronary imaging

In recent years, more advanced intracoronary imaging techniques have been developed to complement the process of coronary angiography in patients undergoing PCI. The use of intravascular ultrasound (IVUS) has led to a better understanding of the in vivo pathophysiology of coronary atherosclerosis and in everyday practice helps the interventionist assess the anatomy of coronary lesions, the results of PCI and the mechanisms of stent failure. IVUS does however give relatively low-resolution images and this has led to the development of a relatively new technique of intracoronary imaging, optical coherence tomography (OCT).

OCT is an optical imaging modality that uses near-infrared light to create high-resolution images of intracoronary tissue microstructure. It uses a flexible fibre-optic catheter with a distal lens for light delivery and enables a high speed 54mm (or 75mm) pull-back through the coronary artery at a speed of 20mm per second. As it uses a light source with a short wavelength (centred at 1.3μm), it reflects (and detects) very small objects including red blood cells, and therefore requires flushing of the vessel with contrast to create a blood-free environment during pull-back imaging. Its major advantage over the alternative modality of intravascular

ultrasound (IVUS) is the very high spatial resolution and therefore its ability to clearly characterise tissue, including lipid-rich plaque, calcium, intimal thickening, thin-capped fibro-atheroma, eroded and ruptured plaques, red blood cell rich thrombus (red thrombus), platelet-rich thrombus (white thrombus) and macrophages (137-142). This degree of detail makes it very useful to assess potential causes of stent thrombosis and restenosis. The major disadvantage of OCT over IVUS is its limited tissue penetration and, therefore, its inability to consistently image the adventitia and assess plaque burden. The main technical differences between IVUS and OCT are shown in table 2.1.

Table 2.1 Technical comparison of optical coherence tomography (OCT) with intravascular ultrasound (IVUS)

	OCT	IVUS
Axial resolution	12-15μm	100-200μm
Beam width	20-40μm	200-300μm
Frame rate	100 frames/sec	30 frames/sec
Pullback speed	20mm/sec	0.5-1mm/sec
Max scan diameter	10mm	15mm
Tissue penetration	1.0-1.5mm	10mm
Lines per frame	500	256

OCT protocol

Following restoration of Thrombolysis in Myocardial Infarction (TIMI) grade 2-3 flow in the culprit coronary artery and ideally before any balloon angioplasty, an OCT pullback was performed whenever possible using the C7XR OCT imaging system (St Jude Medical).

The exclusion criteria for performing OCT were as follows:

- Lack of technical expertise or equipment
- Extreme coronary tortuosity
- Very distal culprit lesion
- Thrombus aspiration unable to re-establish antegrade coronary blood flow

Following administration of intracoronary nitroglycerin (200 micrograms), images were acquired with an automated pullback of the OCT imaging lens through the entire length of the stented coronary artery, including distal and proximal reference vessel sites, at a speed of 20mm per second, whilst injecting intracoronary contrast at a rate of 3-5ml/second to clear the artery of blood. The images were stored digitally and exported in RAW format for subsequent offline evaluation and detailed analysis in the core laboratory in Munich, Germany. I was a member of the analysis team and over a series of meetings in Munich, we analysed the 231 OCT pull-backs acquired from all PRESTIGE centres in Europe.

Coronary intervention was then completed as clinically appropriate.

2.2.3.4. CRF completion

Full written informed consent was sought within 24 hours of admission and a detailed case report form (CRF) was completed. This included demographics, past medical history and drug history. The reports and angiographic images from the initial PCI procedure were analysed in detail by myself, including the indication for PCI, the location, size, extent and complexity of the underlying coronary disease, the type, size and number of stents used, the use of stent post dilatation and whether intracoronary imaging was used. The final angiographic results were also analysed, including the presence of residual coronary disease, untreated coronary dissections, residual thrombus and final TIMI flow. The use of antiplatelet and peri-procedural antithrombotic agents was also noted.

Details of the current admission with ST were also documented, including any changes in past medical history, compliance with antiplatelet medication, the

occurrence of any bleeding events or any recent surgical procedures, as well as the mode of presentation, vital signs, electrocardiogram (ECG) and blood results.

2.2.3.5. Clinical follow-up

Following discharge from hospital, patients were followed up in a dedicated outpatient clinic between 30 and 60 days, during which the incidence of the following clinical 30-day outcomes were documented:

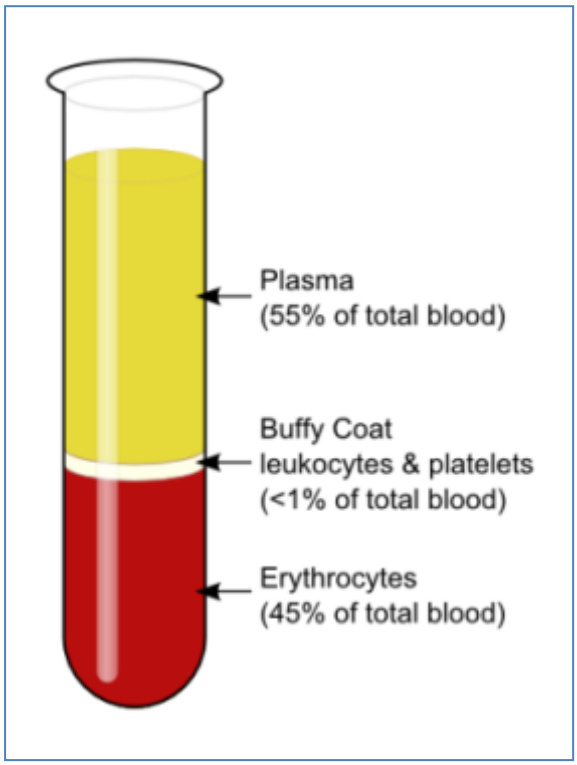
- Stroke
- Recurrent ST (definite or probable)
- Recurrent MI
- Ischaemia-driven ST vessel revascularisation
- Death

Compliance with antiplatelet medication was checked and a 12-lead ECG and transthoracic echocardiogram were performed to assess the left ventricular function (if not already done at the time of ST). Patients had further telephone follow-up annually to 3 years to check clinical outcomes as above.

2.2.3.6. Blood sampling

PFTs were repeated between 30 and 60 days using the VerifyNow® and/or Multiplate® systems (chapter 3). A blood sample was also collected into two 4.5mls 3.2% sodium citrate vacutainer tubes (BD), which were centrifuged within 2 hours of collection at 1,500 RCF (xg) for 15 minutes at room temperature. The plasma was carefully aspirated from both vacutainers using a Pasteur pipette and 1ml was dispensed into each of four 1.5ml flip top Eppendorf tubes. The white buffy coat layer, located in the interface between the erythrocytes and the plasma (figure 2.4) was then carefully aspirated and dispensed in equal volume into two bar-coded 0.5ml 2D matrix tubes (Thermo Scientific) for storage at -80°C for future genetic testing.

Figure 2.4. Separation of blood components after first centrifugation step



The four 1.5ml flip top Eppendorf tubes containing plasma were then centrifuged in a microfuge at 13,000 RCF (xg) for 2 minutes at room temperature. Having identified the platelet pellet at the bottom of the tube, the supernatant was gently aspirated, leaving 100ul of plasma/platelet pellet in the tube. The supernatant (plasma containing microparticles) was dispensed into four bar-coded 1ml 2D matrix tubes (Thermo Scientific) for storage at -80°C and subsequent analysis of thrombin generation potential (chapter 3). The 1.5ml eppendorf tubes containing the plasma/pellet were discarded.

2.2.3.7. Genetic testing

Genetic variations in relation to prothrombotic states and metabolism of antiplatelet drugs are known to increase the risk to patients undergoing PCI (105, 143-145). Several variations have been linked to a reduced effect of antiplatelet therapy and patients carrying these variations are known to have a worse clinical outcome, including a higher risk of ST (146, 147). Although the impact of several variations in cytochrome P450 enzymes has now been well established, there is

agreement that these variations cannot fully explain the diminished response to clopidogrel therapy and poor clinical outcome (144, 148).

Numerous studies have looked at the impact of genetic polymorphisms on clopidogrel metabolites and on platelet function, but few studies have looked at their impact on ST and even fewer have assessed 'non-clopidogrel related' genetic variation. None have been undertaken at the time of definite stent thrombosis. It may be that non-clopidogrel related polymorphisms associated with ST are related to other genes involved in platelet function, metabolism, endothelial function, stem cell turnover and/or inflammation. Large genome-wide association studies (GWAS) would be needed to identify novel genetic variants contributing to the risk of ST.

Although blood samples were taken from all UK PRESTIGE patients as described above and stored for future genetic testing, the DNA analysis has not yet been undertaken as it was not funded in the PRESTIGE grant. A genome wide analysis using the entire European PRESTIGE cohort is currently being planned and I plan to be involved in this under the auspices of Professors Nilesh Samani and Alison Goodall in Leicester.

2.2.4. Retrospective ST cases

Patients who had previously suffered a ST event at the investigational sites, any time from January 2011 onwards, were also included retrospectively, collecting demographic, clinical and angiographic data, as well as a blood sample for PFTs, thrombin generation and subsequent DNA analysis. These patients were identified from the local angioplasty database, contacted and asked to be part of the study.

2.2.5. Control patients

The aim was to recruit five control patients for each ST case, who had undergone PCI without developing ST and these were matched according to the following criteria:

- Timing of PCI (within 1 month of ST case's initial PCI)
- Indication for PCI (stable angina versus ACS)
- Geographical location (PCI at the same initial treating centre)

They were invited by post to take part in the study, with a copy of the patient information leaflet and a return slip. If agreeable, they were asked to attend an outpatient clinic for formal written informed consent, an interview to complete the CRF and blood tests for PFTs, thrombin generation studies and future DNA analysis.

I performed all study related activities at the UHL site, including identifying patients, consenting, blood sampling and processing (including PFTs), thrombus processing, OCT acquisition when present, CRF completion and follow-up. I also co-ordinated all UK satellite sites, processed and stored the blood samples for subsequent genetic studies and entered all UK CRF data on to the online PRESTIGE registry database.

2.3. Results

2.3.1. Recruitment

I recruited a total of 138 patients with "definite" ST and 353 controls, a ratio of 1 to 2.56 in the UK between May 2012 and October 2014. There were between 0 and 6 controls matched per case, as illustrated in figure 2.5. The controls were matched according to the predefined criteria as follows:

- Timing (within 1 month of ST case's initial PCI) 97%
- Indication (stable angina versus ACS) 88%
- Location (PCI at the same initial treating centre) 100%

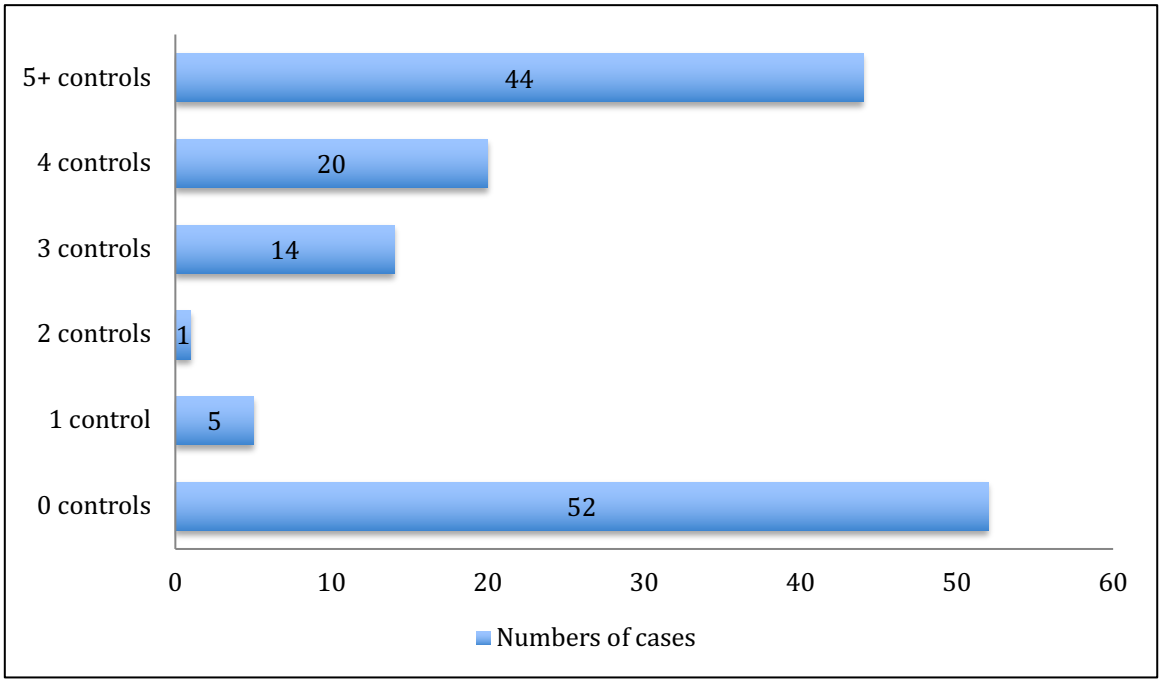
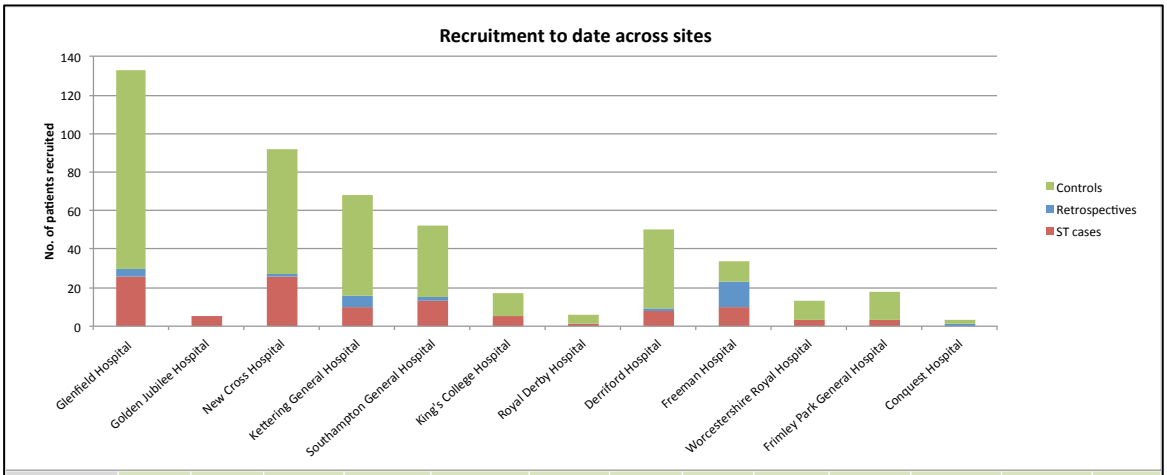


Figure 2.5. Number of controls recruited per case in UK cohort of PRESTIGE study

The monthly recruitment of each UK study site is shown in figure 2.6 and the separate patient groups are shown in figure 2.7.

	Glenfield Hospital (Leicester)	Golden Jubilee Hospital (Glasgow)	New Cross Hospital (Wolverhampton)	Kettering General Hospital	Southampton General Hospital	King's College Hospital (London)	Royal Derby Hospital	Derriford Hospital (Plymouth)	Freeman Hospital (Newcastle)	Worcestershire Royal Hospital	Frimley Park General Hospital	Conquest Hospital (East Sussex)	Monthly Total	Cumulative Total
Joined Study (Approval date)	25/05/2012	26/11/2012	18/12/2012	20/12/2012	31/01/2013	26/02/2013	16/04/2013	25/04/2013	25/04/2013	23/08/2013	25/10/2013	10/12/2013		
May-12	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Jun-12	2	0	0	0	0	0	0	0	0	0	0	0	2	3
Jul-12	2	0	0	0	0	0	0	0	0	0	0	0	2	5
Aug-12	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Sep-12	4	0	0	0	0	0	0	0	0	0	0	0	4	9
Oct-12	2	0	0	0	0	0	0	0	0	0	0	0	2	11
Nov-12	2	0	0	0	0	0	0	0	0	0	0	0	2	13
Dec-12	3	0	0	0	0	0	0	0	0	0	0	0	3	16
Jan-13	4	1	3	2	0	0	0	0	0	0	0	0	10	26
Feb-13	4	0	7	0	0	0	0	0	0	0	0	0	11	37
Mar-13	7	0	3	0	1	0	0	0	0	0	0	0	11	48
Apr-13	2	0	7	5	1	2	0	0	0	0	0	0	17	65
May-13	1	1	7	3	0	0	0	2	1	0	0	0	15	80
Jun-13	8	0	7	1	0	2	1	1	0	0	0	0	20	100
Jul-13	1	0	11	2	9	0	0	5	5	0	0	0	33	133
Aug-13	1	0	10	1	2	0	2	8	1	0	0	0	25	158
Sep-13	1	2	8	1	2	0	2	2	0	0	0	0	18	176
Oct-13	0	0	9	3	6	0	1	2	4	0	0	0	25	201
Nov-13	5	0	6	4	1	0	0	0	0	0	1	0	17	218
Dec-13	12	0	5	4	3	0	0	0	3	1	1	0	29	247
Jan-14	18	0	2	22	7	2	0	4	1	0	1	0	57	304
Feb-14	17	0	3	15	3	0	0	14	2	0	6	0	60	364
Mar-14	19	0	2	2	4	5	0	0	6	4	4	0	46	410
Apr-14	17	0	2	0	7	0	0	11	4	8	3	0	52	462
May-14	0	0	0	2	1	3	0	1	2	0	2	0	11	473
Jun-14	0	0	0	1	4	3	0	0	4	0	0	1	13	486
Jul-14	0	1	0	0	1	0	0	0	1	0	0	0	3	489
Aug-14	0	0	0	0	0	0	0	0	0	0	0	0	0	489
Sep-14	0	0	0	0	0	0	0	0	0	0	0	2	2	491
Total recruited	133	5	92	68	52	17	6	50	34	13	18	3		

Figure 2.6. Monthly recruitment to the UK cohort of the PRESTIGE study



	Glenfield Hospital	Golden Jubilee Hospital	New Cross Hospital	Kettering General Hospital	Southampton General Hospital	King's College Hospital	Royal Derby Hospital	Derriford Hospital	Freeman Hospital	Worcestershire Royal Hospital	Frimley Park General Hospital	Conquest Hospital	Total per patient group
ST cases	26	5	26	10	13	5	1	8	10	3	3	0	**110**
Retrospectives	4	0	1	6	2	0	0	1	13	0	0	1	**28**
Controls	103	0	65	52	37	12	5	41	11	10	15	2	**353**
Total per site	**133**	**5**	**92**	**68**	**52**	**17**	**6**	**50**	**34**	**13**	**18**	**3**	**491**

Figure 2.7. PRESTIGE UK recruitment per site

Of the ST cases, 4.3% presented acutely (within 24 hours), 18.8% sub-acutely (24 hours to 1 month), 8.7% as late presenters (1-12 months) and 68.1% very late (> 1 year). PFTs were carried out in 74.6% and 86.4% of the ST patients and controls respectively. Blood samples were collected and stored for subsequent genetic studies in 89.1% of the ST cases and 98.0% of the control patients. Of the ST cases, 63.8% had thrombus retrieved and 21.7% had OCT imaging.

Table 2.2. Summary of UK PRESTIGE patients' data collection

	ST CASES	CONTROLS
Numbers	**138**	**353**
Data completeness		
Demographic data	138 (100%)	353 (100%)
PFTs	103 (74.6%)	305 (86.4%)
Thrombus	88 (63.8%)	N/A
OCT	30 (21.7%)	N/A
DNA	123 (89.1%)	346 (98%)
ARC timing of ST		
Acute	6 (4.3%)	N/A
Subacute	26 (18.8%)	N/A
Late	12 (8.7%)	N/A
Very late	94 (68.1%)	N/A

2.3.2. Demographics

The characteristics of the patients are summarised in table 2.3. Data are presented as mean ± standard deviation (SD) or number (percentage %), as appropriate. Each factor was compared between the cases and matched controls using conditional logistic regression.

Table 2.3. Summary of characteristics of ST cases and controls in UK cohort of PRESTIGE study

VARIABLES	ST CASES (n=138)	CONTROLS (n=353)	*P-VALUE*
Demographic and clinical profile			
Age at time of original PCI	59.2±11.6	63.2±10.5	***P=0.015***
Body mass index	28.2±6.5	28.7±4.5	*P=0.408*
Male gender	107/138 (77.5%)	270/253 (76.5%)	*P=0.463*
Current smoker	54/131 (41.2%)	73/349 (20.9%)	***P=0.012***
Previous MI	45/133 (33.6%)	76/343 (22.2%)	***P=0.036***
Diabetes mellitus	38/138 (27.5%)	61/351 (17.4%)	***P=0.002***
Hypertension	52/132 (39.4%)	154/349 (44.1%)	*P=0.672*
CHF (LVEF<30%)	0/137 (0%)	3/346 (0.9%)	*P=0.567*
History of malignancy	27/135 (20.0%)	35/347 (10.1%)	*P=0.145*
Renal impairment (creat>175 or eGFR<30)	2/136 (1.5%)	6/348 (1.7%)	*P=0.762*
Peripheral arterial disease	4/137 (2.9%)	11/350 (3.1%)	*P=0.991*
Previous CVA	13/137 (9.5%)	7/348 (2.0%)	***P<0.001***
Atrial fibrillation	8/137 (5.8%)	16/348 (4.6%)	*P=0.507*
Autoimmune disease	7/138 (5.1%)	17/347 (4.9%)	*P=0.540*
Thromboembolic antecedents	14/132 (10.6%)	19/348 (5.5%)	***P=0.006***
Indication for PCI			
>STEMI	44/131 (33.6%)	117/348 (33.6%)	*Matched variable*
>NSTEMI	39/131 (29.8%)	81/348 (23.3%)	
>UA	11/131 (8.4%)	26/348 (7.5%)	
>Stable angina	37/131 (28.2%)	124/348 (35.6%)	
Antiplatelet therapy at time of randomisation			
On DAPT	46/137 (33.6%)	114/353 (32.3%)	*P=0.444*
>Aspirin	116/137 (84.7%)	325/353 (92.1%)	***P=0.009***
>Clopidogrel	41/137 (29.9%)	96/353 (27.2%)	*P=0.302*
>Prasugrel	6/137 (4.4%)	25/353 (7.1%)	*P=0.251*

>Ticagrelor	4/137 (2.9%)	7/353 (2.0%)	*P=0.773*
PPI use	57/136 (41.9%)	151/343 (44.0%)	*P=0.500*
Bleeding episode in month prior to randomisation	6/132 (4.5%)	5/351 (1.4%)	***P=0.059***
Non-cardiac surgery in 90 days prior to randomisation	9/131 (6.9%)	10/350 (2.9%)	***P=0.007***
Angiographic features			
Total lesion length (mm)	26.0±18.6	25.8±17.0	*P=0.343*
Reference vessel diameter (mm)	3.1±0.5	3.1±0.5	*P=0.357*
Stented segment			
>LMS	1/128 (0.8%)	10/349 (2.9%)	*P=0.396*
>LAD	58/128 (45.3%)	160/349 (45.8%)	*P=0.399*
>Cx	19/128 (14.8%)	88/349 (25.2%)	***P=0.026***
>RCA	55/128 (43.0%)	145/349 (41.5%)	*P=0.433*
ACC lesion classification C	40/116 (34.5%)	120/350 (34.3%)	*P=0.397*
Calcification	30/116 (25.9%)	78/333 (23.4%)	***P=0.061***
Tortuosity	17/116 (14.7%)	40/334 (12.0%)	***P=0.072***
PCI of SVG	3/138 (2.2%)	6/353 (1.7%)	*P=0.350*
PCI for ISR	3/131 (1.5%)	11/352 (3.1%)	*P=0.343*
PCI of CTO	3/132 (2.3%)	13/349 (3.7%)	*P=0.847*
Multi-vessel disease	68/129 (52.7%)	197/348 (56.6%)	*P=0.316*
Ostial lesion	12/126 (9.5%)	30/347 (8.6%)	*P=0.628*
Bifurcation lesion	24/129 (18.6%)	73/346 (21.1%)	*P=0.626*
PCI characteristics			
No of stents initially implanted	1.7±1.0	1.7±1.0	*P=0.331*
Total stent length (mm)	36.3±22.5	34.1±21.2	*P=0.792*
Average stent diameter (mm)	3.0±0.5	3.1±0.4	***P=0.088***
No of pts with BMS	34/136 (25.0%)	69/353 (19.5%)	*P=0.954*
No. of pts with DES	106/136 (77.9%)	297/353 (84.1%)	*P=0.686*

>1st generation	45/136 (33.1%)	69/353 (19.5%)	***P=0.013***
>2nd generation	63/136 (46.3%)	220/353 (62.3%)	*P=0.198*
>3rd generation	4/136 (2.9%)	14/353 (4.0%)	*P=0.716*
>2nd/3rd generation	67/136 (49.3%)	234/353 (66.3%)	*P=0.223*
Overlapping stents	42/128 (32.8%)	109/349 (31.2%)	*P=0.991*
Bifurcation stenting (2-stent vs 1-stent)	9/23 (39.1%)	10/72 (13.9%)	*P=0.733*
Stent post dilatation	76/125 (60.8%)	202/347 (58.2%)	*P=0.452*
Use of IVUS/OCT	10/129 (7.8%)	14/352 (4.0%)	*P=0.118*
Post PCI TIMI flow<3	4/120 (3.3%)	7/346 (2.0%)	*P=0.110*
Residual dissection	2/120 (1.7%)	2/345 (0.6%)	*P=0.197*
Residual prox stenosis >50%	4/117 (3.4%)	12/345 (3.5%)	*P=0.879*
Residual distal stenosis >50%	13/117 (11.1%)	27/344 (7.8%)	*P=0.109*
Visible thrombus at end of procedure	2/115 (1.7%)	6/334 (1.8%)	*P=0.424*
Use of GPI	43/126 (34.1%)	83/349 (23.8%)	*P=0.178*
Use of bivalirudin	11/124 (8.9%)	40/349 (11.5%)	*P=0.817*

In summary, the ST patients were younger, had more smokers, more patients with previous MI, diabetes mellitus, previous strokes and more thromboembolic antecedents. Less ST patients were on aspirin at the time of presentation, had more bleeding episodes in the month preceding randomisation and a higher incidence of non-cardiac surgery in the 90 days prior to randomisation. In the ST group, there were more circumflex artery stents, more coronary calcification, tortuosity, smaller stent diameters and more first generation DES.

To identify significant risk factors for ST, a multivariable conditional logistic regression was performed. All variables with $p<0.1$ in the univariate analysis (highlighted in table 2.3 above) were entered into the model, and model selection was conducted based on a backward approach. Statistical analyses were conducted in SAS version 9.4. All reported p-values were two-tailed and

considered statistically significant at $p<0.05$. The variables found to be significant are shown in table 2.4 below.

Table 2.4. Variables with significant difference between ST cases and controls in UK cohort of PRESTIGE study

VARIABLES	ODDS RATIO	95% confidence interval	*P-VALUE*
Age	0.97	0.94-0.996	*0.027*
Diabetes mellitus	2.35	1.22-4.53	*0.011*
Thromboembolic antecedents	2.87	1.12-7.39	*0.029*
Aspirin use	0.28	0.12-0.65	*0.003*
Non-cardiac surgery in 90 days prior to randomisation	6.81	1.83-25.34	*0.004*
Cx stenting	0.32	0.14-0.72	*0.006*
1st generation DES	4.97	1.77-13.95	*0.002*

2.3.3. Optical coherence tomography

Optical coherence tomography (OCT) imaging was carried out in 30 out of the 138 UK ST patients (21.7%). OCT acquisitions for each individual patient were reviewed together with all European sites' OCT images at expert panel core lab meetings in Munich, Germany (Tom Adriaenssens, Takashi Akasaka, Fernando Alfonso, Robert A. Byrne, Giulio Guagliumi, Michael Joner, Nikesh Malik and Vasile Sirbu). Panellists were blinded with respect to timing of ST and type of stent, as well as demographic, clinical and other procedural details. The only information available was whether the OCT pull-back was acquired before or after thrombectomy, balloon dilatation or further stent implantation.

The first assessment was whether the OCT pull-back was of sufficient quality to allow further meaningful analysis. Reasons for exclusion were:

- insufficient image quality due to poor clearance of blood
- missed region of interest with incomplete stent visualisation
- excessive remaining thrombus obscuring the underlying stent assessment
- the presence of artefacts precluding the analysis

Non-analysable frames were defined as those with less than a total of 45 degrees of visible lumen border (e.g. due to the presence of thrombus or side branches). Stent struts located across the ostia of side branches were excluded from the analysis of coverage and apposition. Three UK OCT pull-backs were excluded from analysis, one due to the presence of excess thrombus obscuring visualisation of the underlying vessel, the second was only done post PCI, making it impossible to ascertain the underlying mechanism of ST and the third had inadequate clearance of blood from the vessel.

The remaining 27 OCT pull-backs were qualitatively assessed in both longitudinal and cross-sectional views for the dominant finding at the time of ST. If no dominant factor could be identified as the likely underlying mechanism for ST, this was labelled as ‘no dominant factor’. Additional findings assessed to be of lesser relevance were listed as contributing factors. In case of disagreement, decision was made by consensus.

Possible dominant and contributing factors are listed in table 2.7 below. Edge dissection, proximal or distal to the stented segment, was considered significant if it involved the intima and/or media with a circumferential extent of at least 1/3 of the vessel contour and a longitudinal extent greater than 3mm. Severe under-expansion was described as a stent area <80% of the mean of the proximal and distal reference vessel area. Severe restenosis was defined as the presence of >50% diameter stenosis in the stented segment. Stent struts were considered uncovered if any part of the strut was visibly exposed to the lumen. Mal-apposition was considered present when the axial distance between the strut’s surface to the luminal surface was greater than the strut thickness (including polymer, if

present) including a correction factor to account for strut blooming artefact. Extra-stent cavities were defined as the presence of an outward bulge in the luminal vessel contour between apposed struts, with a maximum depth of the bulge area being greater than 1/3 of the lumen diameter. Neoatherosclerosis was defined by the presence of one or more of the following: lipid-laden tissue within the stent, defined as a signal-poor region with diffuse border and light signal attenuation, possibly masking deep strut detection; thin-capped fibroatheroma (TCFA), defined as plaque with lipid-laden tissue with a fibrous cap thickness ≤65μm at the thinnest measured point, or neointimal calcification, characterised by a signal-poor region with sharp demarcation with the overlying neointima. Neotherosclerosis was considered a significant factor for ST if there was evidence of plaque rupture or in association with TCFA adjacent to the site of maximum thrombus burden. Finally, thrombus was defined as an intraluminal protruding mass with irregular borders with or without adherence to stent struts or luminal tissue.

A separate frame-by-frame and lesion-based analysis was carried out using a standardised protocol in the dedicated core lab. This involved more detailed quantitative and morphometric analyses of OCT cross-sections at 1mm intervals along the entire target segment.

The results of the qualitative analysis are summarised in table 2.5, figure 2.8 and figure 2.9 with representative OCT frames for each dominant mechanism shown in figure 2.10.

Table 2.5. Summary of qualitative analysis of OCT imaging in ST patient from UK cohort of PRESTIGE study

OCT FEATURE	Contributing factor	Dominant factor
Total	**27**	**27**
Stent overlap	7 (25.9%)	0
Stent fracture	0	0
Bifurcation	2 (7.4%)	0
Distal disease	1 (3.7%)	0
Proximal disease	2 (7.4%)	1 (3.7%)
Distal edge dissection	0	0
Proximal edge dissection	0	0
Severe under-expansion	11 (40.7%)	3 (11.1%)
Severe in stent restenosis	4 (14.8%)	1 (3.7%)
Uncovered struts	20 (74.1%)	9 (33.3%)
Mal-apposition	12 (44.4%)	3 (11.1%)
Extra-stent cavity	6 (22.2%)	1 (3.7%)
Neoatherosclerosis	5 (18.5%)	4 (14.8%)
No dominant factor	5 (18.5%)	
Red thrombus	15 (55.6%)	
White thrombus	25 (92.6%)	

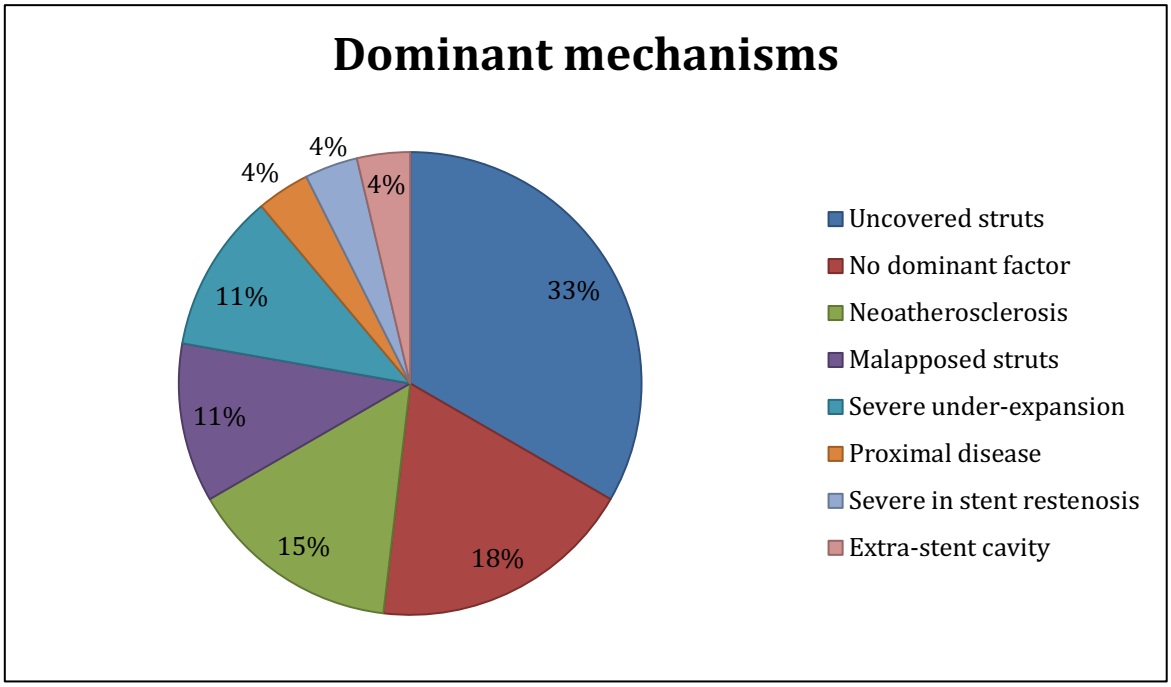


Figure 2.8. Dominant mechanisms identified on OCT imaging for UK PRESTIGE ST patients

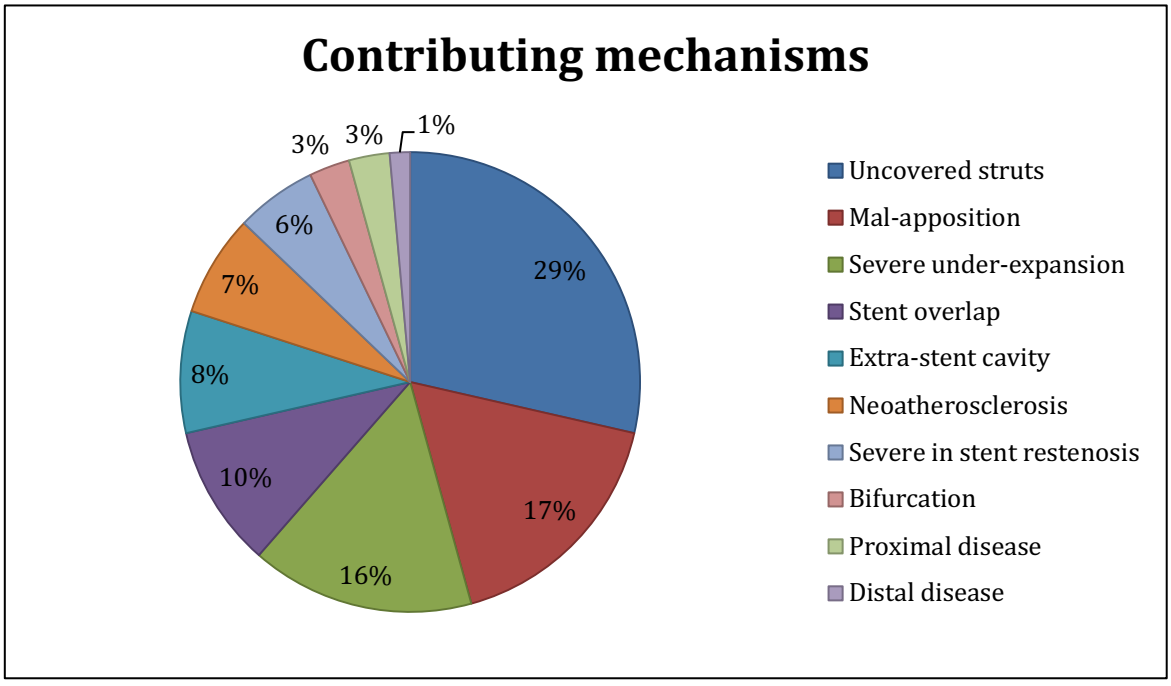


Figure 2.9. Contributing mechanisms identified on OCT imaging for UK PRESTIGE ST patients

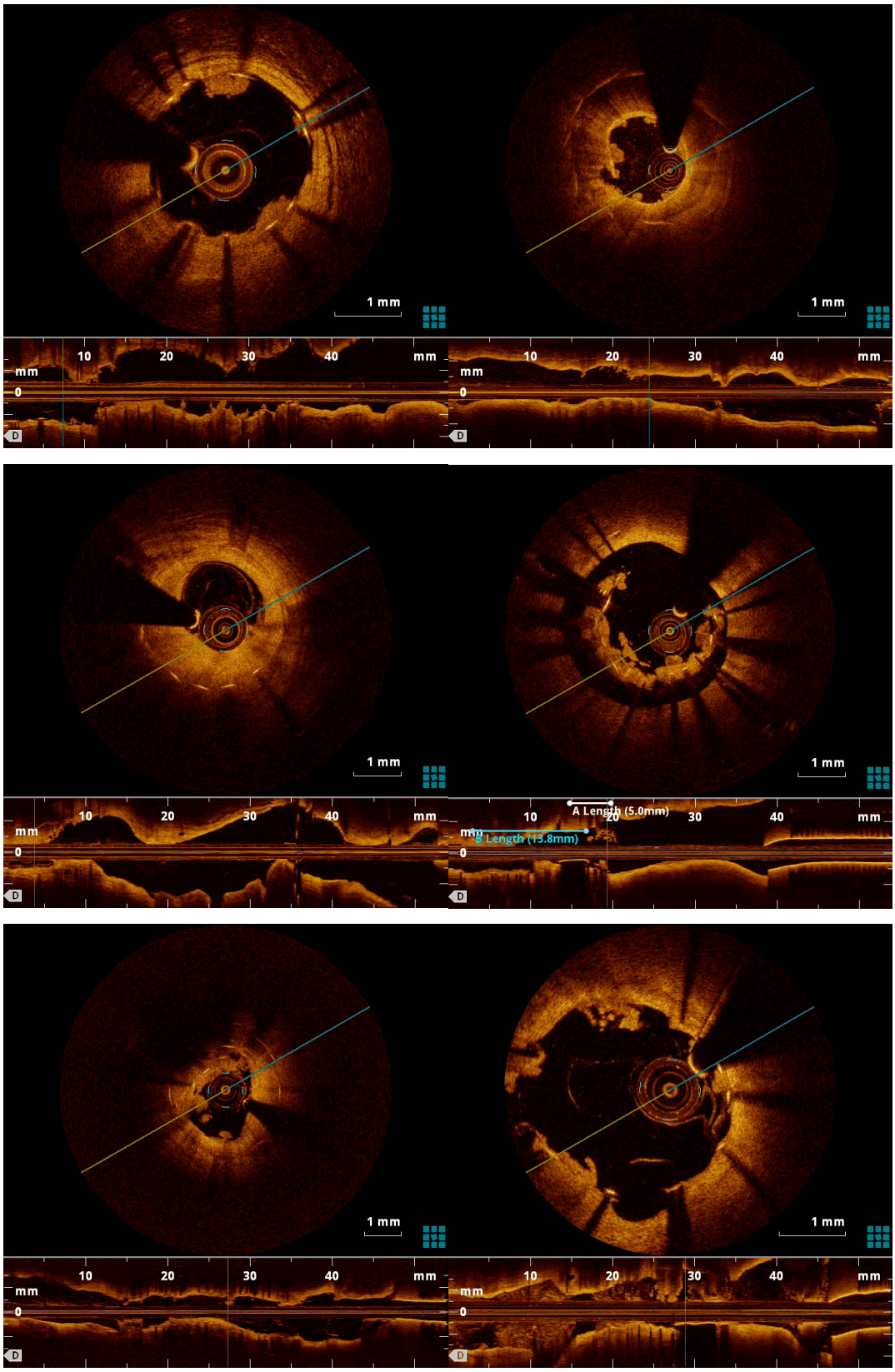


Figure 2.10. OCT examples from UK PRESTIGE ST cases: uncovered struts (top left), neoatherosclerosis (top right), severe in stent restenosis (middle left), malapposition (middle right), severe under-expansion (bottom left) and extra-stent cavities (bottom right)

The dominant underlying mechanisms on OCT for early ST (acute and sub-acute) and very late ST (>1 year after PCI) are shown separately in figures 2.11 and 2.12 below. There was only one OCT pull-back analysable for the late ST time-point (1 month-1 year) and there was no dominant underlying cause identified on this, although 'severe in stent restenosis' and 'stent overlap' were seen as contributing factors.

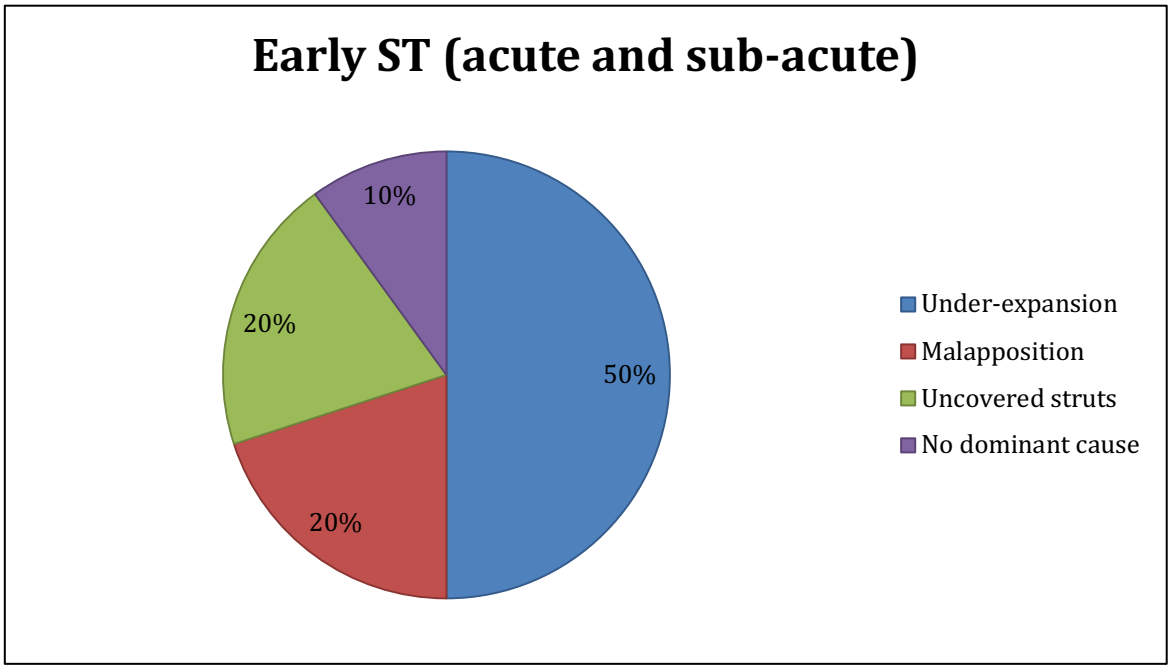


Figure 2.11. Dominant mechanisms identified on OCT imaging for UK PRESTIGE early ST patients

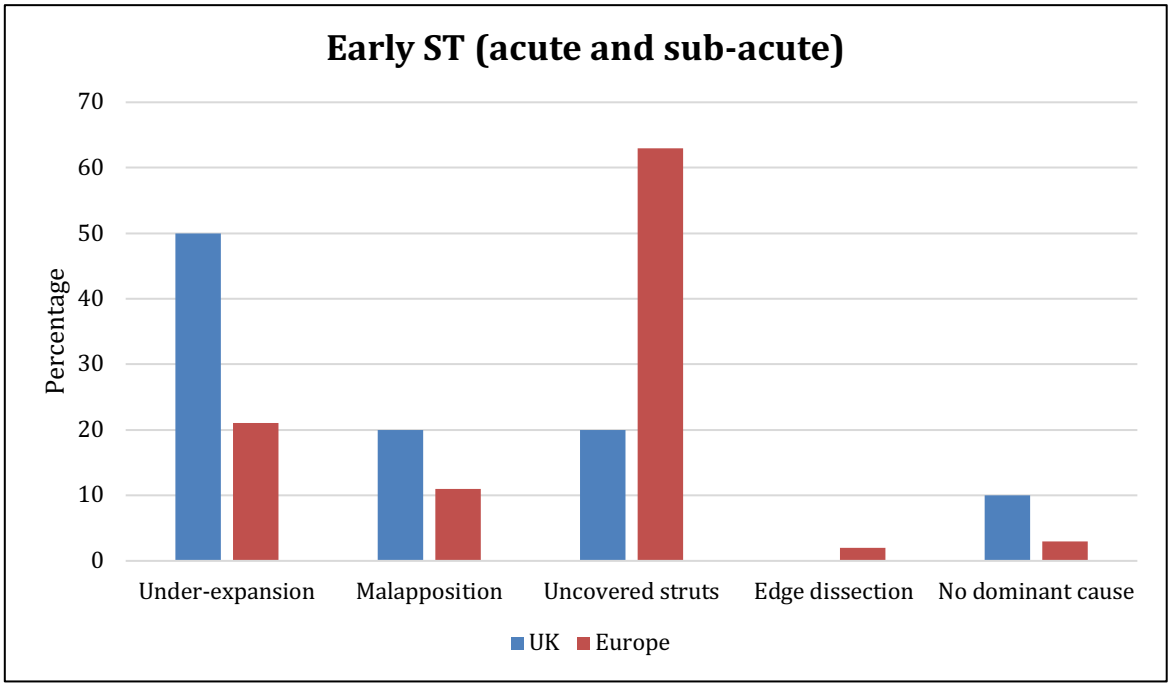


Figure 2.12. Comparison of dominant mechanism identified on OCT imaging for UK and Europe-wide PRESTIGE early ST patients

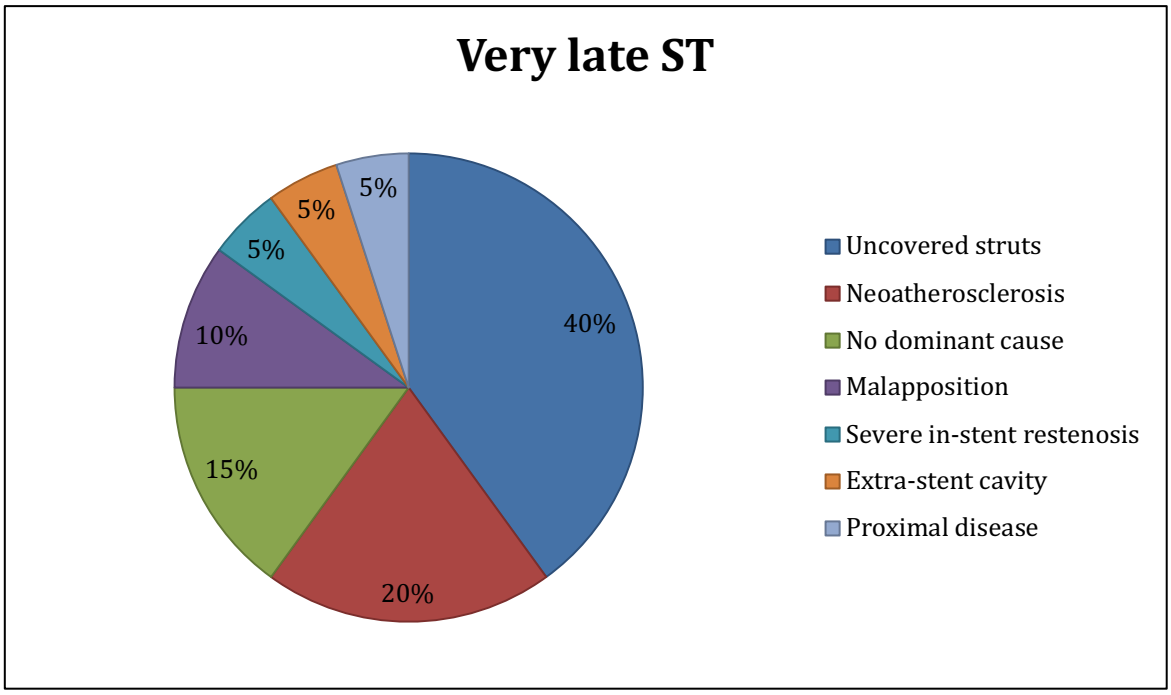


Figure 2.13. Dominant mechanisms identified on OCT imaging for UK PRESTIGE very late ST patients

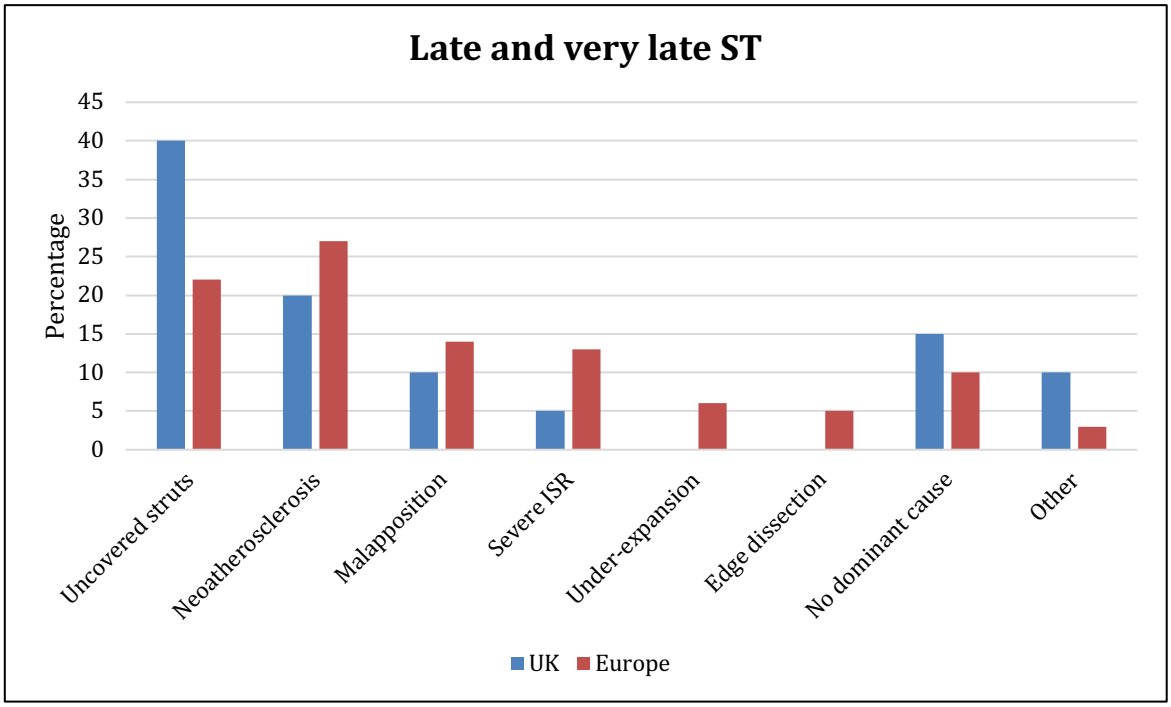


Figure 2.42 Comparison of dominant mechanism identified on OCT imaging for UK and Europe-wide PRESTIGE late and very late ST patients

2.3.4. Outcome of PRESTIGE ST patients

Patients with ST in the UK cohort of the PRESTIGE study were followed up in clinic by myself at 30-60 days with further annual follow-up by telephone up to 3 years. The 30-day and 1-year outcomes are shown in table 2.6 below.

Table 2.6. Outcomes of ST patients in UK cohort of PRESTIGE study

OUTCOME	30 DAY	1-YEAR
Stroke	1/118 ***(0.85%)***	4/103 ***(3.88%)***
Recurrent ST (definite/probable)	5/116 ***(4.31%)***	9/102 ***(8.82%)***
Recurrent MI	4/116 ***(3.45%)***	7/103 ***(6.8%)***
Ischaemia driven TVR	2/115 ***(1.74%)***	5/104 ***(4.81%)***
Death	8/135 ***(5.93%)***	14/132 ***(10.61%)***

2.4. Conclusions

Using a coordinated multi-centre approach, I collected multiple modalities of data from patients with definite ST, the majority of whom (68%) presented at the 'very late' (>1 year) time-point post stenting. This includes detailed demographic and clinical data, along with platelet function testing and intracoronary imaging with OCT analysis. In addition, control patients were recruited using limited matching criteria. Although the initial aim had been to match 5 controls per ST case, this proved difficult and there were anything from 0 to 6 controls per case.

Given the ongoing risk, albeit relatively small, and associated poor clinical outcomes, as illustrated by the high rate of recurrent ST (9% at 1 year) and mortality (>10% at 1 year) in this study, it is important to identify those patients at highest risk of ST. Although previous studies have identified multiple risk factors for ST (28-30, 32, 33, 149, 150), this study certainly adds to the understanding of the risk profile of patients with ST, especially those presenting at the 'very late' time point and in those patients with newer generation DES. Such factors can be used to emphasise the importance of optimal PCI techniques and potentially target higher risk patients for more potent or prolonged dual antiplatelet therapy, as discussed in chapter 5.

2.4.1. Study limitations

The first limitation of this study was the matching process. Despite the very limited number of matching criteria, it proved difficult to recruit the planned 5 controls for each ST case. This certainly reduced the statistical power in the final analysis and it may have been more realistic and favourable to match a fewer number of controls for each ST case.

Secondly, a complete dataset could not be collected for all patients. Although the vast majority of demographics and clinical characteristics were available, not all of the UK satellite sites had access to all of the investigative modalities. OCT, which was available at only 5 out of the 12 UK sites, was performed in only 22% of ST cases. In addition, there was no control group with OCT imaging, which

ideally would have consisted of patients who had undergone stent implantation without developing ST. This limits the ability to determine the association of observed OCT features with the clinical presentation of ST. Despite this, the OCT findings in this study are concordant with previous OCT case-control studies of a smaller size (151).

2.4.2. Demographics and clinical characteristics

The ST group of patients were younger, they had a higher prevalence of diabetes mellitus, more thromboembolic antecedents (e.g. deep vein thrombosis, pulmonary embolism and transient ischaemic attacks), were less likely to be taking aspirin at the time of randomisation, had a higher rate of non-cardiac surgery in the 90 days prior to randomisation, were less likely to have a culprit circumflex artery lesion and had more use of 1st generation DES. Some of these factors, including younger age, diabetes mellitus and the lack of aspirin therapy were also found to be associated with ST in the Dutch Stent Thrombosis Registry (33).

In addition, there were several other factors, which may emerge as statistically significant in the larger European cohort of the PRESTIGE study. Reassuringly, unlike in the Dutch registry, the use of DES was not associated with an increased risk of ST. This is likely to be due to the lower proportion of 1st generation DES in the PRESTIGE study. As expected however, from numerous earlier studies and as described in chapter 1, the use of 1st generation DES was significantly higher in the ST group.

The reason for younger patients being at a higher risk for ST may be due to the fact that they potentially have a longer period post stenting in which to develop ST. Theoretically, the presence of a permanent metallic stent with or without a permanent polymer, especially with possible incomplete endothelial coverage, continues to pose a risk of ST throughout the life of an individual. The latest time point in the UK cohort of the PRESTIGE study was a very late ST just under 15 years after the initial stent implantation. Another possibility could be that the older age group of patients with ST have a potentially higher risk profile with multiple

co-morbidities and therefore may present as a fatal out of hospital event. In the absence of angiographic or autopsy evidence of ST, these cases would be missed in this study, given the ARC definitions of 'definite ST'.

The higher rate of non-cardiac surgery in the 90 days prior to randomisation in the ST group may in part be explained by the potential need to omit DAPT therapy peri-operatively. In fact, 44% of those patients who had undergone non-cardiac surgery in the 90 days prior to developing ST were on neither aspirin nor a $P2Y_{12}$ antagonist at the time of randomisation. Only 44% were on a single antiplatelet agent and 11% were on DAPT. Premature cessation of DAPT, the definition of which is still widely debated, is known to increase the risk of ST (56). In the Dutch registry (33), the lack of clopidogrel therapy at the time of ST in the first 30 days after the index PCI was strongly associated with ST (HR 36.5, 95% CI 8.0 to 176.8), as was the cessation of clopidogrel therapy between 30 days and 6 months after the initial PCI (HR 4.6, 95% CI 1.4 to 15.3) and even after 6 months (HR 5.9, 95% CI 1.7 to 19.8).

The reason for less circumflex artery lesions in the ST group is unclear. This may to some extent be due to under-recognition of acute lateral/posterior territory ST elevation myocardial infarcts in the ST group compared to anterior and inferior STEMIs, which are generally more evident on ECG.

2.4.3. Optical coherence tomography

The aim of this arm of the PRESTIGE study was to gain a better understanding of the underlying pathophysiological mechanisms leading to ST. The use of high-resolution intravascular imaging with OCT was shown to be a feasible diagnostic tool in patients presenting acutely with ST in this study. However, the proportion of patients undergoing OCT in the UK was limited only to OCT-capable centres. In those centres that did have OCT, patients were excluded for various reasons, including unfavourable coronary anatomy (e.g. extreme tortuosity, very distal lesion), inability to restore TIMI 2-3 flow, excessive thrombus and haemodynamic instability. In some cases, the reason for not performing OCT was not

documented. Despite the relatively small numbers of OCT pull-backs, some conclusions can certainly be drawn.

In a selected UK patient population presenting with both early and very late ST of predominantly second generation DES, the rate of uncovered stent struts, malapposition, under-expansion and neoatherosclerosis was high.

For the early time point (acute and subacute ST), under-expansion, followed by malapposition and uncovered struts, were the most frequent dominant features found on OCT. The finding of stent under-expansion is in keeping with the known association between acute PCI procedural results and ST (33). Residual stenosis within the stented segment or a small minimal stent area is a well-recognised independent predictor of ST (36) (152). Indeed a recent case-control study, the CLI-THRO study (153), found a more than two-fold higher rate of stent under-expansion in patients with ST as compared to patients undergoing routine surveillance without clinical events. Malapposition, the relevance of which in isolation is still unclear, has also been found in other reports (154, 155). Flow disturbance due to non-streamlined flow along malapposed stent struts has recently been shown to play a role in the acute thrombogenicity of stents (156), as also suggested by the *in vivo* acute ST model described in chapter 4 of this thesis. The likelihood is that other predisposing factors, such as sub-optimal response to antiplatelet agents, diabetes and renal impairment, play a combined role with some of these OCT features to increase the risk of ST. Regarding the observation of uncovered stent struts, whilst this is not an unexpected finding in patients in the early phase after stent implantation, this perhaps emphasises the inherent thrombogenicity of exposed stents when neointimal healing and re-endothelialisation are incomplete. It also emphasises the need for careful stent sizing and adequate post dilatation.

For patients presenting with very late ST, the dominant OCT features were uncovered struts, followed by neoatherosclerosis, malapposition and to a lesser frequency, severe in stent restenosis. The findings of a higher rate of both uncovered and malapposed struts are consistent with a previous report by Guagliumi et al (151), who compared ST patients with matched DES control

subjects undergoing routine repeat intravascular imaging who did not experience LST for ≥3 years, as well as with pathological findings of delayed arterial healing after DES implantation (157). The observation of severe in-stent restenosis highlights the association between ST and in-stent restenosis as part of a spectrum of stent failure (22, 158). This link can be explained by the deceleration of flow within the restenosed section of the stent causing a more procoagulant state (159).

Another important finding in patients presenting with very late ST in this study was neoatherosclerosis. Although the diagnosis of this relatively recently recognised phenomenon is still somewhat debated, these findings are in keeping with results from autopsy studies in patients with late stent failure that found the presence of neoatherosclerosis in as many as 30% of selected autopsy cases (160). Although the prevention and treatment of neoatherosclerosis remains an important unmet clinical need that requires further investigation, the link between the development of in-stent neoatherosclerosis and native coronary atherosclerosis suggests a significant role for secondary prevention measures, including high does statins, smoking cessation and optimal blood pressure and diabetes control (161).

Similar to this study, Taniwaki et al (155), who reported on OCT findings in 64 patients with mostly late and very late ST, found neoatherosclerosis, as well as malapposition, uncovered struts and stent under-expansion in a significant proportion of cases. In patients presently acutely with ST, intravascular imaging with OCT identifies a number of potentially important mechanical features related to stent-vessel interaction, as well as tissue characterisation. Optimisation of stent deployment techniques, including the use of intravascular imaging at the time of PCI, may reduce the risk of ST by minimising the rate of under-expansion and malapposition. Future studies are needed to investigate the prevention and management of neoatherosclerosis.

2.4.4. Outcomes

In the UK cohort of the PRESTIGE study, 3 out of the 138 ST patients were lost to follow-up at 30-60 days and 6 out of 138 at 1 year. The cumulative rates of recurrent ST (8.8%) and mortality (10.6%) at one year are somewhat lower than that reported in the literature, with mortality rates ranging from 11% to 42% in studies between 2001 and 2012 (162). This may, in part, be due to contemporary treatment of ST in this study, with the latest generation of devices and partly guided by detailed intravascular imaging, as well as PFTs, which may have led to more tailored antiplatelet therapy. At 30-60-day follow-up, 72% of ST patients were on either prasugrel or ticagrelor, as opposed to clopidogrel. Further annual follow-up of ST cases in this study is continuing up to 3 years and when the overall results of the full European cohort of the PRESTIGE study is published, this will certainly provide more contemporary outcome data.

Chapter 3: Platelet function testing and thrombin generation studies

3.1. Introduction

As part of the PRESTIGE clinical study (PREvention of Stent Thrombosis by an Interdisciplinary Global European effort), blood was collected for platelet function testing (PFT) from both ST cases and control patients recruited at UK sites able to perform these studies (see chapter 2). The control patients had previously had PCI without developing ST within the same time period as the ST cases and were matched according to timing of initial PCI (within 1 month of ST case's initial PCI), indication (stable angina versus acute coronary syndrome) and geographical location (PCI at the same treating centre). In addition, blood was collected from the University Hospitals of Leicester NHS Trust (UHL) cohort, as described in chapter 2, for analysis of thrombin generation potential. These studies are described in detail in this chapter.

3.1.1. Platelet function testing

Platelets play a central role in the development of thrombotic occlusions in coronary arteries, including stent thrombosis. Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ receptor antagonist has markedly reduced the rate of atherothrombotic events in patients with ACS and those undergoing PCI, particularly with stenting. $P2Y_{12}$ receptor antagonists inhibit adenosine diphosphate (ADP)-induced platelet activation and the addition of one of the first $P2Y_{12}$ receptor antagonists, clopidogrel, to aspirin therapy has been shown to be superior to aspirin alone in the prevention of atherothrombotic events (including stent thrombosis, MI and death) in patients with ACS and those undergoing PCI (163-168). The inhibitory effect of both aspirin and $P2Y_{12}$ receptor antagonists on platelet activation can be measured ex vivo using platelet function assays. Up to 30% of patients treated with clopidogrel exhibit suboptimal inhibition of platelet activation (62), with so-called *high-on treatment platelet reactivity* (HPR) and these patients are at a heightened risk of developing atherothrombotic events including ST (169-172). Variability in response to the antiplatelet effects of aspirin

has also been described, but its effect on thrombotic events is less certain. As already explained in chapter 1, the newer $P2Y_{12}$ antagonists, prasugrel and ticagrelor, are more potent and faster acting than clopidogrel, with less variability in platelet response (107, 108, 173, 174). However, these drugs have a higher rate of bleeding events and are significantly more expensive than clopidogrel (102, 109).

Various platelet function tests (PFTs) can be used to measure the individual response to antiplatelet therapy. The two types of assays used in the PRESTIGE study were VerifyNow® and Multiplate® and these are both summarised below.

3.1.1.1. VerifyNow®

The VerifyNow® system (Accumetrics) is a turbimetric-based optical detection point-of-care device that uses citrated whole blood and is able to measure the response of platelets to aspirin and $P2Y_{12}$ receptor antagonists using different cartridges containing the relevant agonists (see below). The assay measures the rate and extent of changes in light transmittance caused by platelets binding to fibrinogen-coated beads in whole blood samples in response to agonists. Samples with inhibited platelets produce low levels of light transmittance while samples with normally functioning platelets deliver a higher level of light transmittance (figure 3.1).

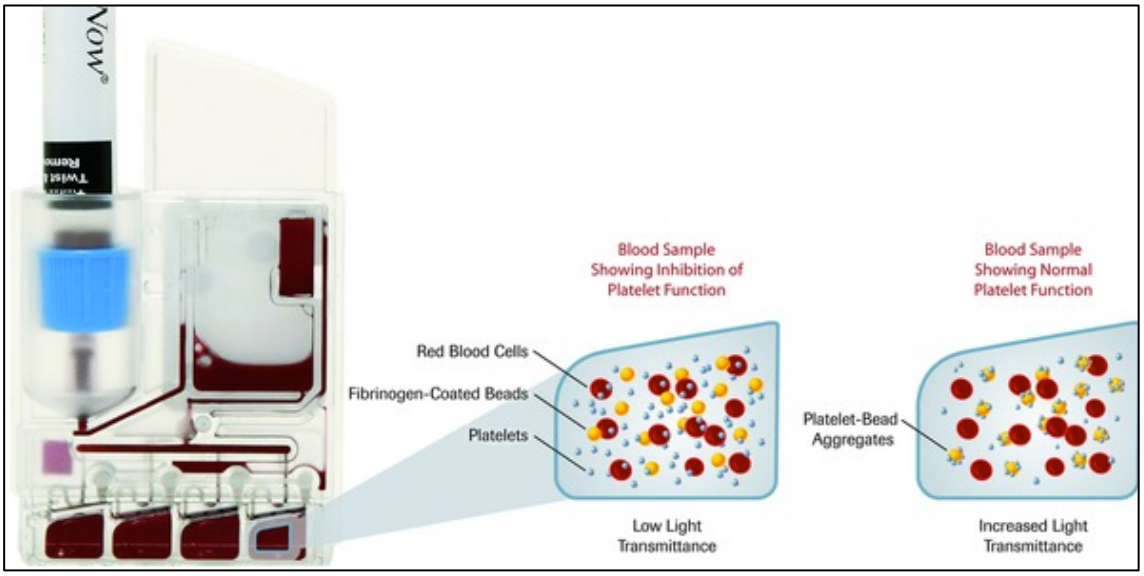


Figure 3.1. The VerifyNow® system

Aspirin binds to COX-1 enzyme to inhibit the conversion of arachidonic acid (AA) to thromboxane A2, which activates platelets. The VerifyNow aspirin test uses AA as the agonist and gives a result of Aspirin Reaction Units (ARU) to indicate the amount of thromboxane A2-mediated platelet activation and aggregation. The commonly used cut-off value to indicate a therapeutic response to aspirin is an ARU<550 (175).

The $P2Y_{12}$ cartridge contains two chambers, one with ADP as the agonist and prostaglandin E1 (PGE1) as an antagonist. PGE1 increases intra-platelet cyclic adenosine monophosphate (AMP) and reduces the action of the $P2Y_{12}$ receptor on activation, thus making the assay more specific for the effects of ADP on the $P2Y_{12}$ receptor. The second chamber contains thrombin receptor-activating peptide (iso-TRAP) as the agonist, which is used to determine the baseline platelet reactivity. Results are reported as $P2Y_{12}$ reaction units (PRU), a BASE value and a percentage inhibition. The lower the PRU, the more inhibited the platelets are from ADP-induced aggregation. Various cut-offs have been used to describe a sub-optimal response to $P2Y_{12}$ receptor antagonists; the most commonly used are PRU≥208 or PRU≥235 (169). The VerifyNow results correlate well with what is considered to be the “gold standard” of platelet function testing, namely light transmission aggregometry (LTA) (176-179).

3.1.1.2. Multiplate®

The Multiplate® platelet function analyser (Roche Diagnostics) is a laboratory-based assay that uses impedance aggregometry to test platelet response to agonists in hirudinised whole blood. It is not near-patient testing as with VerifyNow. In each test cell, there are two parallel pairs of electrodes to which activated platelets adhere. The agonists used in each of three test cells are ADP, AA and TRAP to test the platelet response to $P2Y_{12}$ antagonists, aspirin and a positive control respectively. An increase in platelet adhesion and aggregation is measured as an increased electrical resistance between each pair of electrodes in the individual cells (figure 3.2). This is converted and reported as arbitrary aggregation units (AU) and is plotted against time to give a velocity (AU/min) and area under curve (AUC) in units (U).

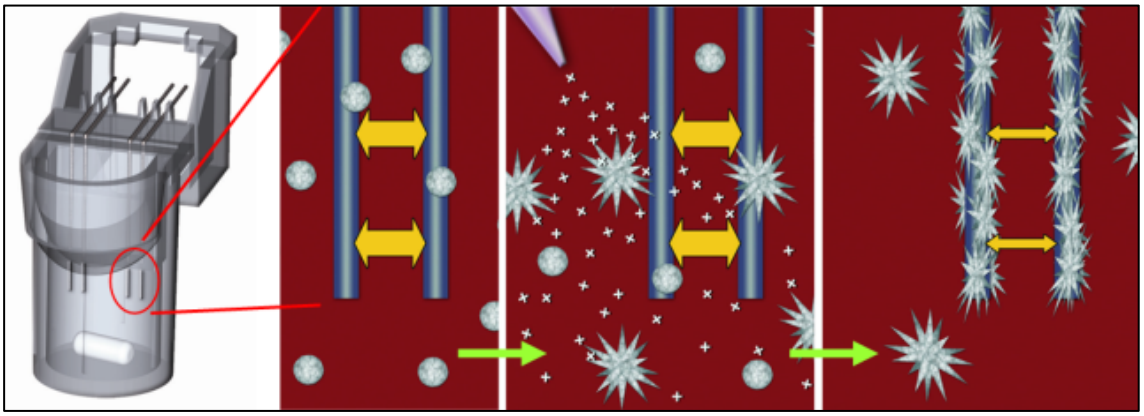


Figure 3.2. Multiple electrode aggregometry showing platelets in blood sample in Multiplate test cell adhering to electrodes following addition of agonist, causing an increase in impedance between each pair of electrodes

The consensus cut-off for the ADP test to indicate HPR is an AUC of ≥46U (≥468AU/min) (169). This is less well described for the aspirin test, but an AUC of <30U is generally considered to indicate an adequate response to aspirin. The results from the Multipate® assay correlate well with LTA (180).

3.1.2. Thrombin generation studies

It is well recognised that platelets play a major role in the development of thrombus within stents, as highlighted by the most important predictor for ST, DAPT discontinuation (33). It is however less clear to what extent the coagulation system, which is closely linked to the haemostatic effects of platelets, is involved in the underlying pathophysiology of ST.

Key to the process of thrombus formation at the site of vascular injury is the generation of thrombin, which not only plays a central role in the coagulation cascade, regulating both procoagulant and anticoagulant responses, but is also involved in the process of atherosclerosis and atherothrombosis, through the activation of protease-activated receptors (PARs) (181, 182). By activating PAR-1 and PAR-4 on platelets, thrombin acts as a potent platelet agonist, inducing platelet degranulation and aggregation (183) (figure 3.3).

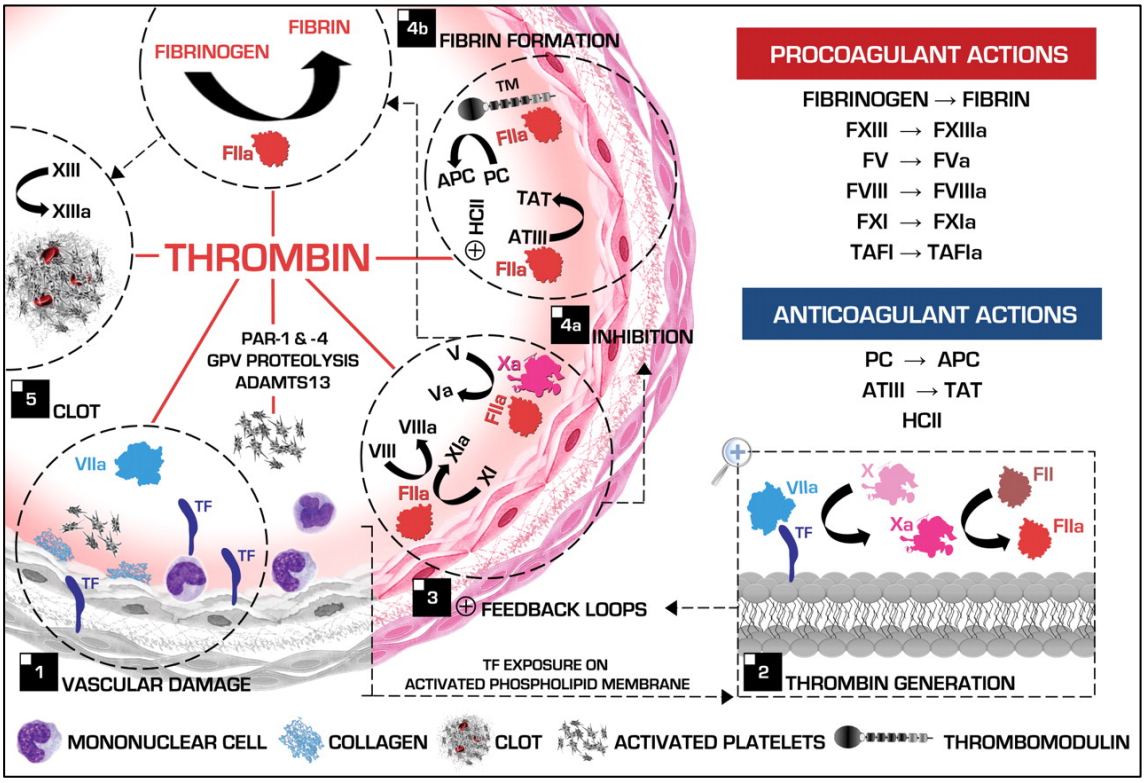


Figure 3.3. The actions of thrombin in the coagulation cascade (reproduced from Borissoff et al. (184)); TF, tissue factor; FIIa, thrombin; TM, thrombomodulin; PC, protein C; APC, activated protein C; ATIII, antithrombin; TAT, thrombin-antithrombin complex; TAFI, thrombin-activatable fibrinolysis inhibitor; HCII, heparin cofactor II; GPV, glycoprotein V

In the context of ST, this process may occur at a site of incomplete stent apposition, incomplete re-endothelialisation, chronic inflammation, adjacent vessel injury, or plaque rupture within an area of neoatherosclerosis. Although antiplatelet and antithrombotic agents at the time of PCI and a period of dual antiplatelet therapy after stenting have significantly reduced the risk of further thrombotic events, ST remains a devastating complication of PCI, with an ongoing incidence of 0.5 to 1.0% per year (34-37), and a high recurrent ST rate of about 15% (28, 185-187).

Thrombin generation is regulated by the endogenous levels of coagulation factors in the blood. These vary considerably between individuals and the overall resultant ability to generate thrombin (and therefore the risk of thrombosis) can be measured as the endogenous thrombin potential (ETP) using the Calibrated Automated Thrombinogram (or CAT) assay (188). This study aimed to analyse

whether patients presenting with ST have an enhanced level of thrombin generation as compared to control patients who have undergone PCI without developing ST. This would potentially provide another therapeutic target to further improve outcomes post stenting. Several recent clinical trials have already evaluated the addition of oral antithrombotic agents, (factor Xa inhibitors and direct thrombin antagonists), to antiplatelet therapy following PCI in acute coronary syndromes (ACS) (189-196). The obvious challenge in implementing a combined anticoagulant and antiplatelet strategy is balancing the bleeding risk with an improvement in ischaemic outcomes. Results from the ATLAS ACS-TIMI 51 (196) trial suggest that the addition of low dose rivaroxaban (an oral factor Xa inhibitor) to standard antiplatelet therapy may achieve this balance when used in carefully selected patients (197).

3.2. Methods

3.2.1. Platelet function testing

Blood was collected for PFTs from ST patients prospectively recruited to the PRESTIGE study at three time points (as described in chapter 2), acutely on presentation with ST (ideally prior to loading with DAPT), 24 hours later and between 30 and 60 days following the event. For retrospective ST patients and controls, PFTs were done on one occasion at the time of recruitment to the study. PFTs were carried out using the VerifyNow® and/or Multiplate® assays depending on the individual capabilities at each UK PRESTIGE site. All blood sampling and PFTs were done by myself for the UHL site.

3.2.1.1. VerifyNow®

Blood was transferred or collected directly in to two 2mls vacuette® tubes containing 3.2% sodium citrate (Greiner Bio-One) after discarding the first 5mls of blood collected (to avoid haemostatic activation) and before filling the EDTA tube for a FBC (to avoid contamination). These samples were then incubated at room temperature for between 10 and 120 minutes for the $P2Y_{12}$ assay and between 30 and 120 minutes for the aspirin assay. The tubes were inverted at

least five times to ensure adequate mixing just prior to testing using the appropriate cartridge in the VerifyNow® instrument. Having performed the electronic control (EQC) with the EQC device in the VerifyNow® instrument and entered the patient details, including PRESTIGE number, the appropriate cartridge was inserted into the instrument and one of the blood sample tubes was inserted into the sample well of the assay device. Having recorded the results, the process was repeated for the $P2Y_{12}$ and aspirin assays (see appendix…. for SOP).

3.2.1.2. Multiplate®

Blood was transferred or collected directly in to two 3mls Multiplate® hirudin blood tubes (Roche Diagnostics), after discarding the first 5mls of blood collected, and incubated at room temperature for 30 to 180 minutes. The tubes were gently inverted once to ensure adequate mixing just prior to testing in the Multiplate analyser. Having connected the sensor cables on the Multiplate analyser and performed the electronic control, the patient details, including PRESTIGE number, were entered. Three test cells were then inserted and connected to the sensor cables for each of the three agonists, ADP, AA and TRAP. After filtering and pre-warming 25mls of 0.9% sodium chloride solution, 300ul of this solution was pipetted into each test cell, followed by 300ul of the hirudinised blood sample and these were incubated for 3 minutes. Following this, 20ul of each of the test reagents, ADP, AA and TRAP, was added to the appropriate test cell and these were incubated for a further 6 minutes. The resulting aggregation curves were recorded for each agonist.

3.2.2. Thrombin generation studies

Blood for thrombin generation studies was sampled, as described in chapter 2, from PRESTIGE patients recruited at the UHL site only. This included ST cases and controls.

Venous blood was taken from an antecubital vein using a 21-gauge needle in to two 4.5mls 3.2% sodium citrate vacutainer tubes (BD) and these were centrifuged, as described in chapter 2, at room temperature at 1,500 RCF (xg) for 15 minutes.

The supernatant was collected and centrifuged again at 13,000 RCF (xg) for 2 minutes, a step that removes residual platelets from the plasma. The platelet poor plasma supernatant was then aliquoted in to 1ml tubes and stored at -80ºC until analysed.

Blood samples that had been taken acutely from patients at the time of ST were excluded from the analysis, due to the potential effect of parenteral antithrombotic agents administered during PCI. Thus, samples were taken at least 30 days after the procedure. In addition, samples from patients on vitamin K antagonists, direct thrombin inhibitors and factor Xa inhibitors were also excluded.

Thrombin generation was measured in platelet poor plasma (PPP) of patients using the Calibrated Automated Thrombogram (CAT) assay. This is an in vitro assay that reflects the overall capacity of the plasma to generate thrombin by measuring the total amount of thrombin generated. Coagulation was activated by the addition of tissue factor (TF), phospholipids (PLs) and calcium chloride ($CaCl_2$) and thrombin activity was measured via the conversion of a low-affinity fluorogenic substrate for thrombin added to the plasma. Four parameters were measured and analysed using this assay (figure 3.4):

1. The ***lag time*** (clotting time), the time between the addition of the fluorogenic thrombin substrate and the beginning of thrombin generation
2. The ***endogenous thrombin potential (ETP)*** or area under the curve (AUC), which is a measurement of the total amount of thrombin generated
3. The ***peak thrombin***, which is the maximum thrombin concentration at any one time point
4. The ***time to peak (ttpeak)***, which is the time to reach maximum thrombin concentration

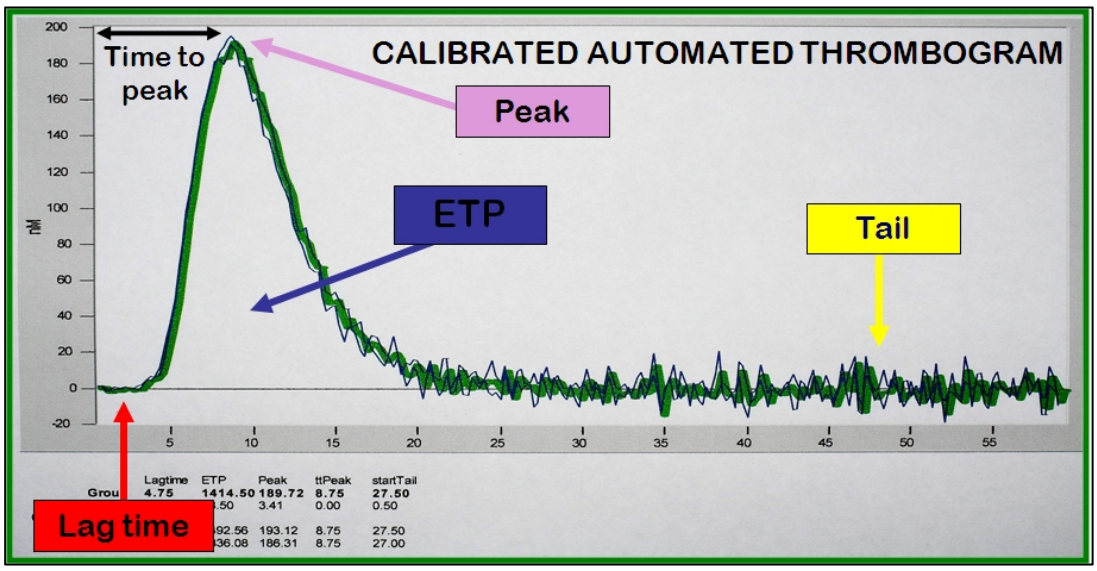


Figure 3.4. Thrombin generation curve and parameters (reproduced from Practical-Haemostasis.com); ETP, endogenous thrombin potential

Thrombin generation measurements were performed on the PPP of the ST patients and control patients. With each assay, an aliquot of pooled PPP, collected from healthy donors, was run alongside for assay standardisation from control donors. Three different trigger reagents were used for each sample to study different aspects of the coagulation cascade:

- Platelet poor plasma (PPP) reagent (5pM TF/4μM PL) – to test overall coagulation in the sample
- Platelet rich plasma (PRP) reagent (1pM TF) – to test for the contribution of procoagulant microparticles
- Microparticle (MP) reagent (4μM PL) – to test for the contribution of TF-positive microparticles

The assays were carried out using a 96-well round-bottom plate. 20 μl of pre-warmed trigger solution (PPP reagent, PRP reagent or MP reagent) was added to the 'sample wells' and 20 μl of thrombin calibrator was added to the 'calibrator wells'. These were mixed and then 80 μl of PPP was added to each 'sample' and 'calibrator' well. Plates were loaded in the Fluoroskan Ascent plate reader (Thermo-Fisher Scientific, Cramlington, UK) equipped with Thrombinoscope software (Thrombinoscope, Synapse b.v., Maastricht, The Netherlands) version

3.0.0.26 and the samples were mixed for 5 seconds. Thrombin generation was triggered upon automatic addition by the dispenser of calcium chloride ($CaCL_2$) together with the fluorogenic substrate (Fluo-Substrate, 2.5mM of Z-Gly-Gly-Arg-AMC in 0.1M $CaCl_2$). The readings were made in a microtiter plate fluorometer at 37ºC. Sample mixtures were excited at a wavelength of 390nm and the emission was measured at 460nm. Each thrombin generation measurement was corrected against a thrombin calibrator containing α2-macroglobulin/thrombin complex. Samples were analysed in duplicate and measured continuously for 60 minutes.

Statistical analyses were performed using PRISM for Mac, version 6 (GraphPad Software).

3.3. Results

3.3.1. Platelet function testing

Out of the 138 ST cases, 37 patients (26.8%) had platelet function testing (PFTs) carried out at the acute time point on presentation with ST, out of which 31 (22.5%) had a VerifyNow test performed, 18 (13.0%) had Multiplate testing and 12 (8.7%) had both tests. At the 24-hour time point 57 patients (41.3%) had PFTs performed, out of which 46 (33.3%) had a VerifyNow test, 20 (14.5%) had Multiplate and 9 (6.5%) had both.

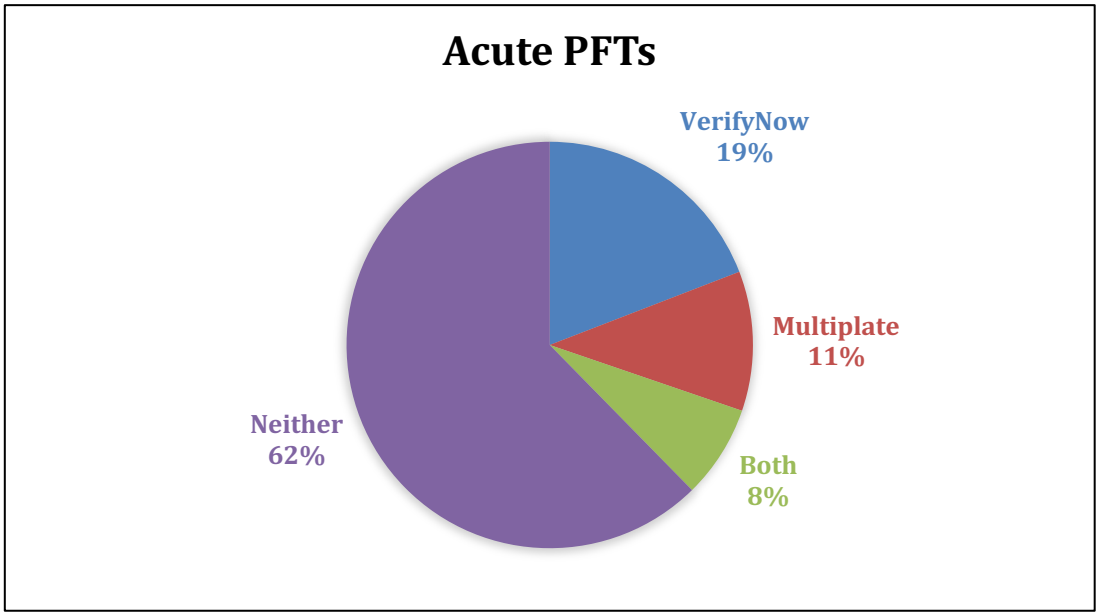


Figure 3.5 Platelet function studies undertaken acutely in PRESTIGE ST patients. PFTs, platelet function tests

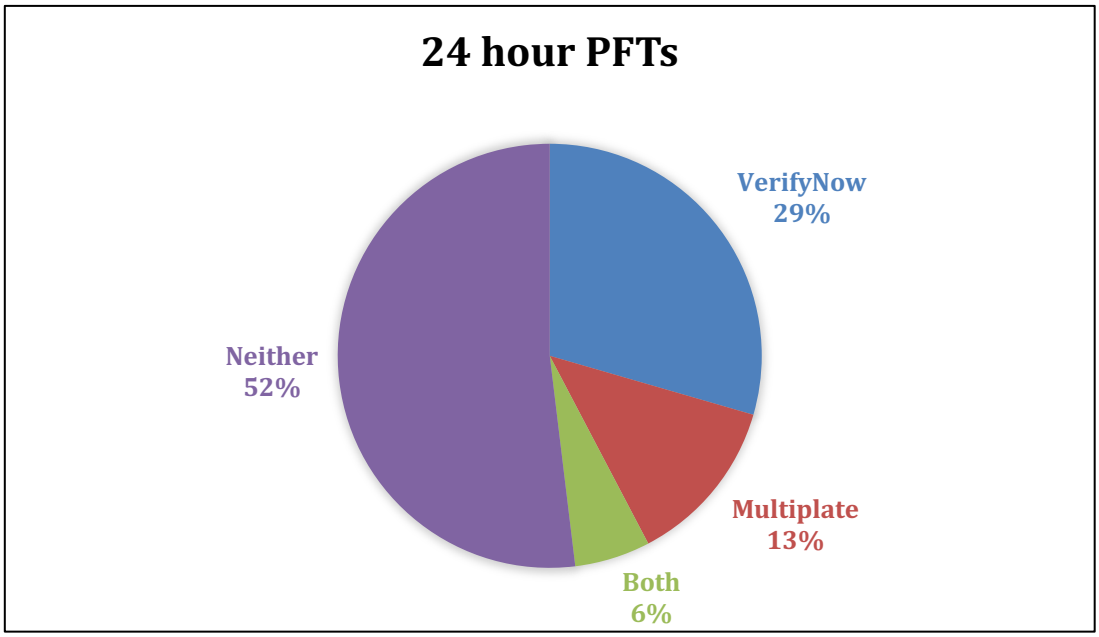


Figure 3.6 Platelet function studies undertaken at 24 hours in PRESTIGE ST patients. PFTs, platelet function tests

Out of the 37 patients that did have PFTs performed acutely, only 11 (30%) were already taking a $P2Y_{12}$ receptor antagonist at the time of presentation. Of these 11 patients, 7 were taking clopidogrel, 4 of whom had high on treatment platelet reactivity (HPR) defined by VerifyNow as a PRU≥208 or by Multiplate as an ADP AUC≥46U. Two patients were on prasugrel, 1 of whom had HPR and 2 were on ticagrelor, 1 of whom also had HPR.

Out of the 37 patients, 32 (86%) were taking aspirin at the time of acute blood sampling and 5 (16%) had HPR, defined by VerifyNow as an ARU≥550 or by Multiplate as an AUC≥30U.

Due to the low numbers of patients having PFTs analysed at the earlier time points and only a minority of them actually taking DAPT at the time of presentation, no further analysis of these PFTs was undertaken.

87 ST patients (63.0%) had PFTs done at 30-60-day follow-up and these were compared to the PFTs in the 305 patients (86.4%) in the control group, as shown in table 3.1 below. These have been analysed using conditional logistic regression for the matched case/control data. Data are presented as mean ± standard deviation (SD) or number (percentage %), as appropriate. A two-tailed probability value of $p \leq 0.05$ was considered statistically significant.

Table 3.1. Platelet function data for ST cases at 30-day follow-up and controls in UK cohort of PRESTIGE study

	ST CASES n=138	CONTROLS n=305	*P-VALUE*
PFTs	87/138 (63.0%)	305/353 (86.4%)	*N/A*
>VerifyNow	67/87 (77.0%)	294/305 (96.4%)	*N/A*
>Multiplate	41/87 (47.1%)	113/305 (37.0%)	*N/A*
>Both	21/87 (24.1%)	102/305 (33.4%)	*N/A*
IN THOSE WITH PFTS			
On $P2Y_{12}$ antagonist	75/87 (86.2%)	113/305 (37.0%)	***P<0.001***
>Clopidogrel	23/87 (26.4%)	85/305 (27.9%)	*P=0.267*
>Prasugrel	40/87 (46.0%)	23/305 (7.5%)	***P<0.001***
>Ticagrelor	12/87 (13.8%)	5/305 (1.6%)	***P<0.001***
On Aspirin	79/80 (98.8%)	286/305 (93.8%)	*P=0.081*
Hb (g/l)	136.7±16.9	139.4±15.3	*P=0.105*
Hct (%)	41.0±4.5	41.5±4.3	*P=0.205*
Platelet count	263.0±73.5	238.1±64.4	*P=0.062*
VerifyNow Baseline	267.0±42.5	262.3±46.2	*P=0.345*
Multiplate TRAP AUC	98.9±37.9	109.2±27.6	*P=0.375*
IN THOSE WITH PFTS AND ON $P2Y_{12}$ ANTAGONIST			
PRU	88.7±76.2	137.1±91.0	***P=0.002***
ADP AUC (U)	30.1±19.8	33.9±24.2	*P=0.357*
PRU≥208	6/64 (9.4%)	26/104 (25.0%)	***P=0.022***
AUC≥46U	5/30 (16.7%)	9/29 (31.0%)	*P=0.385*
HPR	9/73 (12.3%)	28/110 (25.5%)	***P=0.028***
IN THOSE WITH PFTS AND ON ASPIRIN			
ARU	443.6±77.3	440.7±63.4	*P=0.873*
ASPI AUC	22.2±26.5	15.2±19.8	*P=0.077*
ARU≥550	8/67 (11.9%)	23/272 (8.5%)	*P=0.953*
AUC≥30U	3/33 (9.1%)	7/104 (6.7%)	*P=0.168*
HPR	9/79 (11.4%)	25/283 (8.8%)	*P=0.739*

In summary, both the ST group and control patients were well matched for baseline haemoglobin, haematocrit and platelet count, as well as VerifyNow baseline and Multiplate TRAP controls. As expected, a significantly larger proportion of ST cases than controls were on a $P2Y_{12}$ antagonist when platelet function tests were performed at 30-60 days follow-up. This is because the majority of ST cases will have been prescribed DAPT at the time of their event and subsequently. A similar proportion of ST cases and controls were on clopidogrel, but a larger proportion of the ST cases were on either prasugrel or ticagrelor when followed up at 30-60 days.

The mean PRU on a $P2Y_{12}$ antagonist was lower in the ST patients (88.7±76.2 vs 137.1±91.0, p=0.002), with a lower rate of HPR when using the VerifyNow assay (9.4% vs 25%, p=0.022), with however a similar mean antiplatelet response and HPR rate when using the Multiplate assay. The overall rate of HPR on any $P2Y_{12}$ antagonist was significantly lower in the ST group (12.3% vs 25.5%, p=0.028).

The PFT data for the individual $P2Y_{12}$ receptor antagonists are shown in table 3.2 below. Due to relatively small numbers in each group, a conditional logistic regression could not be undertaken. However, the ST group has been compared to the control group as a whole. Continuous data are presented as mean ± standard deviation (SD) and group comparison of continuous data was performed using an unpaired Student's t test. Categorical variables are presented as number (%), with differences between the groups tested using a $\chi 2$ test. A two-tailed probability value of $p \leq 0.05$ was considered statistically significant.

Table 3.2. Platelet function data for individual $P2Y_{12}$ receptors antagonists for ST cases at 30-day follow-up and controls in UK cohort of PRESTIGE study

	ST CASES	CONTROLS	*P-VALUE*
ON CLOPIDOGREL			
PRU	145.2±81.4	166.6±80.6	P=0.30
ADP AUC (U)	30.5±20.1	41.7±24.3	P=0.32
PRU≥208	4/19 (21.1%)	26/80 (32.5%)	P=0.33
AUC≥46U	2/6 (33.3%)	8/19 (42.1%)	P=0.70
HPR	4/23 (17.4%)	27/82 (32.9%)	P=0.15
ON PRASUGREL			
PRU	78.4±63.4	43.9±41.5	**P=0.04**
ADP AUC (U)	28.1±17.5	21.0±19.3	P=0.39
PRU≥208	2/33 (6.1%)	0/20 (0%)	P=0.26
AUC≥46U	1/17 (5.9%)	1/7 (14.3%)	P=0.50
HPR	3/38 (7.9%)	1/23 (4.3%)	P=0.60
ON TICAGRELOR			
PRU	27.8±29.1	12.8±19.5	P=0.36
ADP AUC (U)	34.9±26.5	14.7±1.5	P=0.24
PRU≥208	0/12 (0%)	0/4 (0%)	P=1.0
AUC≥46U	2/7 (28.6%)	0/3 (0%)	P=0.30
HPR	2/12 (16.7%)	0/5 (0%)	P=0.33

When considering the antiplatelet response to clopidogrel, the mean PRU and ADP AUC were similar in both groups of patients, as well as the HPR rate, which was over 30% in the control group. The mean PRU on prasugrel was however, significantly higher in the ST group when using the VerifyNow assay (78.4±63.4 vs 43.9±41.5, p=0.04), although this increase did not reach statistical significance with the Multiplate assay. Although the HPR rate on prasugrel was low in both groups, there were 3 ST patients and 1 control patient who had a sub-optimal antiplatelet response to prasugrel. With ticagrelor, the mean PRU and ADP AUC were numerically higher in the ST group, but this did not reach statistical significance. There were only 2 patients, both in the ST group, who had a

suboptimal antiplatelet response to ticagrelor, both identified using the Multiplate assay.

The majority of patients in both groups were taking aspirin when PFTs were carried out. The mean ARU was similar in both groups, and although the mean ASPI AUC was numerically higher in the ST group, this difference was not statistically significant. The HPR rate on aspirin was similar in both groups, being just over 11% in the ST group.

3.3.2. Thrombin generation studies

20 patients with definite ST were included, of which 17 were prospectively recruited and 3 retrospectively recruited. Of these ST cases, 2 were acute (within 24 hours), 3 subacute (24 hours to 1 month) and 15 very late (>1 year) after the initial PCI. There were no late (1 month to 1 year) ST cases in this cohort. Cases were compared to 98 matched control patients, who had undergone PCI without developing ST.

3.3.2.1. Baseline characteristics

The baseline characteristics of both groups are summarised in table 3.3. Continuous data are presented as mean ± standard deviation (SD) and group comparison of continuous data was performed using an unpaired Student's t test. Categorical variables are presented as number (%) with differences between groups tested using a χ^2 test. A two-tailed probability value of $p \leq 0.05$ was considered statistically significant.

Table 3.3. Baseline characteristics of ST cases and control patients

	ST CASES (n=20)	CONTROLS (n=98)	*P-VALUE*
Demographic and clinical profile			
Age at time of original PCI (mean±SD)	58.4±11.3	64.2±10.2	***P=0.02***
Male gender	16 (80%)	74 (76%)	*P=0.67*
Current smokers	8 (40%)	27 (28%)	*P=0.27*
Previous MI	9 (45%)	25 (26%)	*P=0.08*
Diabetes mellitus	5 (25%)	12 (12%)	*P=0.14*
Hypertension	10 (50%)	42 (43%)	*P=0.56*
CHF (LVEF<30%)	0 (0%)	2 (2%)	*P=0.52*
Active malignancy	1 (5%)	1 (1%)	*P=0.21*
Renal impairment	0 (0%)	0 (0%)	*N/A*
Indication for PCI			
>STEMI	7 (35%)	20 (20%)	*P=0.16*
>NSTEMI	5 (25%)	32 (33%)	*P=0.50*
>UA	1 (5%)	8 (8%)	*P=0.63*
>Stable angina	7 (35%)	38 (39%)	*P=0.75*
Antiplatelet therapy at time of blood sampling			
On DAPT	18 (90%)	19 (19%)	***P<0.01***
>Aspirin	20 (100%)	93 (95%)	*P=0.30*
>Clopidogrel	3 (15%)	16 (16%)	*P=0.88*
>Prasugrel	11 (55%)	4 (4%)	***P<0.01***
>Ticagrelor	4 (20%)	2 (2%)	***P<0.01***
Angiographic features			
Average lesion length (mm)	17.4±8.0	19.0±12.2	*P=0.60*
Average ref vessel diameter (mm)	3.0±0.3	3.1±0.4	*P=0.32*
Stented segment			
>LMS	0 (0%)	4 (4%)	*P=0.36*
>LAD	9 (45%)	46 (47%)	*P=0.87*

>Cx	3 (15%)	24 (24%)	*P=0.36*
>RCA	8 (40%)	41 (42%)	*P=0.88*
PCI characteristics			
Average no. of stents per pt	3.1±2.1	1.7±1.1	***P<0.01***
No. of pts with DES	17 (85%)	76 (78%)	*P=0.46*
>1st generation	8 (40%)	14 (14%)	***P<0.01***
>Absorbable polymer	1 (5%)	6 (6%)	*P=0.85*
No of pts with BMS	5 (25%)	26 (27%)	*P=0.89*
Use of GPI	6 (30%)	11 (11%)	***P=0.04***
Use of bivalirudin	5 (25%)	13 (13%)	*P=0.18*

The ST cases and control groups were well matched for gender, hypertension, heart failure, active malignancy, renal impairment and indication for initial PCI. There were proportionately more smokers, patients with previous MI and diabetes mellitus in the ST group, but none of these differences were statistically significant. However, the ST group were younger (mean age 58.4±11.3 vs 64.2±10.2 years; p<0.01) and as expected more of the ST patients were on DAPT (90% vs 19%; p<0.01) and the more potent $P2Y_{12}$ receptor antagonists, prasugrel (55% vs 4%; p<0.01) and ticagrelor (20% vs 2%; p<0.01), at the time of blood sampling.

The angiographic features, including lesion and vessel size and coronary artery treated, were well matched between the two groups.

The ST group had a higher number of stents inserted (3.1±2.1 vs 1.7±1.1; p<0;01), and a higher proportion of first generation drug eluting stents (40% vs 14%; p<0.01). Finally, there was a higher use of glycoprotein IIbIIIa inhibitors (GPI) in the ST group (30% vs 11%; p=0.04).

3.3.2.2. ST cases and controls

When comparing the PPP of the ST patients and the control patients, there were no statistically significant differences between the two groups in any of the four

parameters used to measure thrombin generation (clotting time, total amount of thrombin generated, peak thrombin concentration and time to peak) with either of the 3 triggering reagents, PPP, PRP and MP (see figures 3.7-3.9).

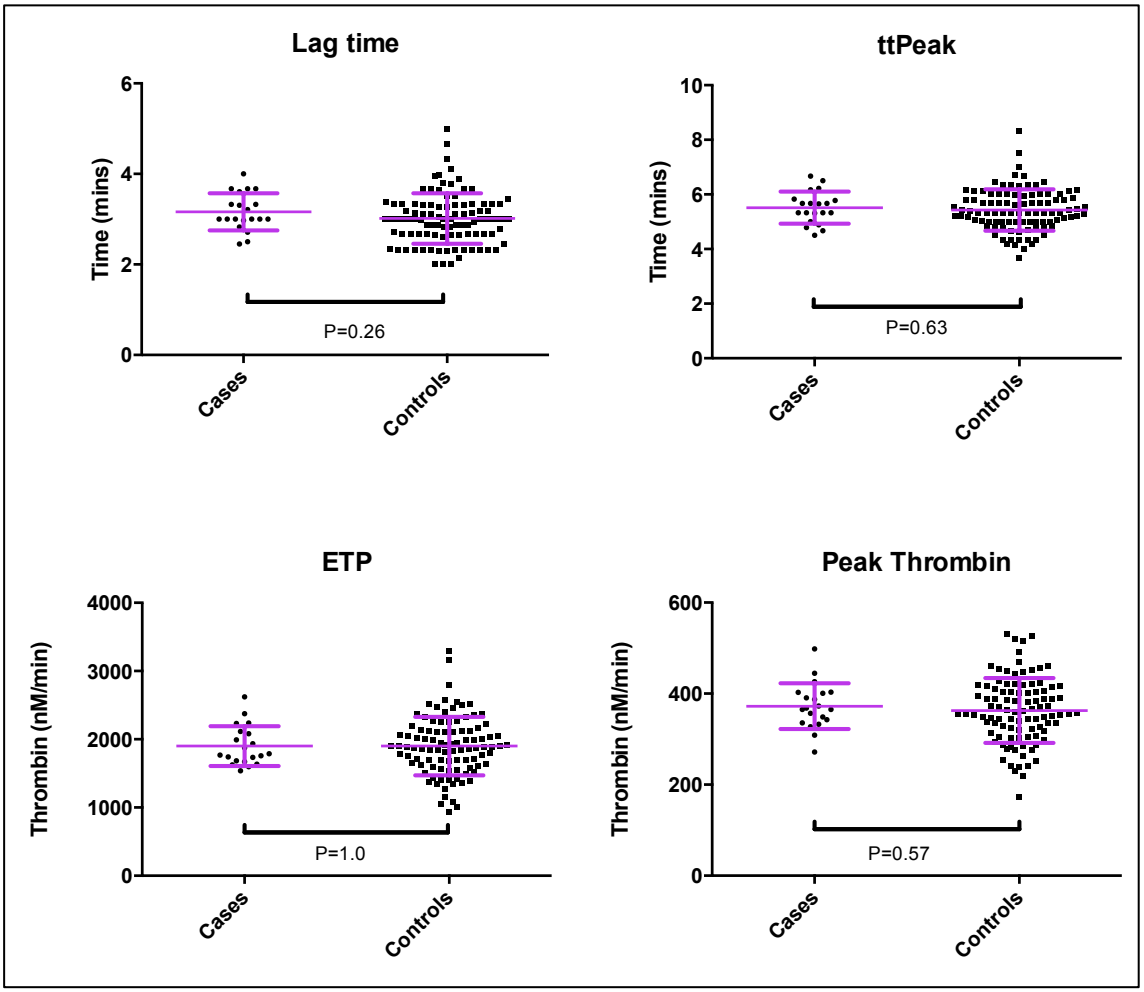


Figure 3.7. Thrombin generation assays comparing ST cases with controls using PPP reagent

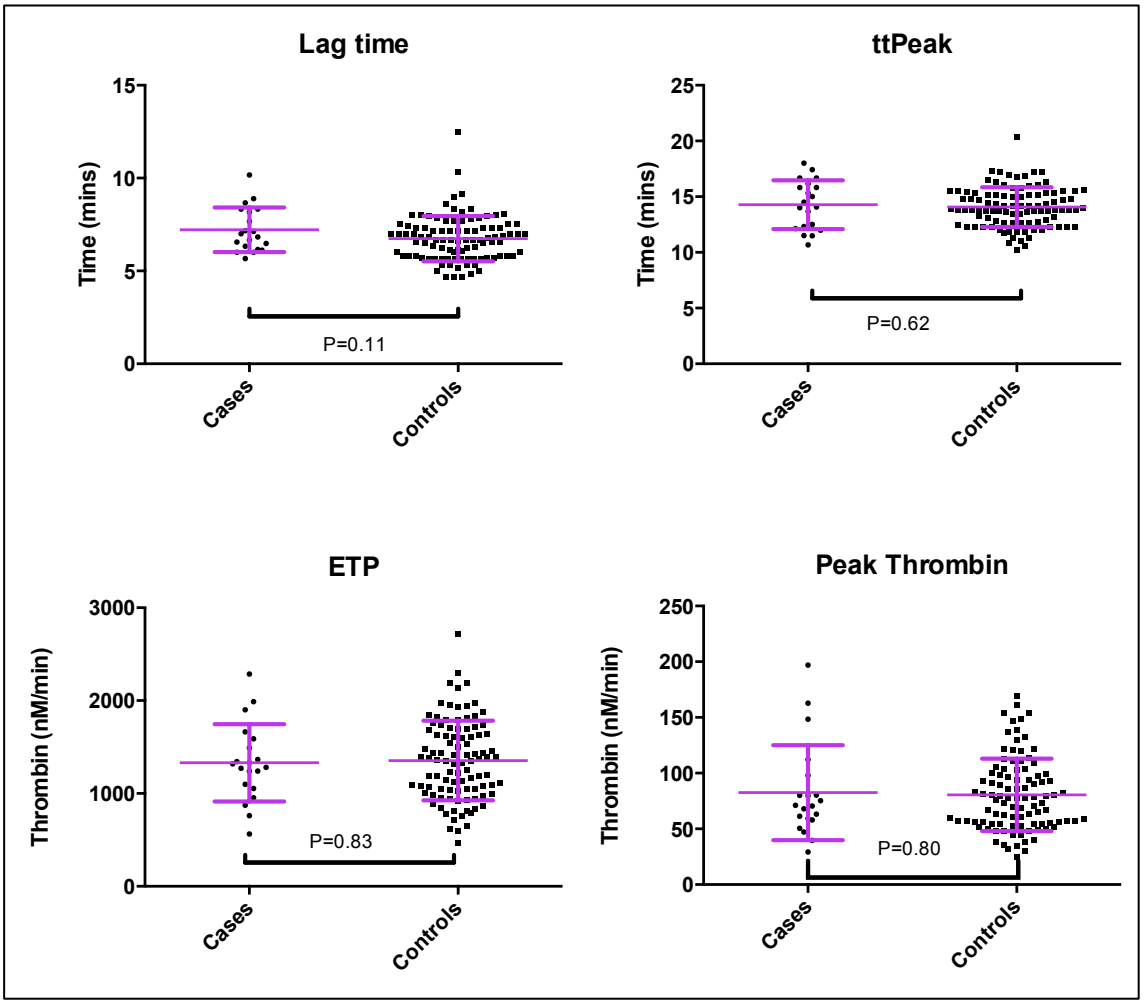


Figure 3.8. Thrombin generation assays comparing ST cases with controls using PRP reagent

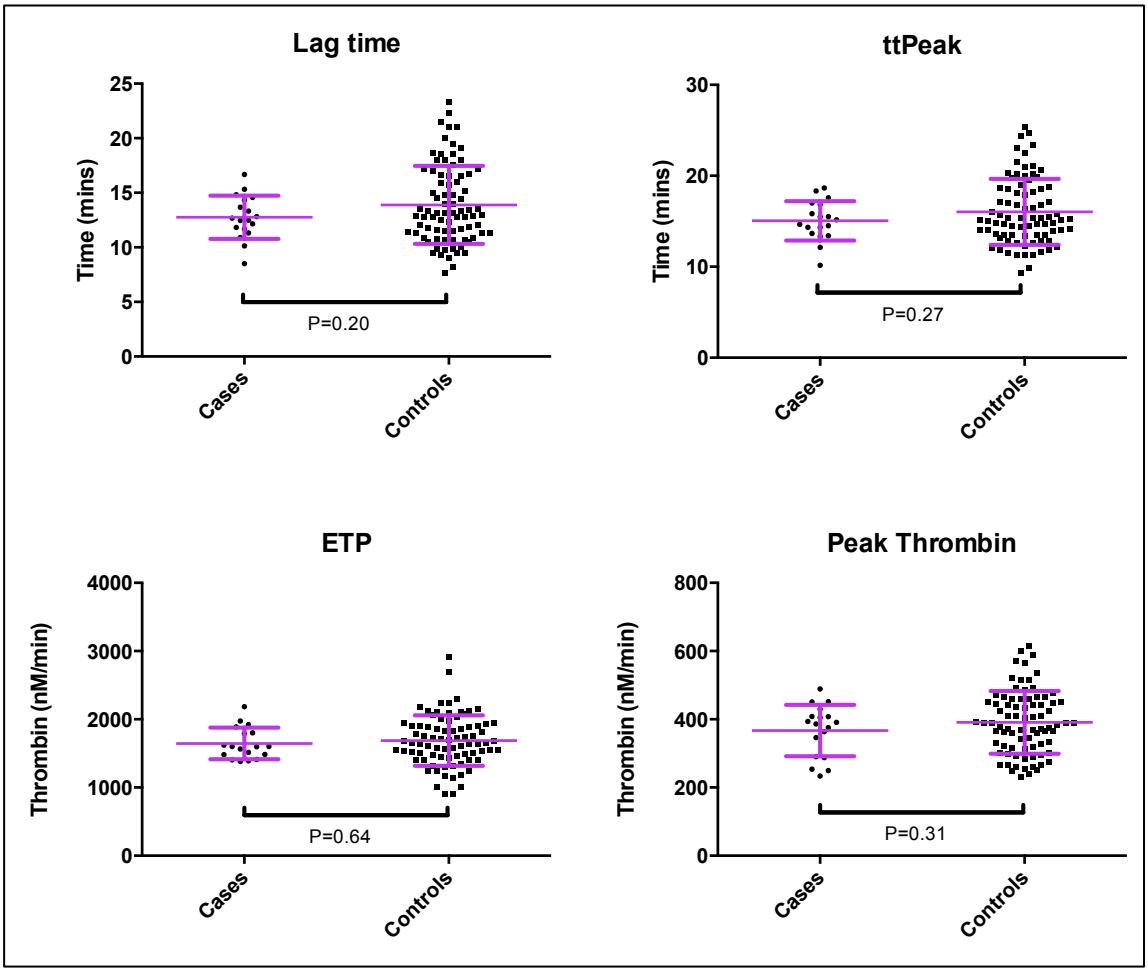


Figure 3.9. Thrombin generation assays comparing ST cases with controls using MP reagent

3.3.2.3. ST cases and controls on dual antiplatelet therapy (DAPT)

When analysing only those patients that were taking both Aspirin and a $P2Y_{12}$ antagonist at the time of blood sampling, there were again no statistically significant differences between the two groups in any of the four parameters used to measure thrombin generation with either of the 3 triggering reagents (see figures 3.10-3.12).

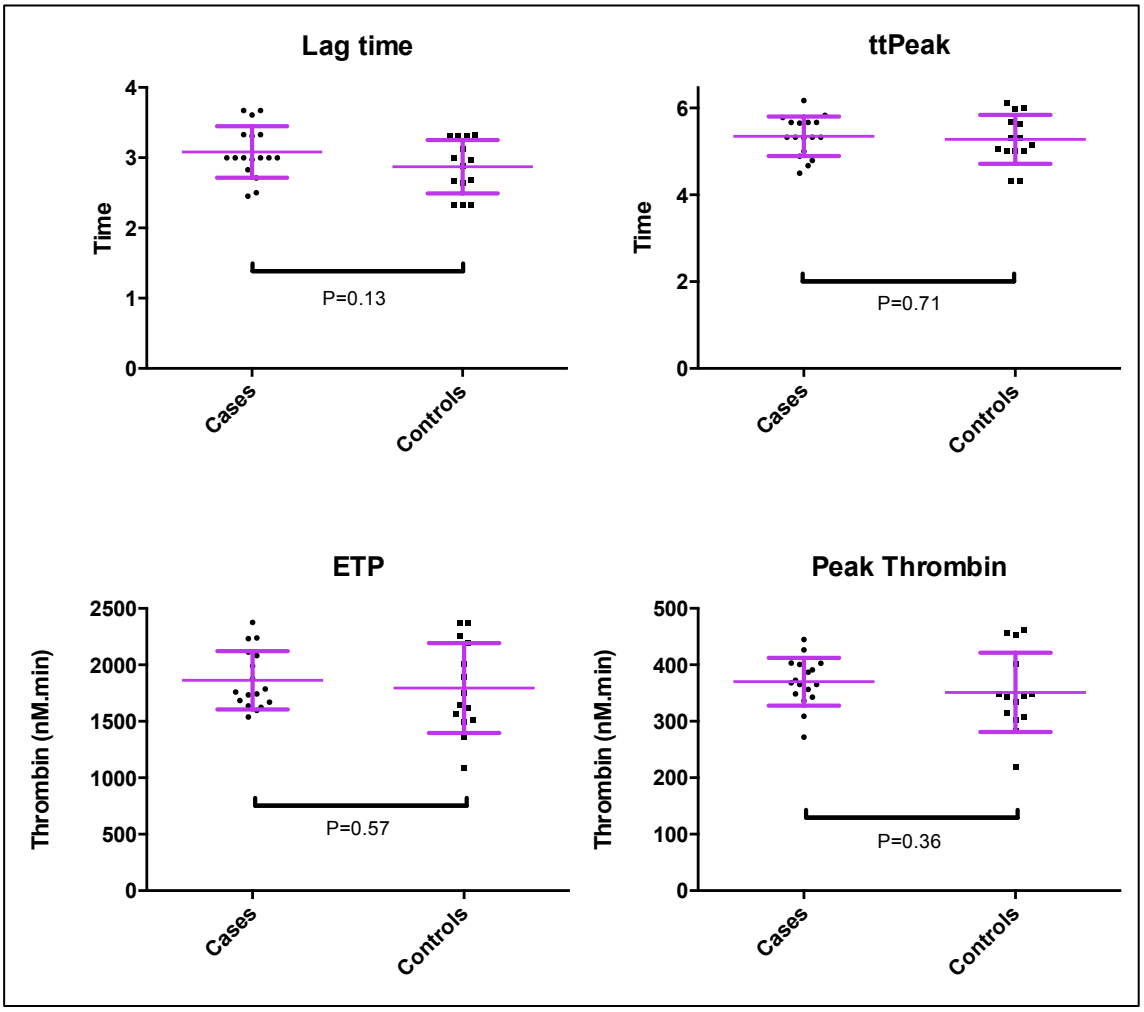


Figure 3.10. Thrombin generation assays comparing ST cases with controls on DAPT using PPP reagent

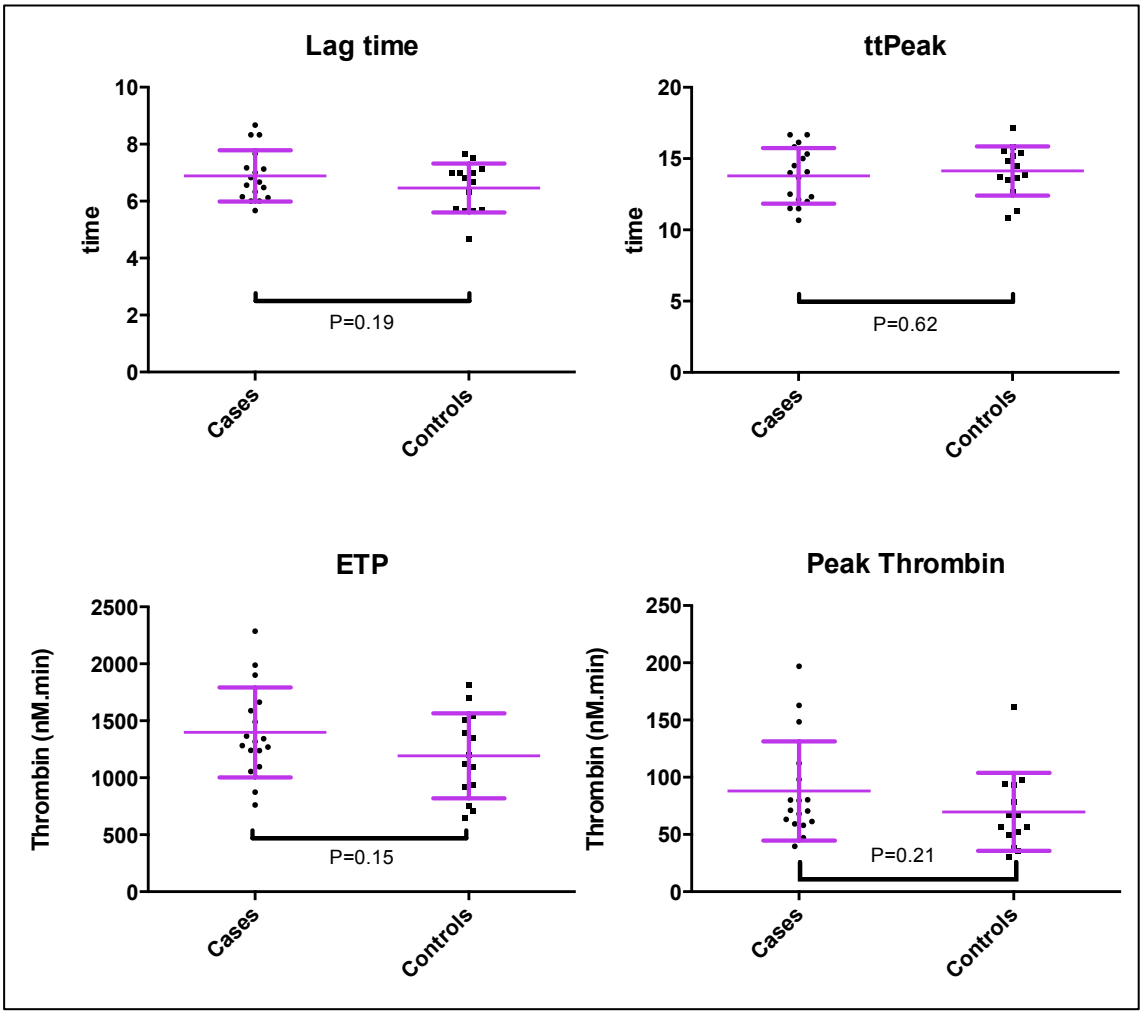


Figure 3.11. Thrombin generation assays comparing ST cases with controls on DAPT using PRP reagent

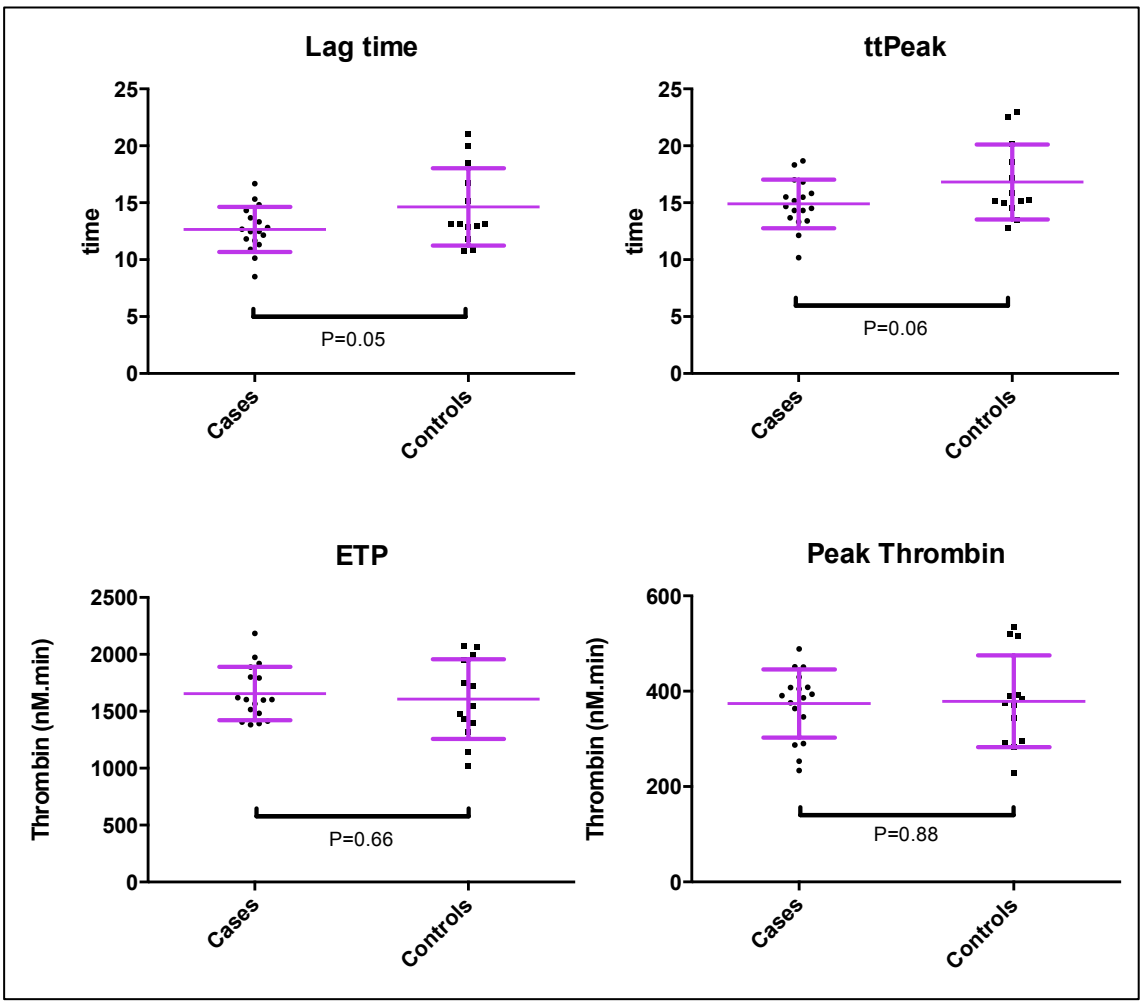


Figure 3.12. Thrombin generation assays comparing ST cases with controls on DAPT using MP reagent

3.3.2.4. Patients with very late ST and controls on DAPT

When comparing the PPP of only the very late ST patients with the controls, both on DAPT, there were again no statistically significant differences between the two groups in any of the four parameters used to measure thrombin generation with either of the 3 triggering reagents (see figures 3.13-3.15).

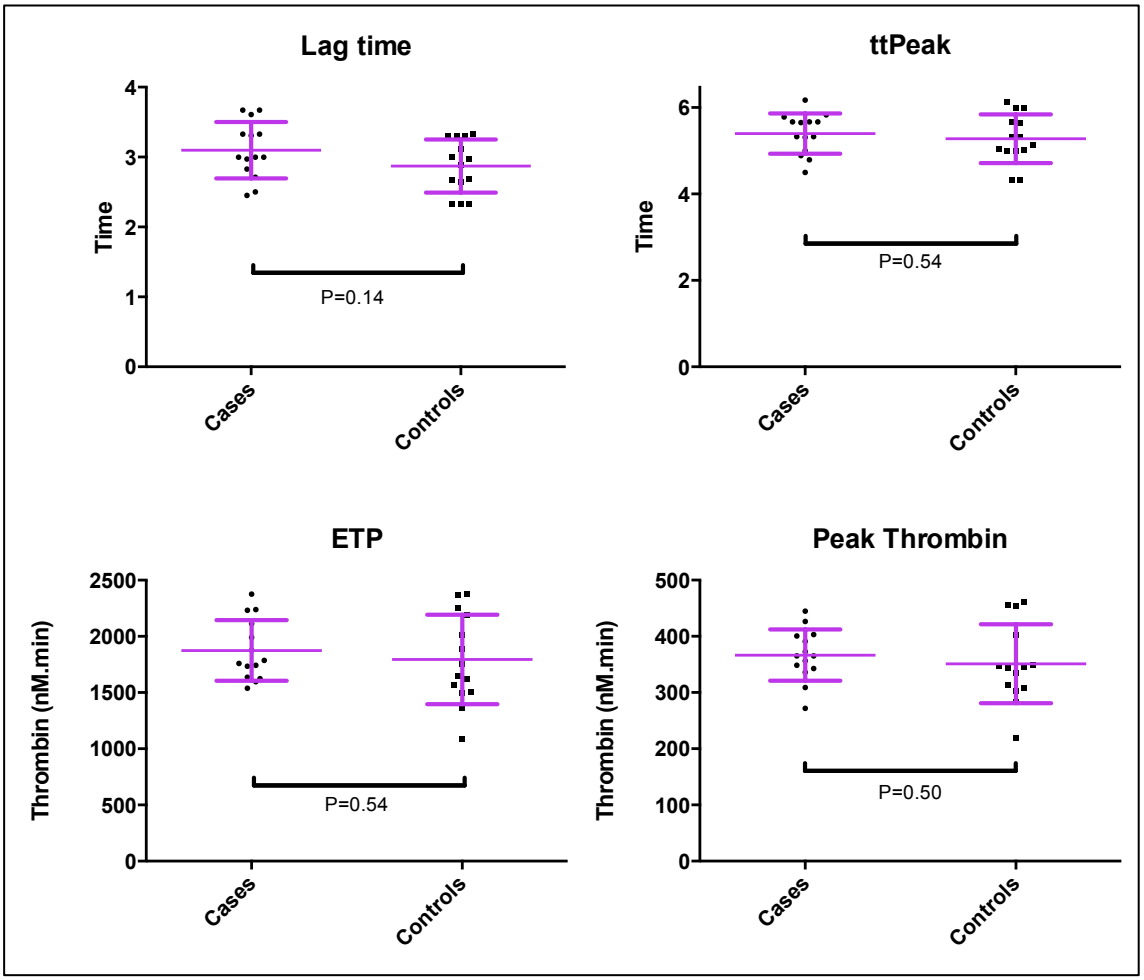


Figure 3.13. Thrombin generation assays comparing very late ST cases with controls on DAPT using PPP reagent

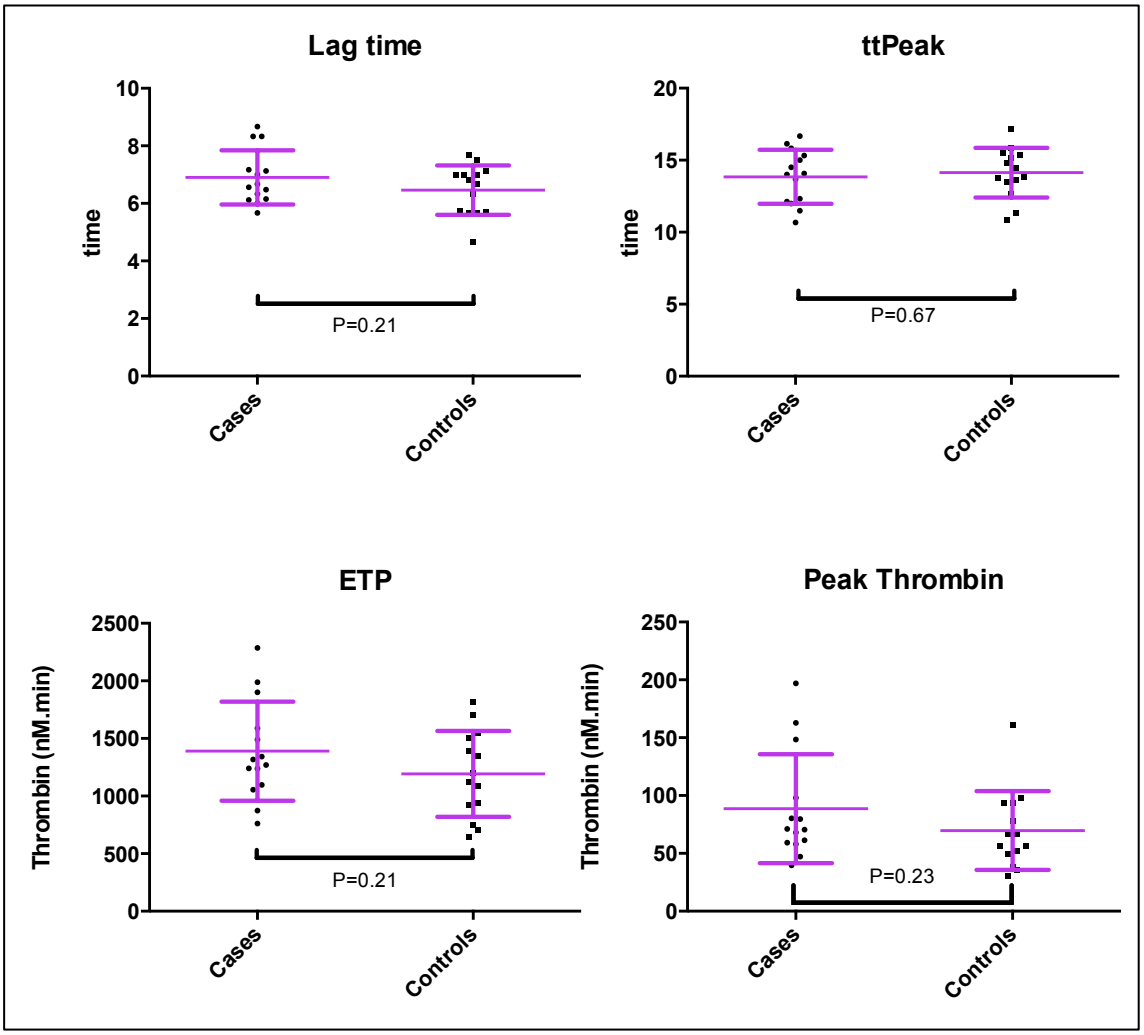


Figure 3.14. Thrombin generation assays comparing very late ST cases with controls on DAPT using PRP reagent

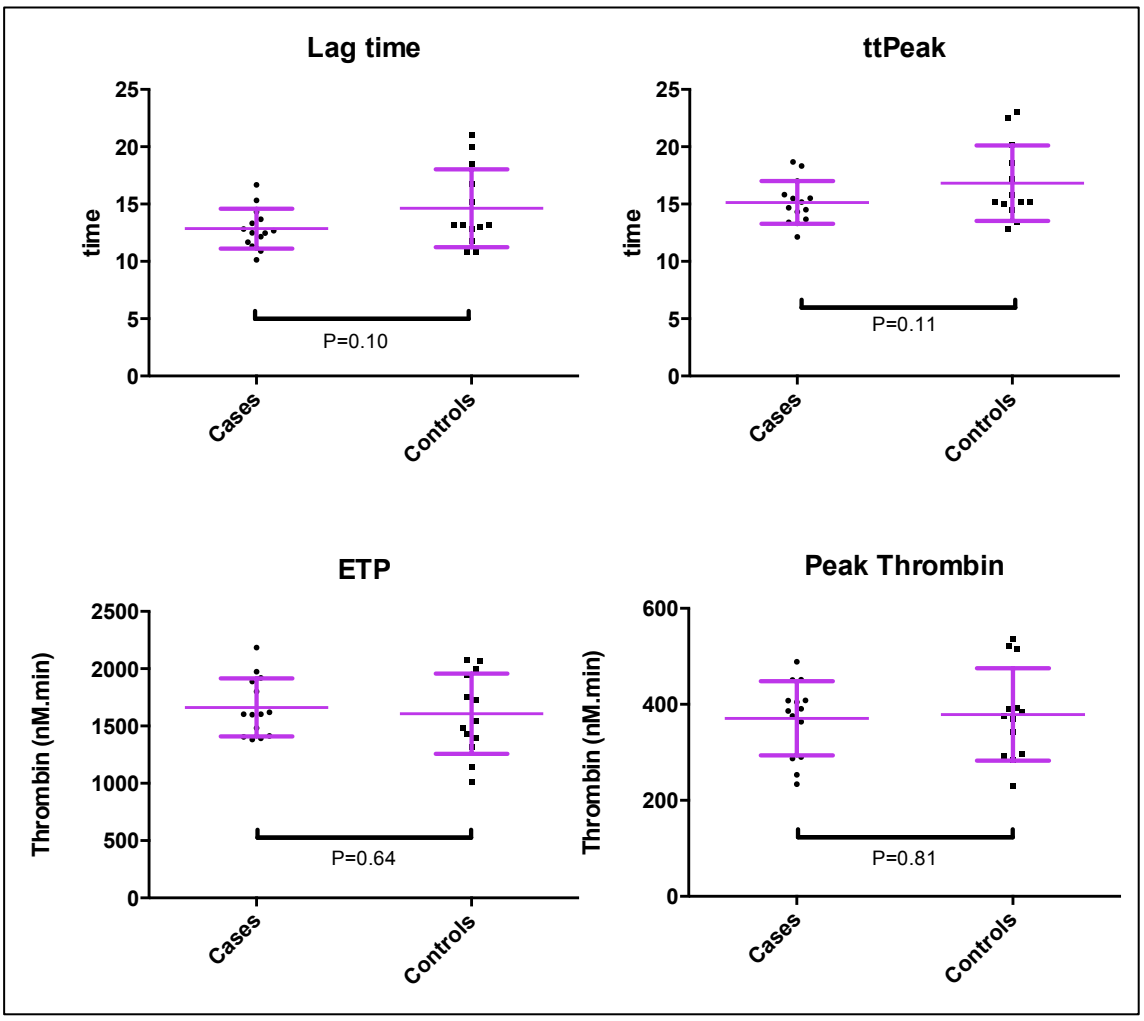


Figure 3.15. Thrombin generation assays comparing very late ST cases with controls on DAPT using MP reagent

3.4. Conclusions

3.4.1. Platelet function testing

PFTs, which were available at 10 out of the 12 sites, were carried out in 75% of ST cases (for at least one time point) and 86% of controls. These were however a mixture of VerifyNow and Multiplate tests, as most sites only had access to one type of assay. This variability in assay used may have reduced the power of the study.

There are limited conclusions that can be drawn from the PFTs that were carried out at the acute and 24-hour time points. Only a minority of patients had PFTs

performed at these earlier time points, with only 26.8% of patients having them done acutely. This was due to a combination of patients presenting out of hours, the use of GPI or bivalirudin, the lack of verbal consent at the time of presentation and the lack of opportunity for acute PFTs in the retrospectively recruited ST cases. Of those that did have PFTs carried out acutely who were already on antiplatelet therapy (n=11), there was a high incidence of HPR to clopidogrel (4/7), prasugrel (1/2) and ticagrelor (1/2). The study did not take in to consideration potential compliance issues and few conclusions can be drawn from such small numbers. At the acute time point, there was also a HPR rate to aspirin of 16%, which may have been under-estimated, as just over half of these patients had already been re-loaded with an additional 300mg of aspirin at least 1 hour prior to acute blood sampling. In addition, some of the patients had also been loaded with a $P2Y_{12}$ receptor antagonist prior to acute blood sampling and the majority had been loaded with DAPT well before the 24-hour time-point.

When comparing PFTs for the ST patients taken at 30-60 days with the control group, the overall rate of HPR on any $P2Y_{12}$ antagonist was significantly lower in the ST group. This can be explained by the fact that a significantly larger proportion of ST patients were taking the more potent agents, prasugrel and ticagrelor. The rate of HPR is known to be significantly lower with these newer agents (198-201). There was a similar HPR rate to clopidogrel in both groups and this is in line with published data, indicating an HPR rate to clopidogrel of about 30% (169, 202). The HPR rate to prasugrel and ticagrelor was very low in both ST cases and controls and other than this, no conclusions can be drawn due to very small patient numbers in each group. The HPR rate on aspirin was also similar in both groups and again in line with published data (203, 204). The relevance and potential clinical implications of this suboptimal response to antiplatelet agents is discussed further in chapter 5.

3.4.2. Thrombin generation studies

In this study, there was no significant difference found in thrombin generation in the plasma of patients with a history of coronary stent thrombosis compared to controls, whether measuring the coagulation cascade (PRP reagent) or testing

for the effect of cell-derived MPs in the plasma (PPP and MP reagents). When the analysis was repeated for only those patients on DAPT, as well as those with very late ST, there were no differences between the two groups.

Of note, the ST group had a statistically significantly higher proportion of patients on the more potent $P2Y_{12}$ receptors antagonists, prasugrel (55% vs 4%, P<0.01) and ticagrelor (20% vs 2%, p<0.01), which may have diminished the generation of thrombin in some of these assays, especially when measuring microparticles. However, the use of PPP should have minimised any such effect (188, 205). Also, the use of aspirin has been shown, in the Glasgow Myocardial Infarction study (206), not to affect any of the thrombin generation assays. However, in one study, assessing the impact of antiplatelet therapy on thrombin generation potential in patients with previous cerebrovascular events (207), the use of clopidogrel did lead to a reduction in the lag time early on after initiation, but this effect was not maintained at 90 days.

Patients with acute myocardial infarction are already known to have a state of plasma hypercoagulability. In a study by Smid et al (208), patients presenting with a first episode of myocardial infarction were found to have an increased rate of thrombin generation both acutely and at 6 months follow-up. There was also a trend towards a higher rate of recurrent ischaemic cardiovascular events in patients with increased levels of thrombin generation. Although these findings are not consistent with ST patients in this study, it certainly warrants further investigation, perhaps with larger numbers, with the aim of improving our understanding of the underlying pathophysiology, as well directing improvements in the acute and more long-term antithrombotic strategy in this high-risk group of patients.

Chapter 4: In-vivo assessment of novel coronary stents

4.1. Introduction

A rabbit iliac model (209) was adapted to investigate the *in-vivo* interaction of coronary stents with the vessel wall and circulating blood. A recovery model was used to assess the biocompatibility of novel prototype RGD peptide coated stents that were designed as part of this PRESTIGE project to theoretically promote re-endothelialisation. The New Zealand White rabbit was chosen, as it is a well-recognised model for the study of vascular biology and produces responses that are similar to the vascular behaviour after angioplasty in humans (210, 211). Moreover, the size of the iliac arteries allows for the use of human adult sized coronary catheters and stents, enabling direct transfer of the technology to future human trials. Contralateral interventional procedures allowed testing of study and control stents in the same animal.

The animals were housed and cared for in the Division of Biomedical Sciences in the new purpose built Central Research Facility in the University of Leicester and all procedures were undertaken in accordance with the Animals (Scientific Procedures) Act 1986 under license from the Home Office in London (project license number 60/4517). (See Appendix for license and approval letters).

4.1.1. Acute stent thrombosis model

The aim of this study was to assess the acute thrombogenicity of novel coronary stents as compared to “standard” coronary stents already widely used in clinical practice. As described in chapter 1, such current generation DES have thinner struts with more biocompatible durable or absorbable polymers, releasing a limus antiproliferative drug. These advances have markedly reduced the rate of ST, as well as restenosis, and the need for repeat revascularisation (24, 212). However still much of the published late ST data reflect the cumulative rates of all ST events occurring from the time of implantation. Indeed, at least 50% of the cumulative incidence of ST is accounted for by early ST rates (figure 4.1). It was

thought valuable therefore to assess the acute ST rates associated with these novel devices.

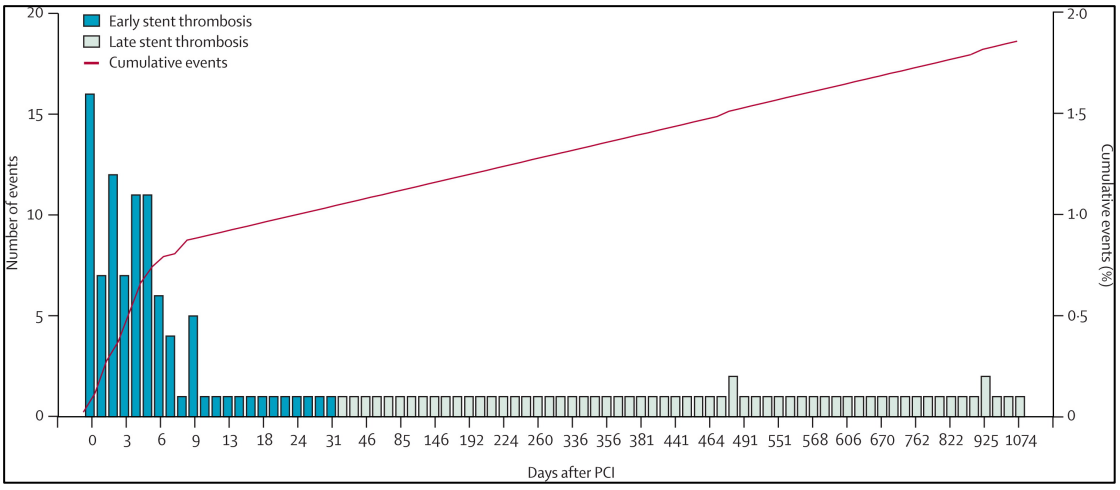


Figure 4.1. Cumulative incidence of ST in sirolimus-eluting and paclitaxel-eluting stents in a large two-institutional cohort study (reproduced from Daemen et al (30))

Furthermore, the permanent presence of metal within the coronary artery is still perceived by some as a limitation of coronary stents and is theoretically associated with an on-going risk of stent thrombosis, especially very late ST when metal is still present. Bioabsorbable coronary stents held out promise to overcome this very late ST incidence. They aim to provide a temporarily scaffold within the vessel, preventing acute closure and recoil, whilst eluting the same antiproliferative drug to reduce neointimal hyperplasia as is used on metal DES, and then completely resorb over 2-5 years.

Although early clinical studies involving the first commercially available drug eluting bioabsorbable vascular scaffold (Absorb BVS) revealed encouraging short and intermediate term safety and efficacy outcomes (129, 213-219), recent registries have indicated higher scaffold thrombosis rates than those reported in contemporary studies of second generation DES (130, 220-222). These data have been supported by the recent 3 year report from the ABSORB 2 trial, where there was not only no improvement in vasomotor tone in the BVS arm, but additionally worrisome excess ST (132). This has been partly explained by inadequate lesion preparation, suboptimal device expansion and a lack of sufficient intracoronary imaging guidance, especially in more complex coronary

lesions. However, the thicker scaffold struts (150μm), which are similar in size to first generation DES, may also play a role, especially in vessels under 2.5 mm in diameter. These data have appeared recently and notably since this MD project was started.

The model described in this chapter was established to assess the acute thrombogenicity of the Absorb BVS as compared to a contemporary second generation DES, the Xience stent, using measures of iliac artery blood flow, platelet adhesion and OCT.

4.1.2. Recovery model

In this second series of experiments, a recovery model was used to assess the healing of novel prototype coronary stents that were specifically bio-engineered as part of the pre-clinical work package of the PRESTIGE study. The aim of this work package, was to try and improve the long-term biocompatibility of DES by targeting platelet adhesion, coagulation and endothelial cell adhesion, in order to reduce the on-going risk of stent thrombosis. Stents were made and provided by the CV Path Institute in Washington, USA, led by our PRESTIGE collaborator, Dr Michael Joner.

4.1.2.1. Bio-engineered stents

The novel stent basic platform was a Biotronik PRO-Kinetic Energy bare metal stent (BMS) composed of cobalt chromium with a strut thickness of 60μm. The stent surface was modified by applying either a monolayer of sulphated chitosan CS60 polymer (RGD-CS60) or 3 bilayers of chitosan/sulphated chitosan CS60 polymer (RGD PLA-CS60). Platelet adhesion was shown to be lower in vitro when the outermost layer of the polymer was sulphated chitosan as compared to when heparin was the outermost layer. Synthetically produced RGD-peptidomimetics, which were shown in vitro to be selective for the endothelial cell receptor αvβ3-integrin, were then coupled to the stent surface, in order to attract vascular endothelial cells with high affinity and specificity and thereby promote functional endothelialisation (figure 4.2).

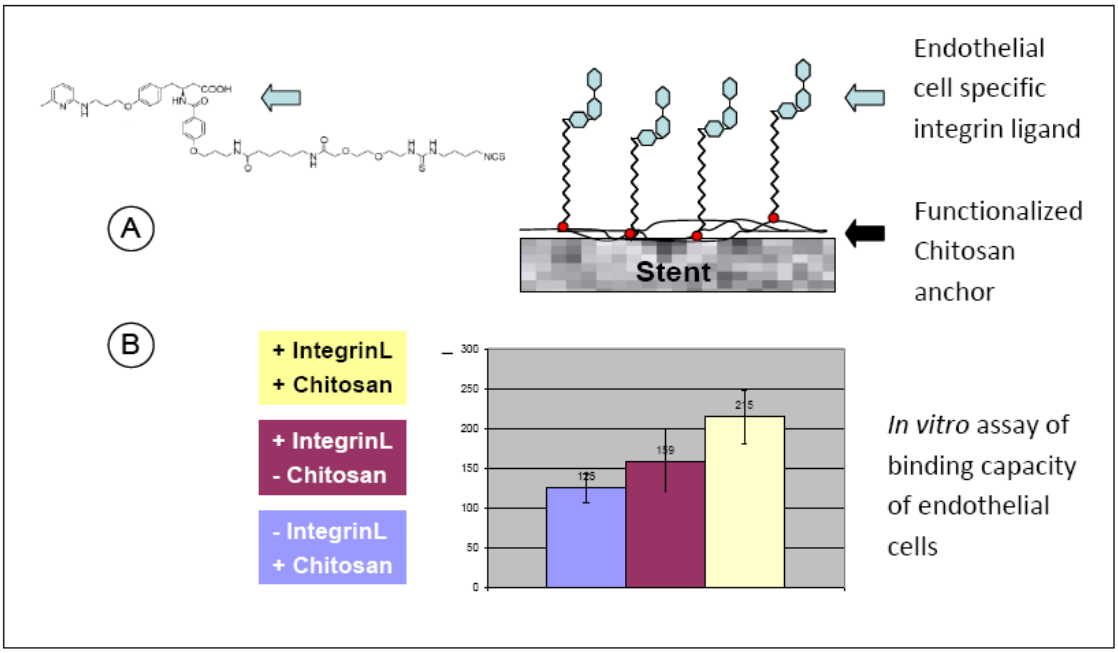


Figure 4.2. Evaluation of the stent surface coating with amino-functionalized chitosan and the coupling of synthetically produced integrin ligands (A). (B) The results show an increased adhesion of endothelial cells on the surface with chitosan-binding (+chitosan) and endothelial cell integrin (+ IntegrinL) as compared to control coatings (with / without ligand and with / without chitosan). (Data from PRESTIGE study work package 2; German Heart Centre, Munich, Riga Stradins University and Neoplas GmbH, Greifswald)

4.2. Methods

4.2.1. Antiplatelet dosing

Animals were administered with aspirin 1mg/kg/day and clopidogrel 1mg/kg/day in flavoured drinking water (to improve palatability) for 5 days pre-operatively in the acute protocol. The initial dosing was based on previous work done by this group (223). These doses were shown on Multiplate impedance aggregometry testing to induce an appropriate antiplatelet response in the rabbits (figure 4.3).

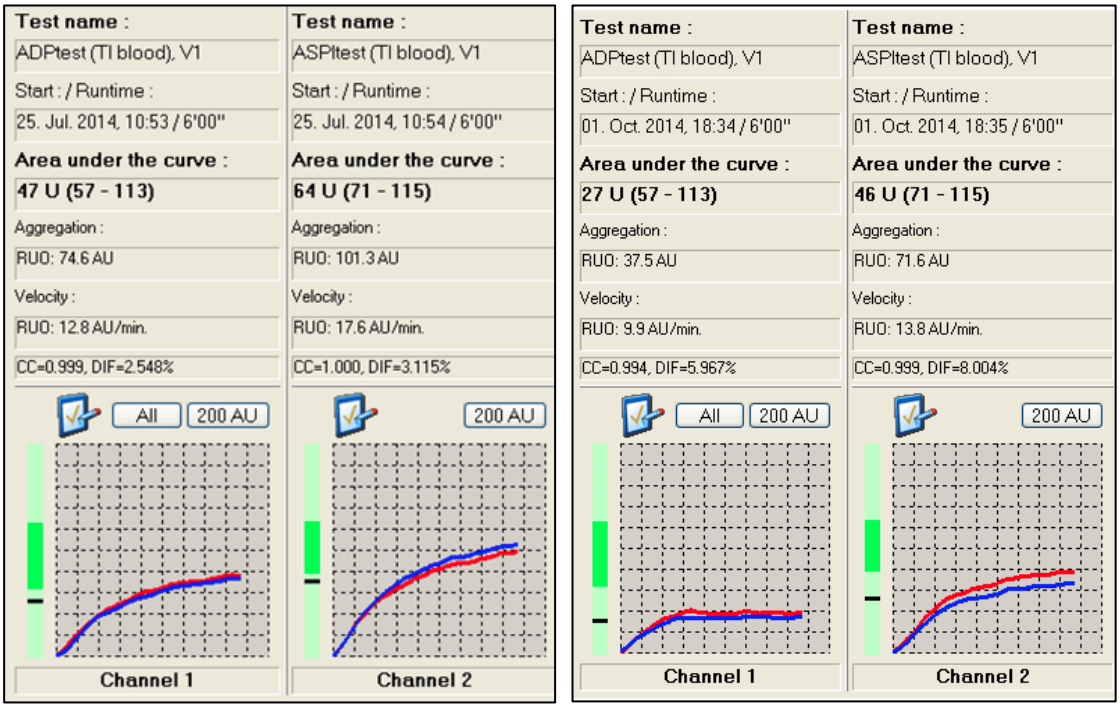


Figure 4.3. Multiplate results in a single rabbit treated with 5 days of clopidogrel 1mg/kg and aspirin 1mg/kg (left panel) compared to clopidogrel 2mg/kg and aspirin 1mg/kg (right panel) showing a reduction in ADP-induced platelet aggregation with the higher dose

Initial blood sampling for platelet function testing was performed without sedation using a 22G butterfly needle in the ear vein after applying EMLA cream 5% (lidocaine 2.5%/prilocaine 2.5%) as topical anaesthetic. Having discarded the first 1ml, 2mls of blood was allowed to drip in to a hirudin blood tube. However, the Multiplate results indicated pre-activation of platelets in these samples (figure 4.4), possibly due to the slow venous blood flow and movement of the animal during blood sampling. Animals were therefore sedated with a combination of ketamine 20mg/kg and xylazine 5mg/kg administered subcutaneously (subsequently reversed with a subcutaneous injection of atipamezole 0.8mg/kg) and blood was taken from the central ear artery using a 22G cannula to reduce the chance of platelet activation (figure 4.5).

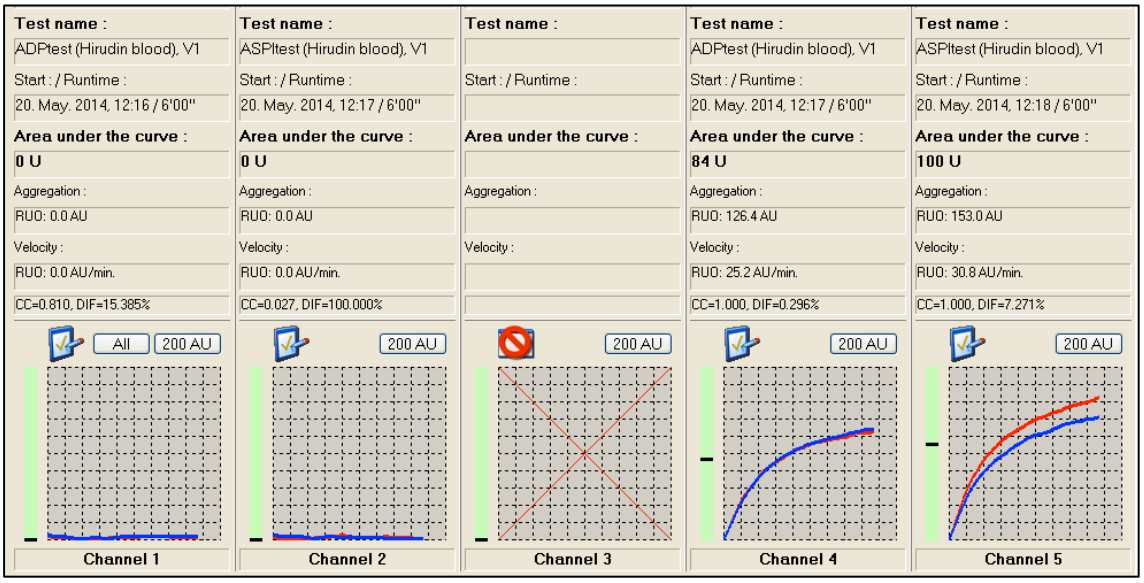


Figure 4.4. Multiplate results of blood taken using cannula in rabbit ear vein (left pair of graphs), with platelets showing no response to ADP or arachidonic acid, and then from same rabbit following sedation and using cannula in central ear artery (right pair of graphs), showing a normal response to stimulation

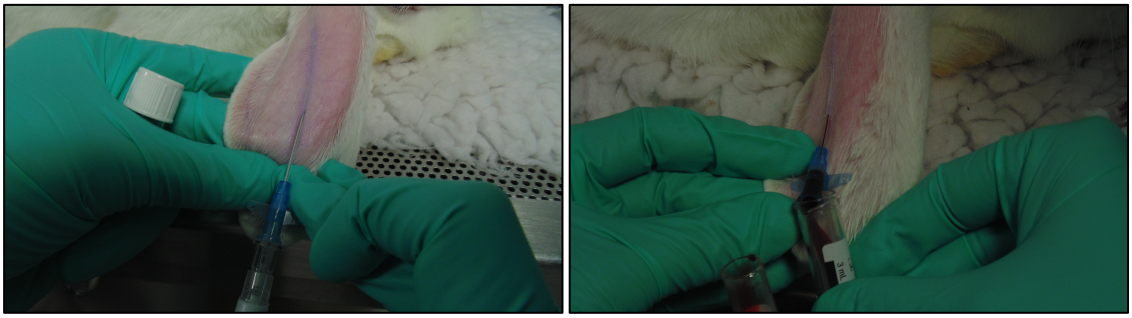


Figure 4.5. Blood sampling from central ear artery for platelet function testing

The blood was tested within 30-180 minutes of collection, using ADP and arachidonic acid as agonists in the Multiplate analyser as described in chapter 2 (section 2.2.3.1.2). A TRAP control test was not used, as rabbit platelets are known not to respond to thrombin receptor peptides that activate human platelets (figure 4.6) (224).

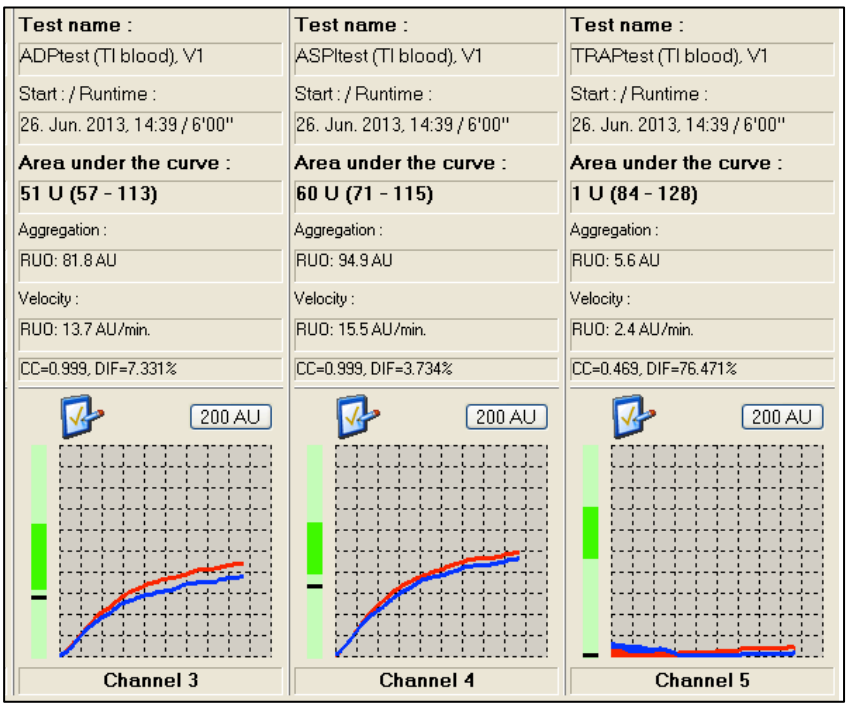


Figure 4.6. Multiplate results of blood taken from rabbit treated with 5 days of clopidogrel 1mg/kg and aspirin 1mg/kg, showing no response of platelet aggregation to TRAP agonist (right panel graph)

Based on previous work with similar models comparing novel stents to controls in a rabbit iliac model (209) and a calculated power analysis (225), the estimated number of animals to be used in the study, assuming a significance level of 5% and a power of 90%, was calculated to be 30, including pilot studies.

Initially, 12 rabbits treated with 5 days of clopidogrel 1mg/kg and aspirin 1mg/kg as part of the acute stent thrombosis model had blood sampling undertaken for platelet function testing and, as already mentioned, these results showed a moderate level of inhibition of ADP- and arachadonic acid (AA)-induced platelet aggregation. Seven rabbits, treated with a higher dose of clopidogrel (2mg/kg) and aspirin at 1mg/kg as part of the recovery model showed a significantly more potent inhibition of ADP-induced platelet aggregation, mean AUC 36.4±6.5 units vs. 50.7±2.3 units (p=0.02, 95% CI -2.1, -26.3) and a similar response to AA, mean AUC 67.0±2.1 units vs. 63.0±9.0 units (p=0.59, 95% CI -19.3, 11. 3) (figure 4.7).

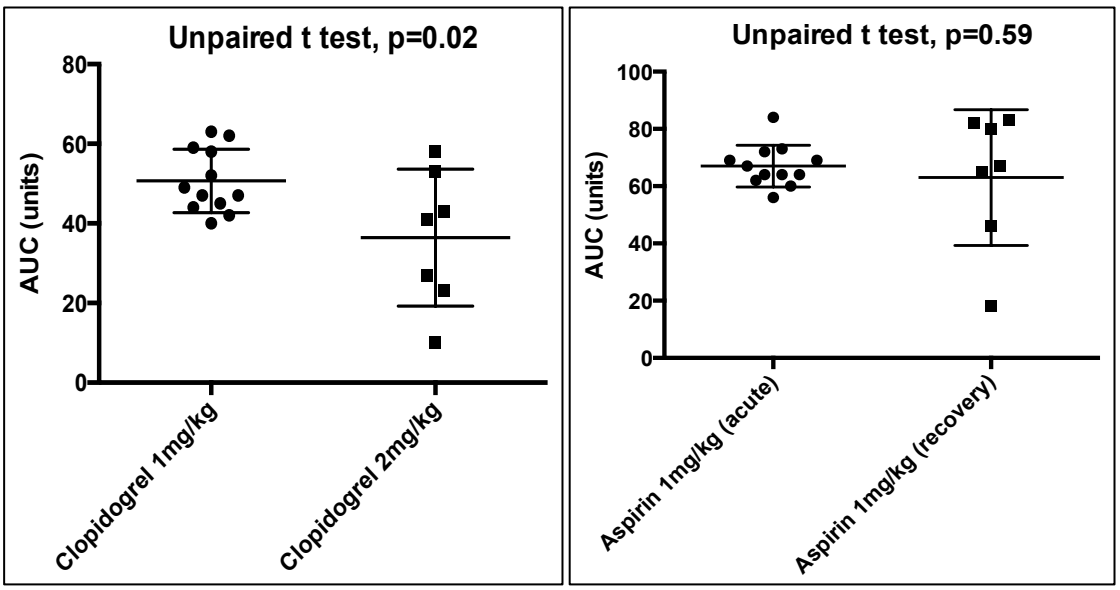


Figure 4.7. Multiplate results from rabbits treated with clopidogrel 1mg/kg vs. 2mg/kg (left graph), using ADP as an agonist, and aspirin 1mg/kg in both groups (right graph), using arachidonic acid as an agonist

Based on the Multiplate results, a higher dose of clopidogrel was therefore used for the recovery model in order to minimise the risk of incidental stent thrombosis due to inadequate DAPT. In this model, aspirin 1mg/kg/day and clopidogrel 2mg/kg/day were given for 5 days pre-operatively and then daily throughout the recovery period until euthanasia.

4.2.2. Angioplasty and stent model

Once the pre-procedural DAPT dosage had been established, studies were undertaken with male New Zealand White rabbits, aged 14 to 18 weeks, weighing 3.0 to 4.0kg. Aspirin and clopidogrel were administered in flavoured drinking water for five days pre-operatively and throughout the recovery period until euthanasia.

Animals were pre-medicated with subcutaneous ketamine 35mg/kg and xylazine 7mg/kg, followed by intramuscular butorphanol 0.1ml/kg and atropine 0.05mg/kg at induction of general anaesthesia with inhaled isoflurane 2%. A combination of inhaled isoflurane 1-3%, oxygen 2-5l/min and nitrous oxide 0.5-2l/min was used to maintain anaesthesia and further ketamine and xylazine administered if

necessary. A 22G cannula was placed in the ear vein for infusion of intravenous fluids (0.9% saline at 5mls/hr), for re-injection of labelled platelets in the acute model (as described below) and for the final overdose agent. Animals were spontaneously ventilated with a face mask, placed on a heating pad at 38°C and had continuous intra-operative monitoring of heart rate, respiratory rate and rectal temperature.

4.2.3. Acute model

In this model, acute thrombogenicity was compared between stents by measuring blood flow through stented iliac arteries, adherence of platelets and ex vivo OCT imaging.

4.2.3.1. Immunofluorescent labelling of platelets

Rabbit platelets were isolated, washed and immunofluorescently labelled, prior to re-injecting pre-operatively to measure adherence to implanted iliac stents. A protocol was developed and optimised as described below.

Preparation of washed platelets from rabbit blood

A long-established method of washing human platelets used by Professor Alison Goodall's group at the University of Leicester was adapted to optimise the process for rabbit platelets, which proved to be particularly sensitive to activation (as demonstrated in the platelet function tests above). The final protocol is detailed below. Additional steps to the existing human blood protocol included collecting arterial blood directly into 1/6 volume acid citrate dextrose (ACD) anticoagulant, adding apyrase to platelet rich plasma (PRP) between each centrifugation step to neutralise ADP released by the platelets, and finally re-suspending the washed platelets in plasma as opposed to pH 7.4 HEPES-buffered saline (HBS). These additional steps minimised platelet activation and desensitisation as demonstrated by flow cytometry analyses described below.

Flow cytometry assays

The process of preparing washed rabbit platelets was validated using flow cytometry to analyse binding of rabbit fibrinogen, amino terminal labelled with fluorescein isothiocyanate (FITC), in response to increasing concentrations of ADP. FITC-labelled rabbit fibrinogen (Molecular Innovations, RbFBGN-FITC) is prepared from fresh rabbit plasma using several chromatographic steps. Plasminogen is depleted by lysine affinity chromatography. It has a peak excitation wavelength of 495nm and a peak emission wavelength of 520nm.

A series of experiments was performed to determine the optimal concentration of FITC-fibrinogen. Using LP3 tubes, a negative control was set up with EDTA, a resting sample with no ADP and then increasing concentrations of ADP (figure 4.8). Duplicate tubes were set up for each concentration of ADP. FITC-labelled rabbit fibrinogen (10mg/ml) was diluted to various concentrations (figure 4.8) with HBS, and 50µl of this solution was added to each tube. 2µl of 150mM EDTA was added to the negative control tube and 5µl of the appropriate concentration of ADP was then added to each tube, except the negative control and the resting sample. 5µl of whole blood or washed platelets was then added to each tube and these were incubated at room temperature for 20 minutes. 450µl of 0.2% formyl saline fixative was then added to each tube and incubated for 10 minutes at room temperature. 50µl of each sample was then transferred to Cyan tubes containing 450µl of 0.2% formyl saline and incubated for another 10 minutes at room temperature. The samples were then analysed in a Cyan flow cytometer using the FITC fibrinogen protocol.

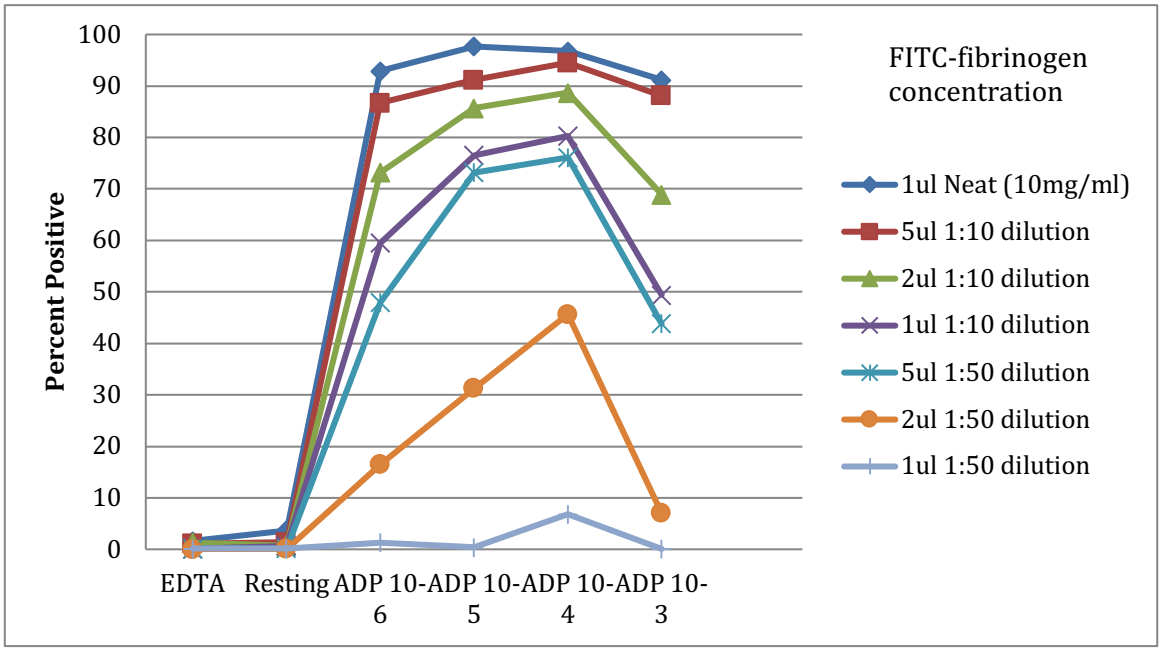


Figure 4.8. Flow cytometry testing increasing concentrations of FITC-fibrinogen and increasing concentrations of ADP agonist with rabbit whole blood (n=1)

The initials experiments indicated that the optimal concentration of FITC-fibrinogen was 1µl of 1:10 dilution and this was used for future experiments as described below. When the flow cytometry assay was initially repeated with washed rabbit platelets re-suspended in plasma, there was no response to ADP, indicating that the platelets had either been pre-activated or desensitised during the washing process (figure 4.9).

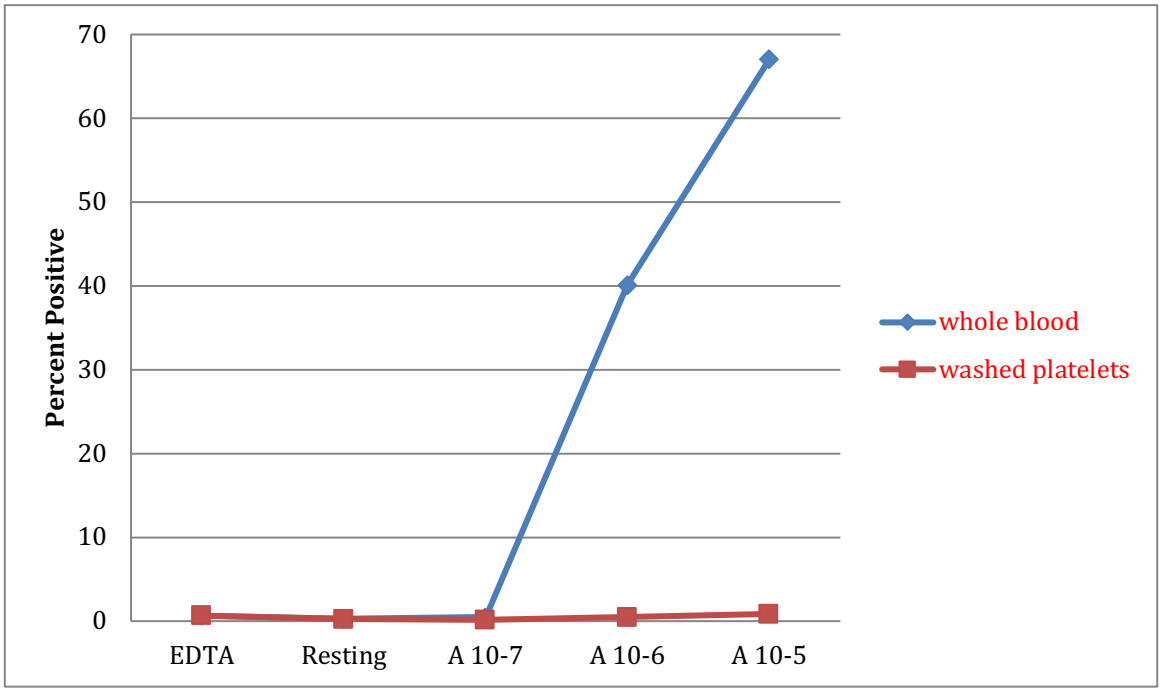


Figure 4.9. Flow cytometry using 1µl of 1:10 dilution FITC-fibrinogen with rabbit whole blood and washed platelets re-suspended in HBS (n=1)

Various conditions were altered in an attempt to avoid activating and desensitising the rabbit platelets during the washing stage, including adding apyrase between each centrifugation step, adding ethylene glycol tetraacetic acid (EGTA) and using pH 6.5 buffer (figure 4.10).

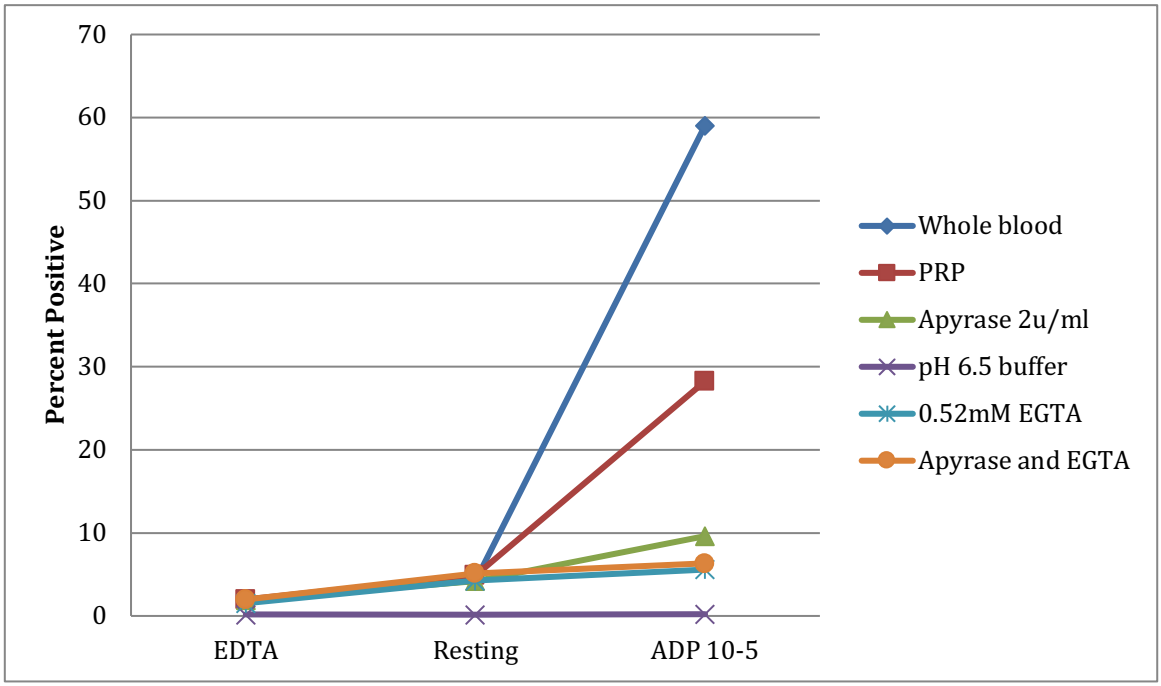


Figure 4.10. Flow cytometry using FITC-fibrinogen assay with rabbit whole blood, PRP and washed platelets with various environmental changes (n=1)

The addition of apyrase, which is a calcium-activated plasma membrane-bound enzyme that, amongst other actions, inhibits ADP-induced platelet aggregation, reduced platelet desensitisation a little and when the final washed platelet pellet was re-suspended in plasma, as opposed to HBS, the response to ADP was significantly better, close to that of whole blood (figure 4.11).

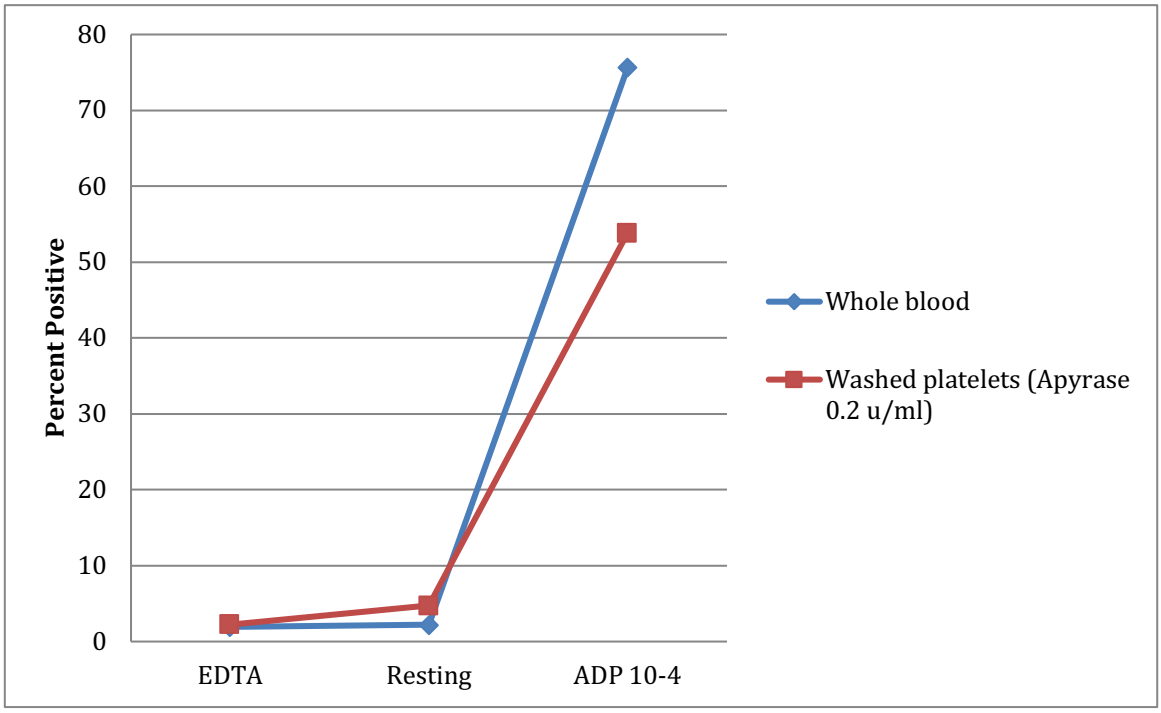


Figure 4.11. Flow cytometry using FITC-fibrinogen assay with rabbit whole blood and washed platelets, with apyrase added between centrifugation steps, and final platelet pellet re-suspended in plasma (n=1)

This response was improved further by omitting the final addition of apyrase in the platelet washing protocol (figures 4.12-4.14), as detailed in section 4.2.3.1.5 below.

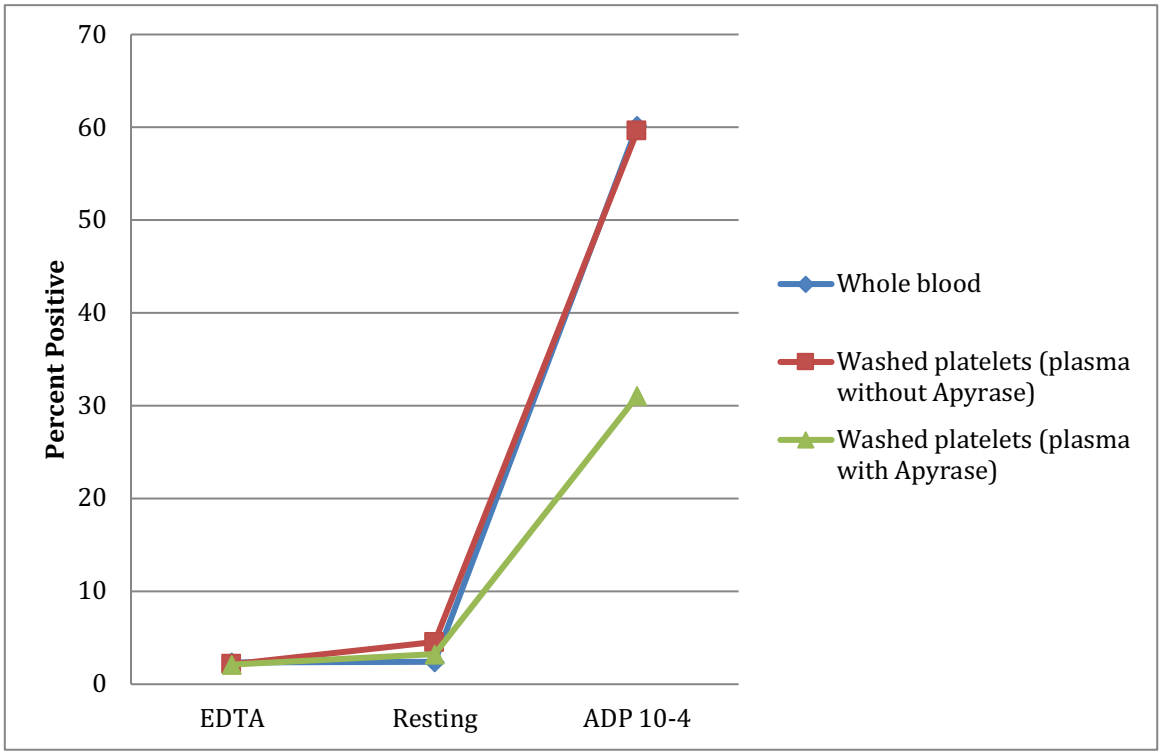


Figure 4.12. Flow cytometry using FITC-fibrinogen assay with rabbit whole blood, washed platelets with and without apyrase added in final step, with final platelet pellet re-suspended in plasma (n=1)

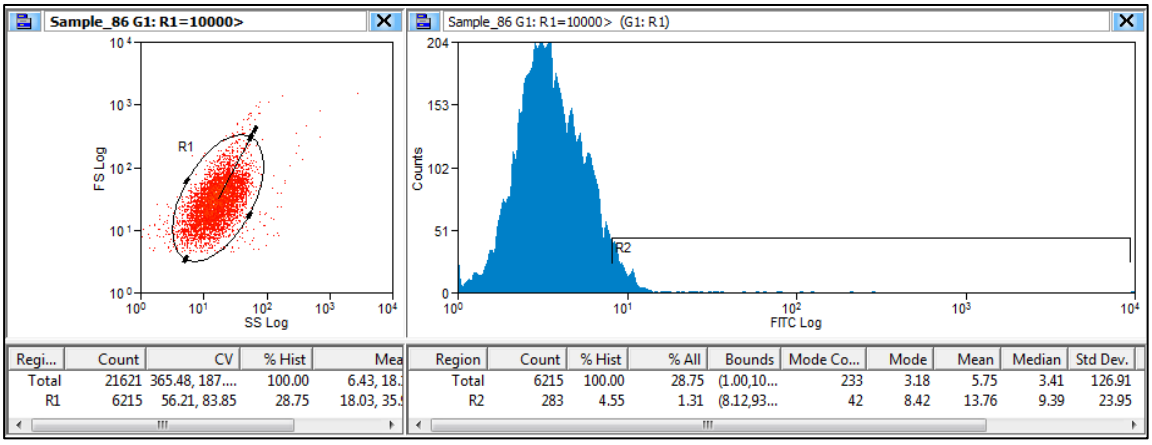


Figure 4.13. Example of flow cytometry with FITC-fibrinogen assay showing resting washed rabbit platelets in plasma

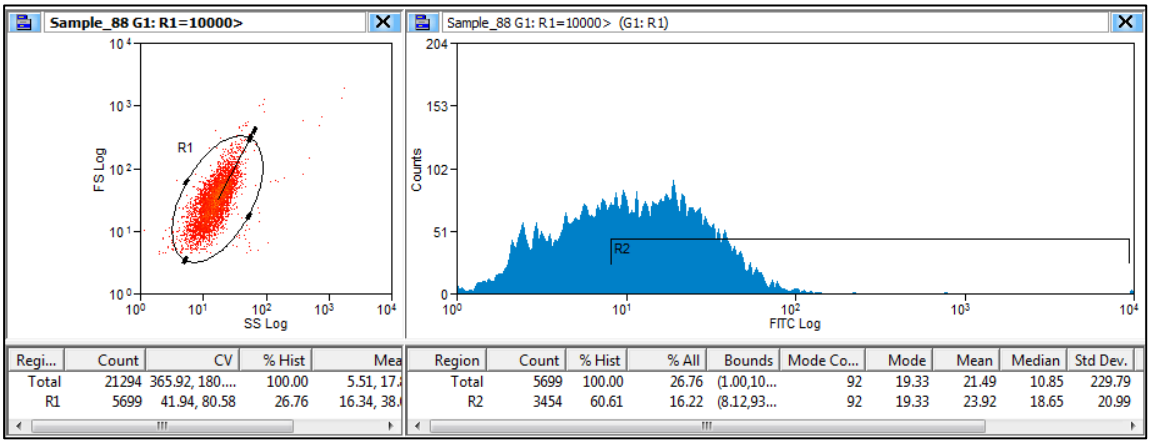


Figure 4.14. Flow cytometry with FITC-fibrinogen assay showing activated washed rabbit platelets (with ADP 10^{-4}M) in plasma

Optimising immunofluorescent labelling

Washed platelets were labelled with Mitotracker Red CMXRos fluorescent dye (ThermoFisher Scientific). This is a red fluorescent dye that stains mitochondria in live cells and is retained after formalin fixation. It has an excitation wavelength of 579nm and an emission wavelength of 599nm. In order to determine the optimal concentration of dye to adequately label the platelets, various concentrations of 1mM stock solution were made up and incubated with rabbit platelets during the washing process, as described in the protocol below. When examined under a fluorescent microscope (Zeiss Axio Observer Z1 microscope), a Mitotracker Red concentration of 1μM (1μl/ml) appeared to label the majority of platelets (figure 4.15). The labelling efficiency on flow cytometry was >98%. When checked with the FITC-fibrinogen assay, there was no significant activation and minimal desensitisation of the washed, labelled platelets (figure 4.16).

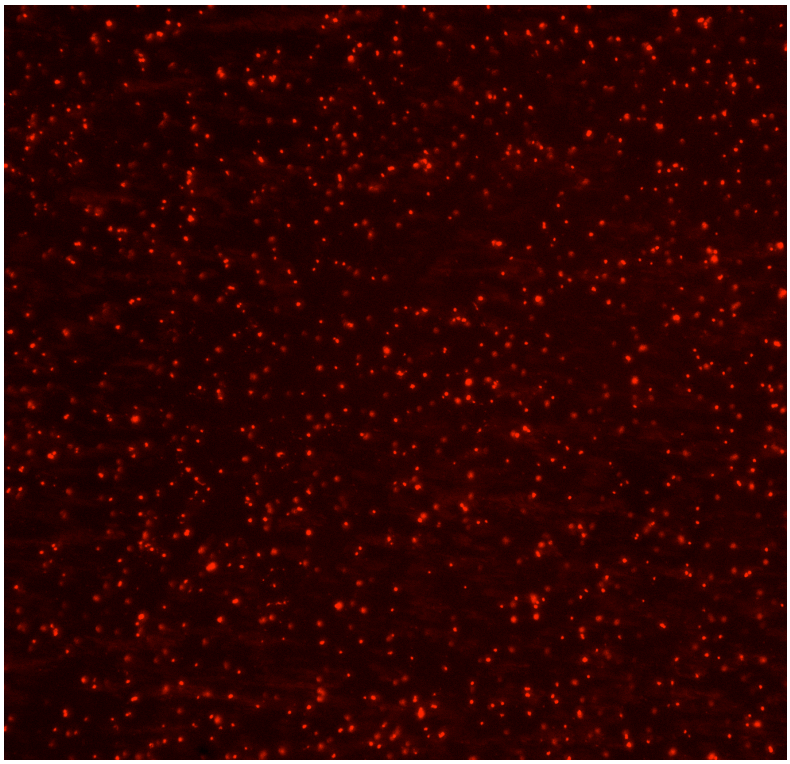


Figure 4.15. Fluorescent microscopy (x20 magnification) showing washed rabbit platelets labelled with 1μM Mitotracker Red fluorescent dye

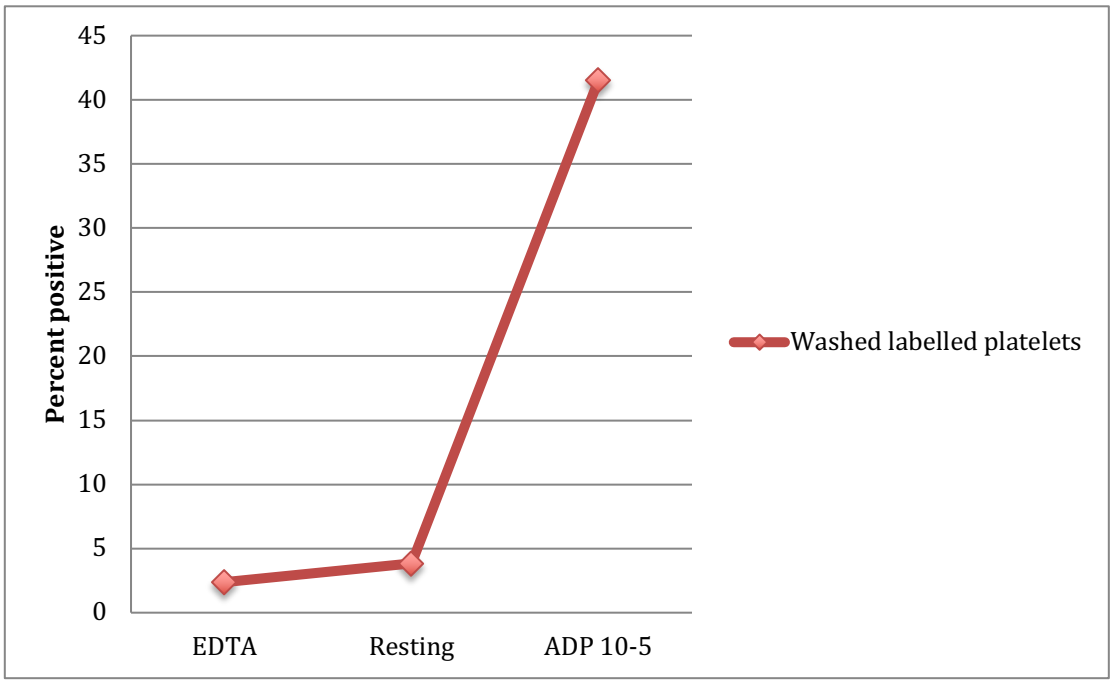


Figure 4.16. Flow cytometry using FITC-fibrinogen assay with washed rabbit platelets, labelled with 1μM Mitotracker Red dye

Chandler Loop model

In order to assess whether labelled platelets could be imaged and quantified when attached to a coronary stent, a Chandler Loop model was used (226) before moving on to live animal work. This is an in vitro artificial circulation mimicking arterial blood flow, involving a column of citrated blood, which is re-calcified and placed in a 45cm length of PVC tubing with a 3mm inner diameter. The tubing is then sealed from end-to-end to form a loop and circulated on a drum at 36 rotations per minute in an incubator set at 37°C for 1.5 hours, resulting in the formation of a thrombus within the column of blood.

300µl of washed, labelled rabbit platelets, re-suspended in plasma were added to 700µl of citrated whole rabbit blood. Having deployed a 3mm BMS within the Chandler Loop, 0.9ml of blood was added to a mixture of 150µl 0.9% saline and 50µl of 0.25M calcium chloride, and 1ml of the recalcified blood was injected into the Chandler Loop and circulated in an incubator at 37°C as described above for 1.5 hours. The resultant thrombus within the stent was examined using fluorescent microscopy (Leica Microsystems DFC450 C) (figure 4.17) and in an IVIS® Spectrum preclinical imaging system (PerkinElmer) to quantify the degree of immunofluorescence and thereby the number of platelets attached to the stent struts (figure 4.18).

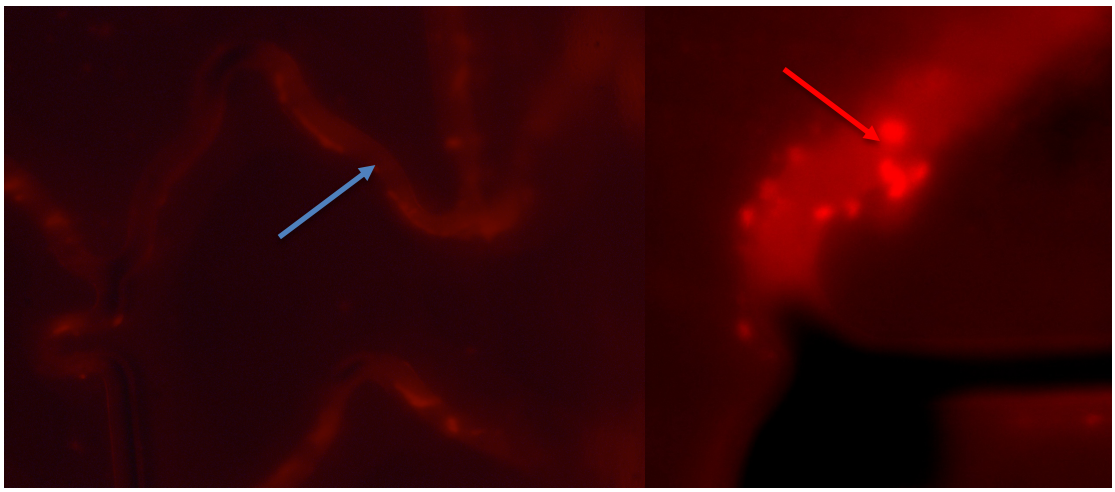


Figure 4.17. Fluorescent microscopy (x5 magnification left panel, x20 magnification right panel) showing labelled platelets (red arrow in right panel) attached to coronary stent struts (blue arrow in left panel)

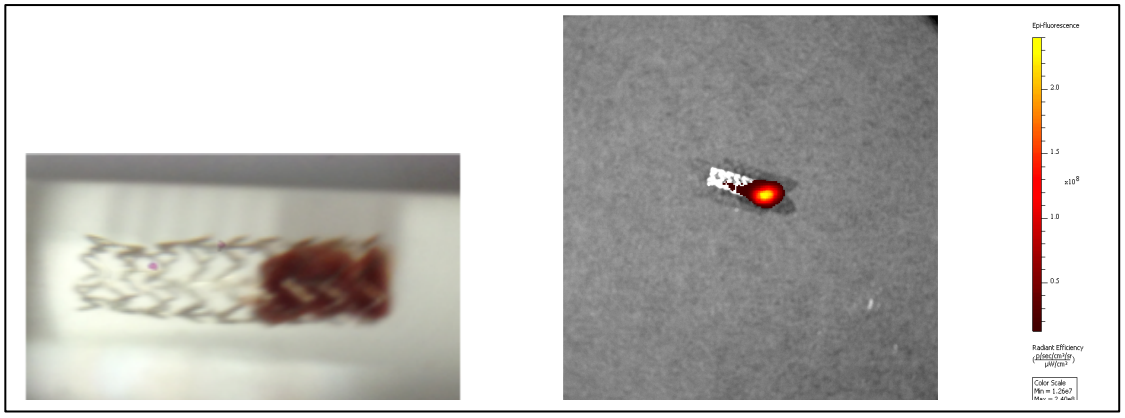


Figure 4.18. Stent thrombosis formed with rabbit blood in Chandler Loop (left) and associated immunofluorescence from labelled platelets imaged in IVIS spectrometer (right)

Final protocol for washing and labelling of rabbit platelets

Having carried out the preliminary experiments to optimise the methods, as described in the sections above, the method for washing and labelling the rabbit platelets was as follows. Following sedation, the central ear artery was cannulated with a 22G cannula and having discarded the first 1ml of blood and taken another 2mls for Multiplate testing, 18mls of blood was collected into 1/6 volume acid citrate dextrose anticoagulant. This was centrifuged at 200g for 15 minutes and 7mls of platelet rich plasma (PRP) was collected and incubated with apyrase 0.2 units/ml (14µl) for 15 minutes. The remaining blood was centrifuged at 600g for 15 minutes to obtain platelet poor plasma (PPP) for later use. Prostacyclin 200ng/ml was then added to the PRP solution and centrifuged at 600G for 15 minutes. The supernatant was discarded and the platelet pellet re-suspended in pH 6 HBS (7mls), to which further apyrase was added. This solution was then incubated with 7µl MitoTracker Red fluorescent dye (1µM concentration) for 30 minutes at room temperature to label the platelets. Finally, further prostacyclin was added before centrifuging at 600g for 15 minutes and re-suspending the labelled platelets in 4mls of PPP for re-injection intra-operatively, 30 minutes prior to angioplasty and stenting. 5µl of this final platelet suspension was kept separately and diluted in 95µl of PPP in order to count the number of

platelets using a haemocytometer to enable the platelet count to be taken into account with immunofluorescence in the IVIS spectrometer. The rabbit's fluid volume was replaced with 20mls of gelofusin infused intravenously over the first 2 hours, followed by maintenance fluids of 0.9% saline 5mls/hour. At the end of the procedure, stented vessels were explanted as described below and the immunofluorescence associated with the stents measured using an IVIS spectrometer. This was then converted in to the actual number of platelets adhering to the stents, by using a known concentration of labelled platelets.

4.2.3.2. Operative procedure

Following induction of general anaesthesia, as described in section 4.2.2, the ventral aspects of both thighs, groin area and abdomen were shaved and cleaned thoroughly with 0.05% chlorhexidine and the animal draped with sterile sheets. Bupivacaine was infiltrated as a local anaesthetic and a transverse groin incision was made over the area of femoral pulsation, below the inguinal ligament. Blunt dissection was used to expose the neurovascular bundle and the superficial femoral artery was freed from surrounding connective tissue. Both right and left femoral arteries were exposed in this way (figure 4.19).

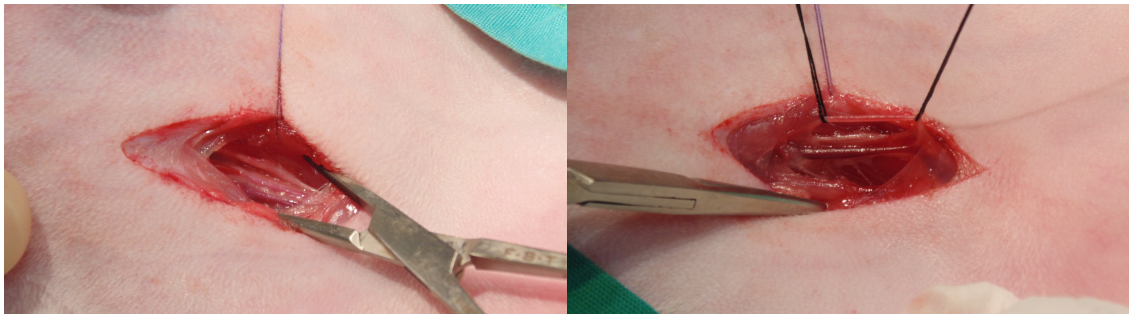


Figure 4.19. Exposure of neurovascular bundle (left panel) and isolation of superficial femoral artery (right panel)

A midline abdominal incision was then made and the peritoneum carefully opened to expose the abdominal contents, which were deflected to one side and covered with warm wet swabs. The bladder was emptied using a needle and syringe. Blunt dissection was used to expose and free the abdominal aorta and both iliac arteries from surrounding connective tissue. Baseline blood flow was

measured using perivascular flow probes placed around both common iliac arteries (figure 4.20).

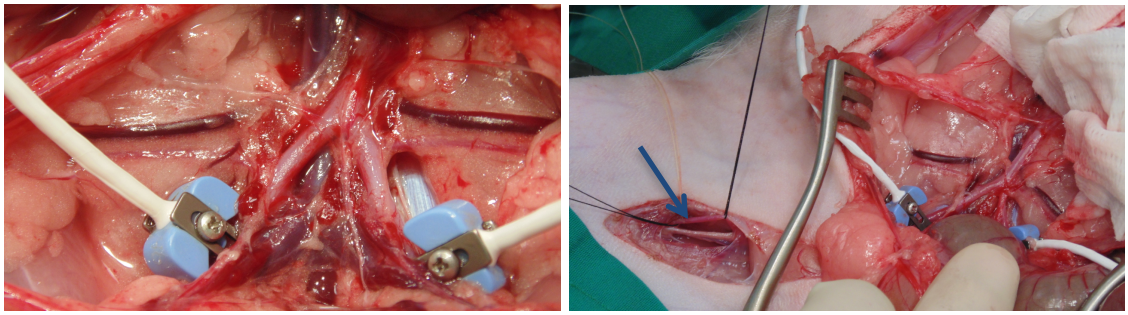


Figure 4.20. Perivascular flow probes placed around both common iliac arteries (left panel); relation to superficial femoral artery (blue arrow) shown in right panel

An arteriotomy was made between 2 ligatures placed loosely around the superficial femoral artery distal to the lateral circumflex branch (figure 4.21) and arterial bleeding was controlled with tension on the proximal ligature.

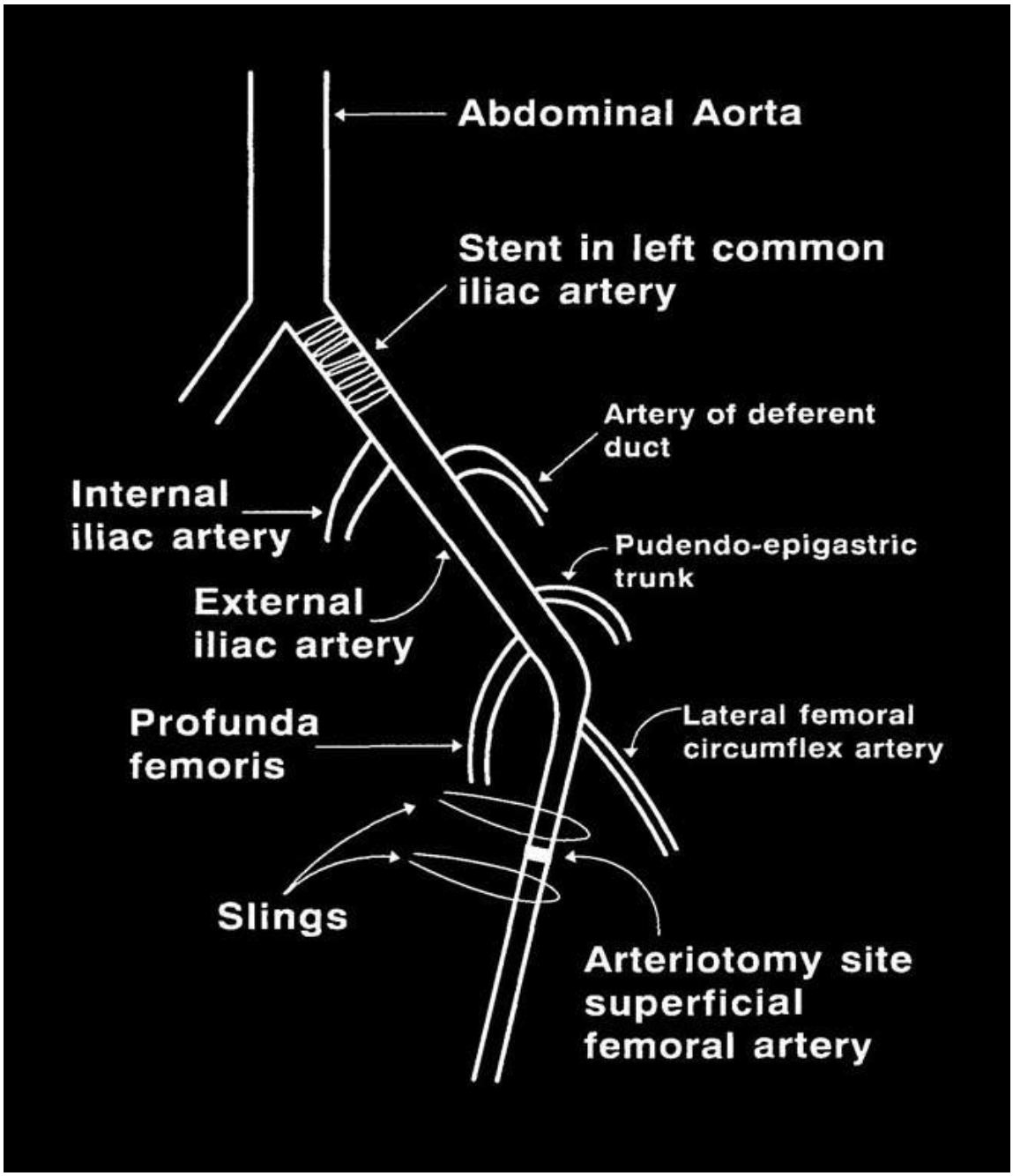


Figure 4.21. Schematic illustration of operative anatomy (illustration from MD thesis by Rajesh Aggarwal)

A 3x12mm non-compliant coronary angioplasty balloon catheter was advanced under direct vision to the proximal common iliac artery just distal to the aortic bifurcation and inflated using a standard indeflator to 12 atmospheres for 60 seconds. This was carried out three times, with a 2-minute recovery period between each inflation, to induce deep arterial injury. The balloon catheter was then removed and a 3x12mm stent (Absorb BVS or Xience DES) was introduced in the same way and deployed at the site of balloon injury at nominal pressure (7

atmospheres for Absorb BVS and 10 atmospheres for Xience DES). Choice of stent could not be blinded. However, in order to avoid any potential selection bias at the time of stent implantation, the side of placement for each type of stent (left or right iliac) was planned in advance, with equal numbers of both stent types on each side.

Following stent deployment, the stent delivery balloon catheter was removed and the superficial femoral artery ligated at the arteriotomy site to reduce flow upstream in the stented iliac artery. The same process was repeated to deploy the comparator stent (Absorb BVS or Xience DES) in the contralateral common iliac artery.

4.2.3.3. Iliac artery blood flow measurement

After allowing flow to stabilise for 10 minutes following arterial manipulation, baseline blood flow, prior to angioplasty, was measured through both common iliac arteries for 2 minutes, using two 3mm precision perivascular flow probes, connected to a T402 Transonic dual channel flow console (Transonic Systems Inc). Following stent implantation and ligation of both superficial femoral arteries, blood flow was measured continuously for two hours, with the flow probes placed bilaterally just distal to the common iliac stents.

Total stent occlusion was defined as a reduction of flow to less than 0.5ml/minute for at least 10 minutes. Cyclical flow variation, thought to be a reliable marker of platelet aggregate formation, and highly predictive of vessel closure after angioplasty (227), was defined as a reduction of flow to less than 0.5ml/minute, with an abrupt restoration of flow to greater than 0.5ml/minute within 10 minutes.

4.2.3.4. Termination of animals and recovery of tissues

Following the two-hour flow measurement, animals were euthanised with an intravenous overdose of pentobarbitone (150mg/kg). The abdominal aorta was cannulated with a 20G cannula and following ligation of the aorta proximally, the distal aortic bifurcation was flushed with 20ml of 0.9% saline. The stented

common iliac arteries were carefully explanted and emersion fixed in 10% neutral buffered formalin (NBF) prior to measurement of immunofluorescence.

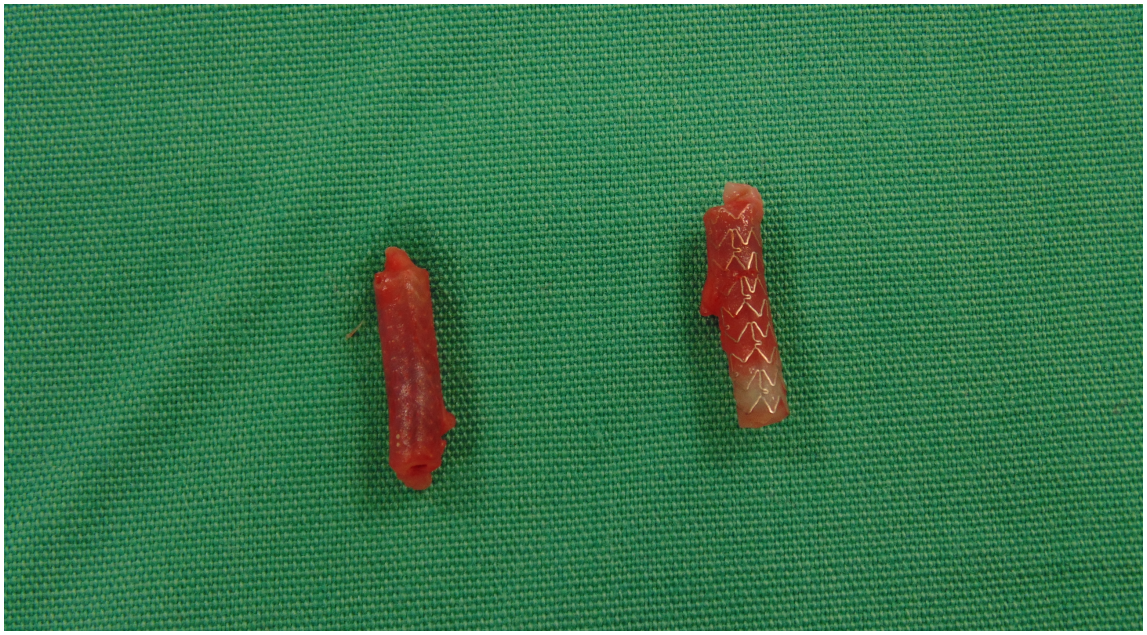


Figure 4.22. Explanted common iliac arteries with Absorb BVS (left) and Xience DES (right)

4.2.3.5. OCT imaging of explanted stents

Having measured immunofluorescence, the stented iliac vessels were re-emersed in NBF prior to OCT examination. Each stent was imaged using a standard C7XR Dragonfly OCT catheter (St Jude Medical), with a pull-back through the stented vessel from distal to proximal. The images were stored for analysis. Each pull-back was graded according to the extent of thrombus within the stent, using the following qualitative scale:

Occlusive	-	complete thrombotic occlusion of the arterial lumen
Luminal	-	encroachment into the lumen without complete occlusion
Minimal	-	minimal thrombus visible on stent struts
None	-	no visible thrombus

4.2.4. Recovery model

4.2.4.1. Operative procedure

This involved a less invasive procedure, with operative dissection limited to bilateral groin incisions to expose the superficial femoral arteries. Preparation of the animal, anaesthesia and intra-operative monitoring was as previously described for the acute model. In order to provide additional analgesia for the recovery period, a subcutaneous injection of meloxicam 0.2mg/kg was given at induction and for a further 2 days post-operatively. As antibiotic cover for the wounds, a pre-procedure dose of enrofloxacin 5mg/kg was given subcutaneously. In addition to the higher dose of clopidogrel, as explained above, heparin 100units/kg was administered intravenously prior to stenting to reduce the risk of thrombus formation during stent implantation.

Using the inguinal ligament as an anatomical landmark, a 3x15mm stent, either a Biotronik PRO-Kinetic Energy BMS or one of the two bio-engineered RGD-peptidomimetic stents, was advanced via an arteriotomy in the superficial femoral artery and deployed at nominal pressure (9 atmospheres) in the region of the common iliac artery. Once again, choice of stent could not be blinded. However, in order to avoid any potential selection bias at the time of stent implantation, the side of placement for each type of stent (left or right iliac) was planned and allocated in advance, with equal numbers of both stent types on each side. Having removed the stent delivery balloon, the superficial femoral artery was ligated. The comparator stent (BMS or bio-engineered stent) was deployed in the contralateral common iliac artery in the same way. Both groin wounds were then closed in layers with 5-0 vicryl sutures and a topical tissue adhesive was applied to the skin incision. This was then sprayed with an Opsite dressing (Smith & Nephew). Anaesthesia was reversed with a subcutaneous injection of atipamezole 1mg/kg at the end of the procedure. Buprenorphine 0.05mg/kg was also given subcutaneously as additional analgesia prior to waking.

During these procedures, particular care was taken to avoid trauma to nerves during dissection of the neurovascular bundle and animals were closely observed

post-operatively for signs of limb paralysis or ischaemia and in follow up for limb gnawing. Animals were maintained for a period of 28+/-2 days.

4.2.4.2. Perfusion fixation and explantation of stented vessels

After 28 days, animals were re-sedated and prepared for surgery aseptically as above. Following infiltration of local anaesthesia, a midline abdominal incision was made, the peritoneum carefully opened and the abdominal organs deflected to one side. Blunt dissection was used to isolate the abdominal aorta and inferior vena cava, as well as the stented iliac arteries. The aorta was cannulated with a 22G cannula for infusion and the vena cava with a 20G cannula for exsanguination.

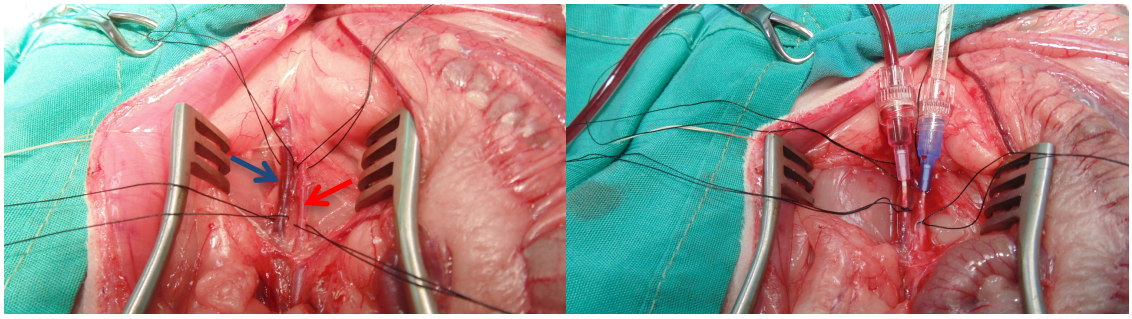


Figure 4.23. Inferior vena cava (blue arrow) and abdominal aorta (red arrow) cannulated (right panel) for perfusion fixation of stented iliac arteries

Having established antegrade flow with heparinised saline via the aortic cannula, the animal was euthanised with an intravenous overdose of pentobarbitone (150mg/kg). The aorto-iliac vessels were flushed in situ at 80-100mmHg with 500mls of heparinised (1 unit/ml) 0.9% saline followed by in situ perfusion fixation with 300mls of 10% NBF. Simultaneous exsanguination was achieved from the cannula in the inferior vena cava. The stented iliac vessels were then carefully dissected, explanted and emersion fixed in 20mls of 10% NBF at room temperature for at least 10 days, before being transferred to 5mls 10% NBF for subsequent shipping to the CV Path Institute in Washington, USA, for detailed histopathological analysis in the laboratory of our PRESTIGE collaborator, Dr Michael Joner. The right iliac artery was marked with a suture tied distally for identification.

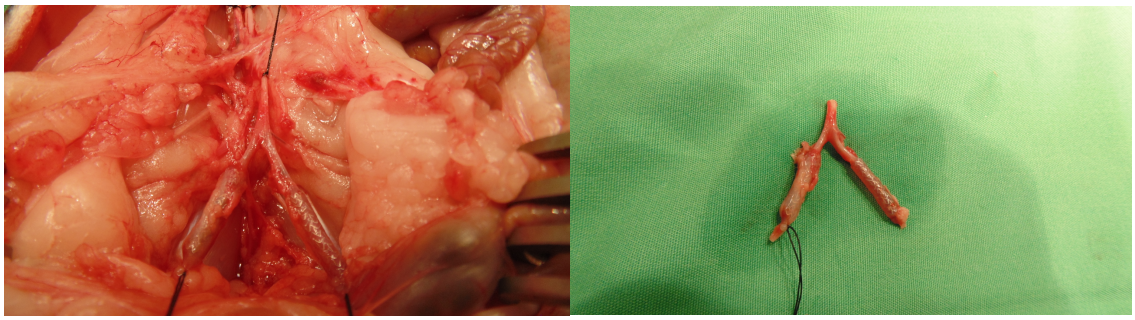


Figure 4.24. Aorto-iliac bifurcation perfusion fixed in situ (left panel) prior to explantation of stented common iliac arteries (right panel) (right iliac artery marked with a suture)

4.2.4.3. Histopathological analysis at CVPath Institute

Radiographic evaluation

Before processing, intact stented iliac vessels were imaged using high contrast digital radiography (Faxitron X-ray Corp, Model LX-60) to locate and assess stent placement. The radiographs were then examined for stent strut fractures and structural integrity.

Sample preparation for light microscopy

The stented vessels were dehydrated in a graded series of ethanol concentrations and embedded in methylmethacrylate plastic. After polymerisation, 2 to 3mm sections were cut from the proximal, mid and distal sections of each implanted stent. Proximal and distal stented ends were taken within 2-3mm of the stent ends. Sections from the stents were cut on a rotary microtome at 6 microns, mounted and stained with haematoxylin and eosin (H&E) and Movat Pentachrome stains. Non-stented reference vessel segments were taken proximal and distal within 2-3mm of the stent ends and processed for paraffin histology. Sections were cut on a rotary microtome at 5 microns, mounted and stained with H&E and Movat Pentachrome stains. All sections, stented and non-stented were examined by light microscopy for the presence of inflammation, thrombus and vessel wall injury.

Morphometry

Histologic sections were analysed with an NIST calibrated microscope system (IP Lab software, Rockville, MD). The cross-sectional areas (external elastic lamina [EEL], internal elastic lamina [IEL] and lumen) of each stented section were measured. Neointimal thickness was measured as the distance from the inner (abluminal) surface of each stent strut to the luminal border. Area measurements were used to calculate vessel layer areas with the following formulae:

Medial Area = EEL Area- IEL Area
Neointimal Area = IEL Area- Lumen Area
% Stenosis = [1- (Lumen Area / IEL Area)] x100

To compare neointimal organisation and healing between stent types, data were collected on each stent section, including strut apposition to the vessel wall, fibrin deposition, calcification, granuloma and giant cell reactions and haemorrhage around the stent struts. These were expressed as a percentage of the total number of struts in each section. A vessel injury score was calculated according to the Schwartz method (228). Overall neointimal inflammation, adventitial inflammation and the presence of fibrin were scored for each section. A detailed description of the scores is shown in table 4.1 below. Endothelial coverage was visually estimated and expressed as the percentage of the lumen circumference covered by endothelium.

Table 4.1. Description of semi-quantitative histology scores

Attribute	Score	Description
Injury score	0	Internal elastic lamina (IEL) intact, endothelium typically denuded, media may be compressed but not lacerated
	1	IEL lacerated, media typically compressed but not lacerated
	2	IEL lacerated, media visibly lacerated, external elastic lamina (EEL) intact but may be compressed
	3	EEL lacerated, typically large lacerations of media extending through EEL, coil wires sometimes residing in adventitia
Neointimal inflammation score	0	<25% struts with fewer than 10 inflammatory cells
	1	Up to 25% struts with greater than 10 inflammatory cells
	2	25-50% struts with greater than 10 inflammatory cells
	3	>50% struts with greater than 10 inflammatory cells
	4	2 or more struts with associated granulomatous inflammatory reactions
Adventitial inflammation score	0	No inflammation or minimal interspersed inflammatory cells anywhere in the adventitia
	1	Mild peripheral inflammatory infiltration or focally present in <25% of adventitial area
	2	Moderate peripheral inflammatory infiltration or focally present in 25-50% of adventitial area
	3	Heavy peripheral inflammatory infiltration or focally present in >50% of adventitial area
Fibrin score	0	No fibrin seen or only small strands
	1	At least 25% of struts involving confluent fibrin that surrounds up to 25% of the strut circumference
	2	At least 50% of struts involving confluent fibrin that surrounds >25% of strut circumference
	3	All struts with confluent fibrin surrounding >50% of strut circumference or 25-50% of struts with confluent fibrin involving >25% of strut circumference with extension or bridging between struts

4.3. Results

4.3.1. Acute stent thrombosis model

Initial pilot studies involved unilateral iliac stenting of eight animals in total. These studies were used to optimise the model, including the anaesthetic protocol, ventilation, heparinisation, size of stents and various procedural technicalities, as summarised in the 'learning points' and 'study limitations' sections of the conclusions. Following the pilot studies, six animals underwent bilateral iliac stenting. These numbers were based on previous work with a similar model and power calculations as described in the methods section. One animal held its breath, urinated and vomited during induction of general anaesthesia and needed to be terminated prior to surgery due to possible aspiration. All of the other six animals were successfully stented with an Absorb BVS on one side and Xience DES on the contralateral side. Blood flow was measured for 2 hours post stenting in all 12 vessels. Immunofluorescence from labelled platelets was measured in all 12 stented vessels and each vessel underwent ex-vivo OCT imaging.

4.3.1.1. Iliac blood flow

Two hours of continuous bilateral iliac flow measurements were obtained from all 6 animals (i.e. 12 vessels) post stent implantation, 6 with Absorb BVS and 6 with Xience DES. Complete stent occlusion occurred in only one implant, which was an Absorb BVS and occurred just over 5 minutes post deployment in rabbit number 5. The contra-lateral Xience DES in the same animal remained patent (see figure 4.25 below).

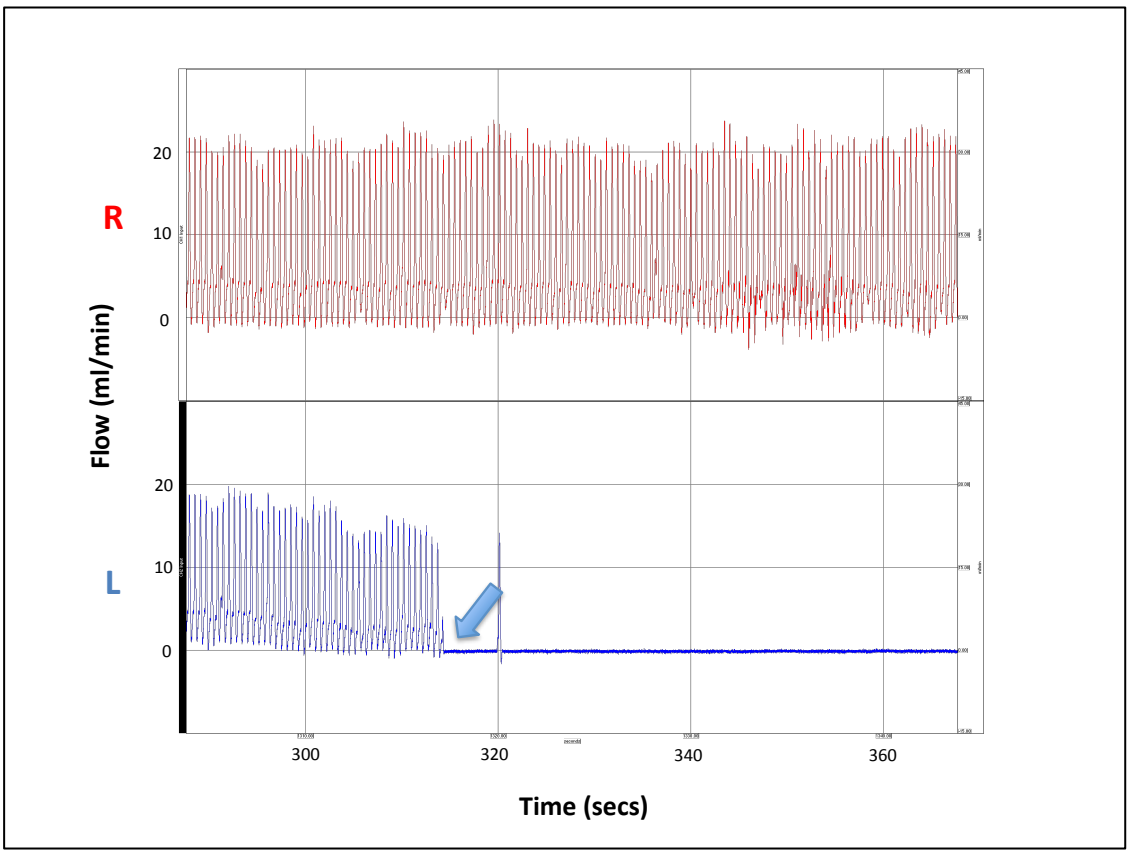


Figure 4.25. Blood flow through both iliac arteries 5 minutes after stenting; complete cessation of flow seen in left iliac artery (blue arrow) with no recovery of flow for the remainder of the 2-hour recording, indicating complete stent occlusion

Cyclical flow variation (CFV) was observed in the iliac arteries implanted with both types of stent. An example of this is shown in figure 4.26 below.

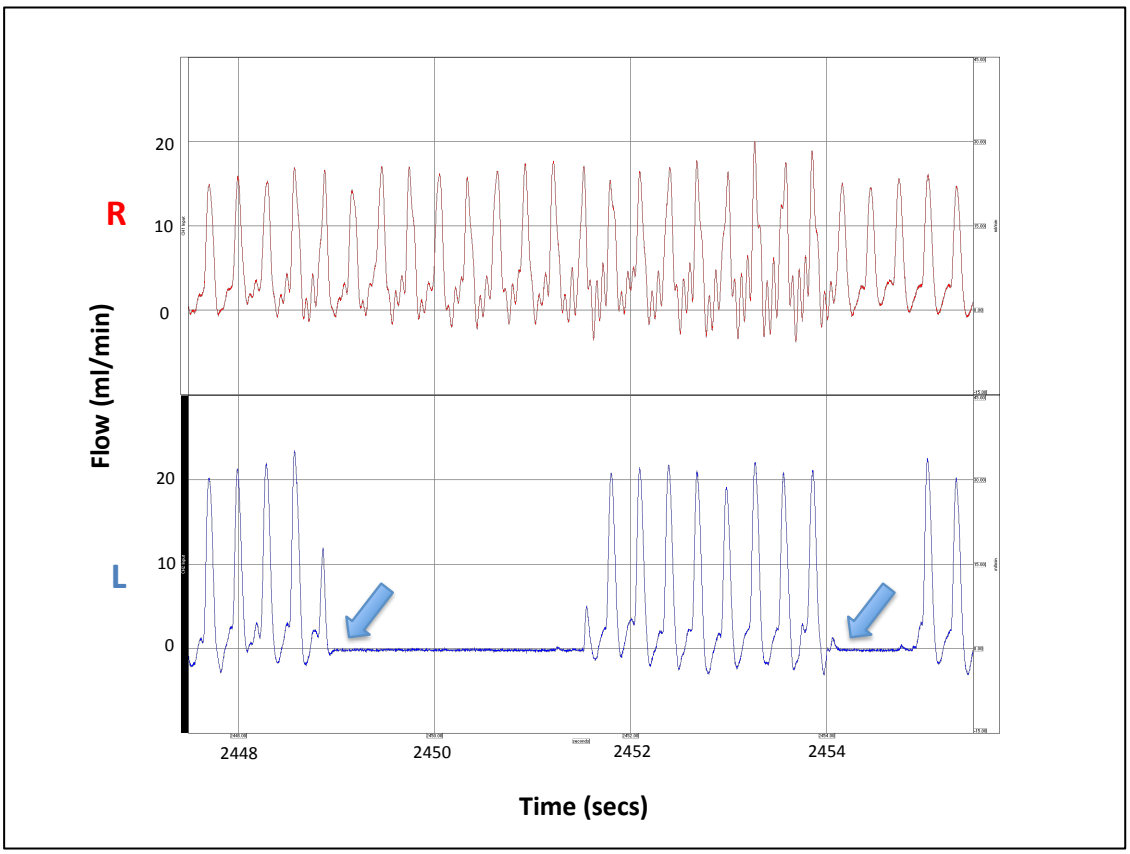


Figure 4.26. Blood flow through both iliac arteries 40 minutes after stenting; episodes of recurrent falls and abrupt recovery of flow seen in left iliac artery (blue arrows), typical of CFV

The overall results are summarised in figure 4.27 below. In three animals, there were numerically more episodes of CFV in the Absorb BVS limb compared to the Xience DES limb. In one animal, there were more episodes of CFV in the Xience DES and in two animals there was no difference in the number of CFV episodes between both types of stent. Although numerically the frequency of CFV was higher in the iliac arteries implanted with an Absorb BVS (16 episodes of CFV) compared to Xience DES (10 episodes of CFV), this did not reach statistical significance (p=0.23).

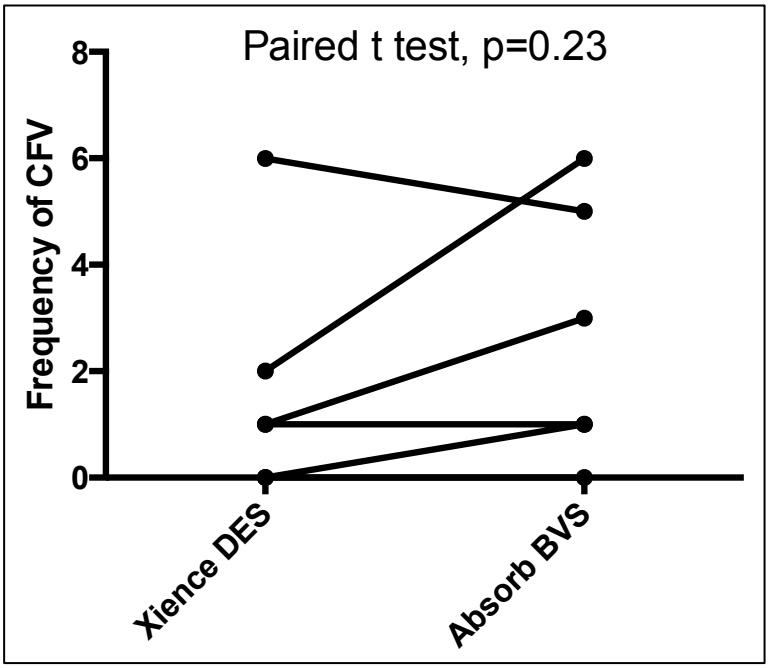


Figure 4.27. Frequency of CFV occurring during 2 hours of continuous bilateral flow measurement in iliac arteries implanted with Xience DES on one side and Absorb BVS on contralateral side

4.3.1.2. Platelet adhesion

Following two hours of flow measurement, each of the six animals had bilateral stented iliac arteries successfully explanted and fixed as described in the methods section. These were placed side-by-side, together with 5µl of the known concentration of labelled platelets from the same animal, in the IVIS spectrometer to measure immunofluorescence (figure 4.28).

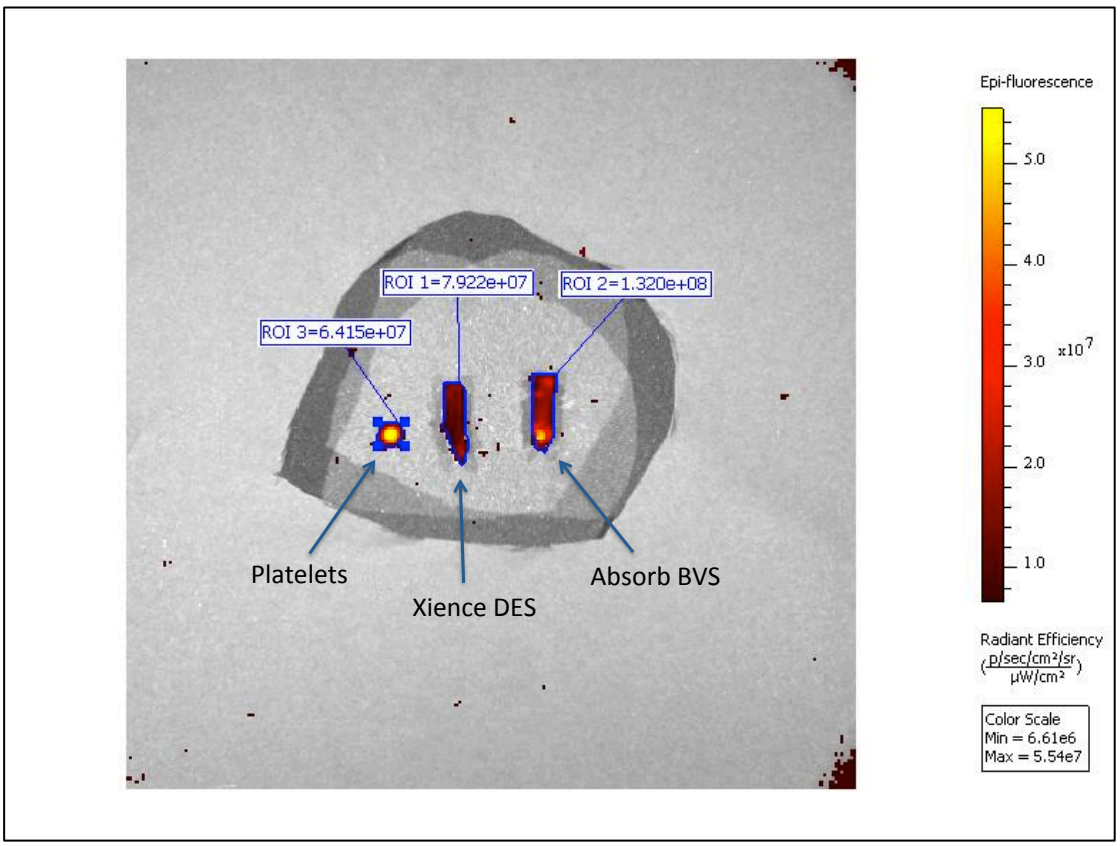


Figure 4.28. Example of comparison of immunofluorescence in known concentration of labelled platelets and explanted iliac arteries with Xience DES and Absorb BVS implanted in a single rabbit (values represent total radiant efficiency emitted from specified regions of interest, ROI)

The number of labelled platelets attached to each stented vessel was calculated using the average number counted (using a haemocytometer) in 0.1µl of washed labelled platelets that had been re-suspended in plasma from the same animal and associating this with the average measured immunofluorescence associated with each stented vessel (see table 4.2 below).

Table 4.2. Immunofluorescence and calculated number of platelets associated with iliac arteries implanted with Xience DES and Absorb BVS in each of six animals in acute ST protocol

	Average platelet count		Average immunofluorescence [(p/sec/cm^2/sr)/μW/cm^2)]			No. of platelets attached to stent	
	In 0.1 μl solution	In 5 μl solution	5μl platelets	Xience DES	Absorb BVS	Xience DES	Absorb BVS
Rabbit 1	220.25	11012.5	2.56 x10^7	6.56 x10^6	1.72 x10^7	***2822***	***7399***
Rabbit 2	126.25	6312.5	2.04 x10^7	1.06 x10^7	1.12 x10^7	***3280***	***3466***
Rabbit 3	166.25	8312.5	2.29 x10^7	1.19 x10^7	1.60 x10^7	***4320***	***5808***
Rabbit 4	137	6850	1.37 x10^7	7.83 x10^6	1.27 x10^7	***3915***	***6350***
Rabbit 5	202	10100	2.74 x10^7	9.26 x10^6	1.07 x10^7	***3413***	***3944***
Rabbit 6	182.25	9112.5	2.38 x10^7	1.09 x10^7	1.68 x10^7	***4173***	***6432***

There were significantly more platelets attached to the iliac arteries implanted with Absorb BVS as compared to those implanted with Xience DES and this increase was consistent in all 6 animals (figure 4.29 below).

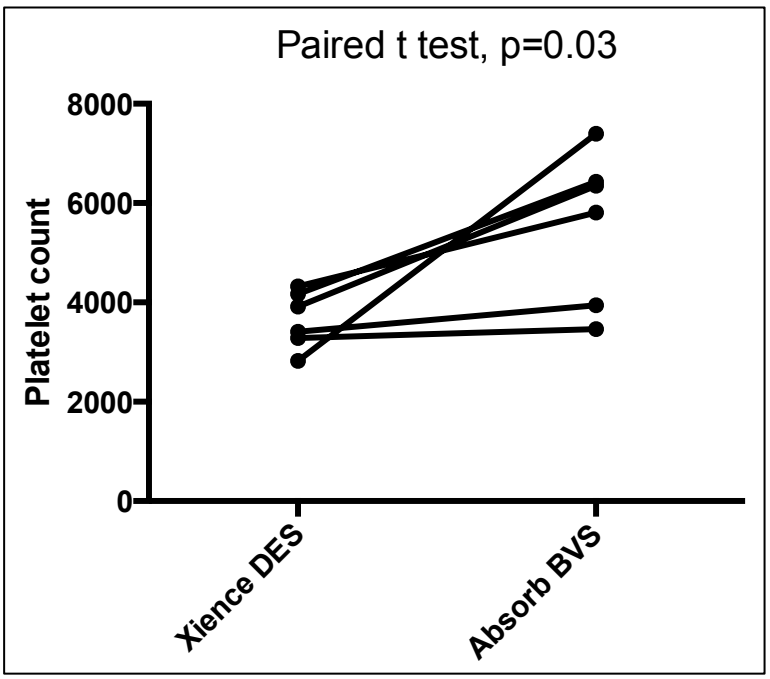


Figure 4.29. Number of platelets associated with iliac arteries implanted with Xience DES on one side compared to Absorb BVS on the contralateral side

4.3.1.3. Optical coherence tomography data

OCT pull-back was successfully obtained through each of the 12 explanted, fixed stented iliac arteries. Varying degrees of thrombus burden were seen in both stent types. Representative still frames from each pull-back are shown in figures 4.30 to 4.35 below, with Xience DES and Absorb BVS stents for each respective animal shown side by side.

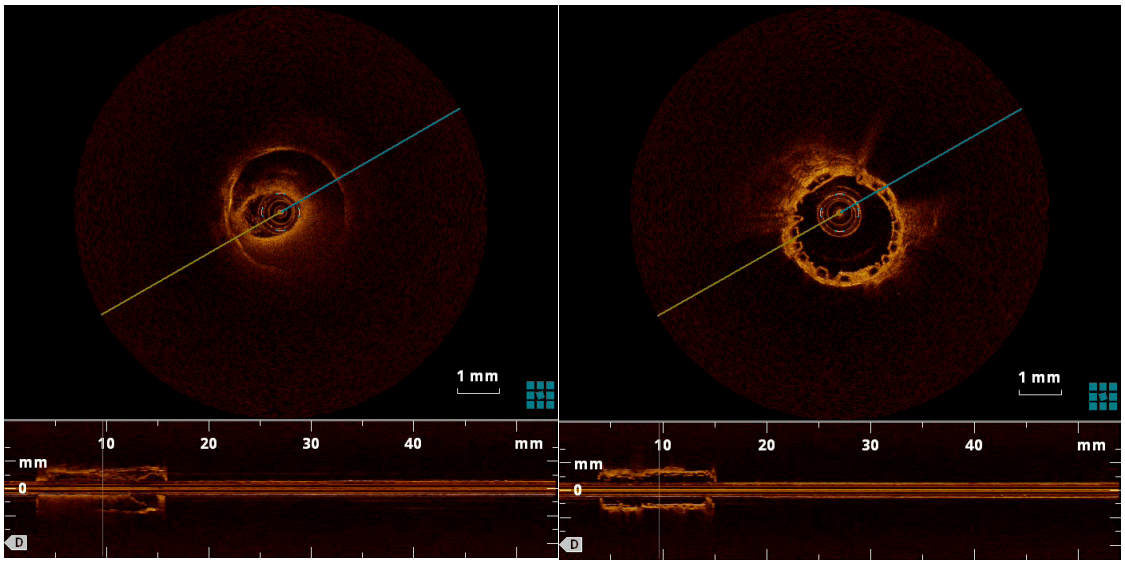


Figure 4.30. Ex vivo OCT frames from rabbit 1 iliac arteries stented with Xience DES, left panel (occlusive thrombus) and Absorb BVS, right panel (minimal thrombus)

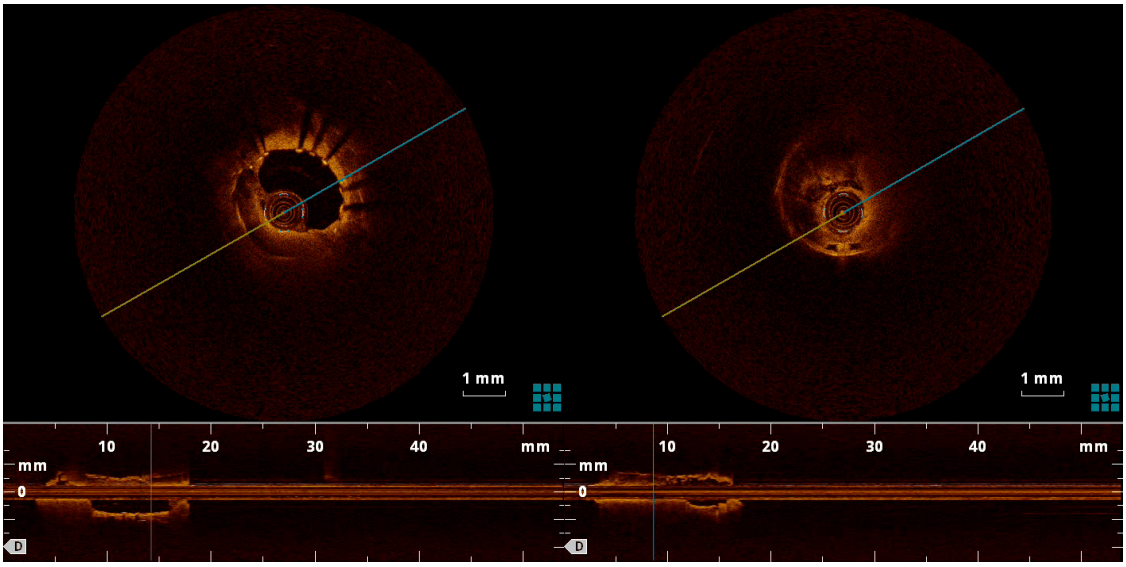


Figure 4.31. Ex vivo OCT frames from rabbit 2 iliac arteries stented with Xience DES, left panel (luminal thrombus) and Absorb BVS, right panel (occlusive thrombus)

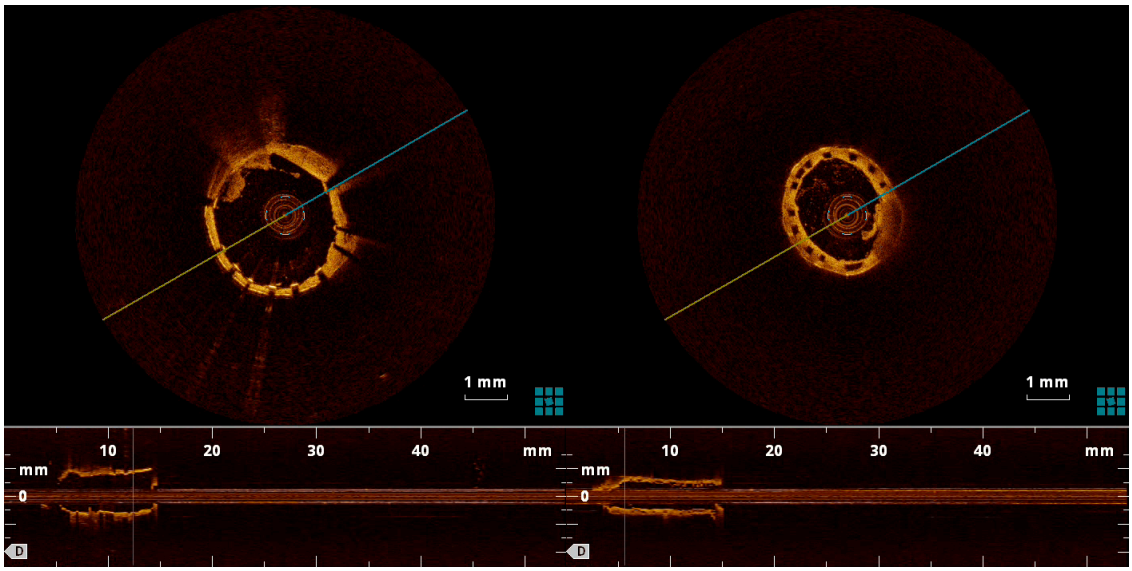


Figure 4.32. Ex vivo OCT frames from rabbit 3 iliac arteries stented with Xience DES, left panel (minimal thrombus) and Absorb BVS, right panel (minimal thrombus)

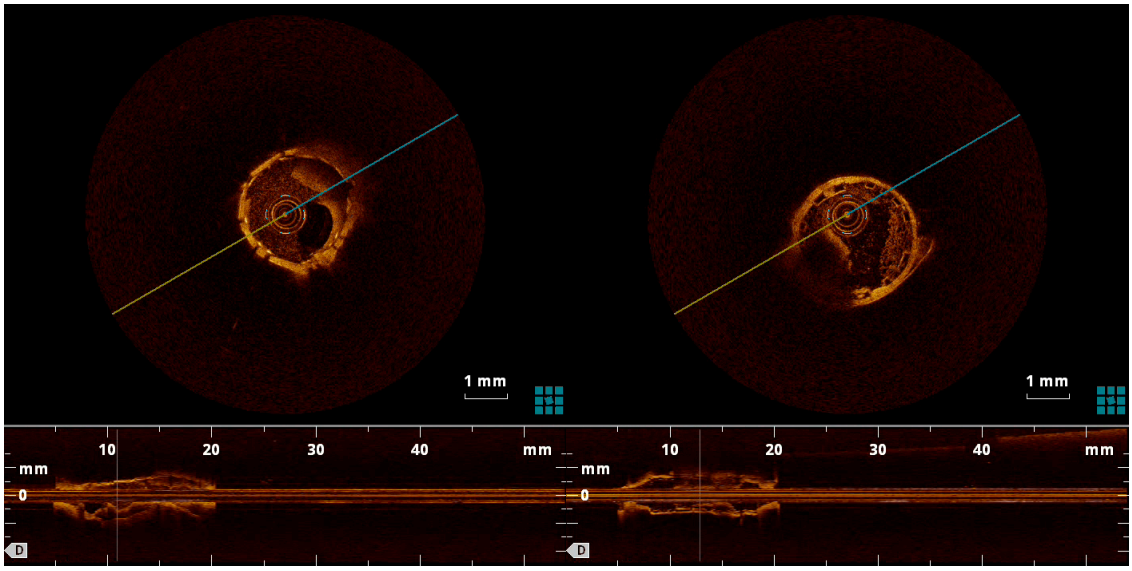


Figure 4.33. Ex vivo OCT frames from rabbit 4 iliac arteries stented with Xience DES, left panel (luminal thrombus) and Absorb BVS, right panel (luminal thrombus)

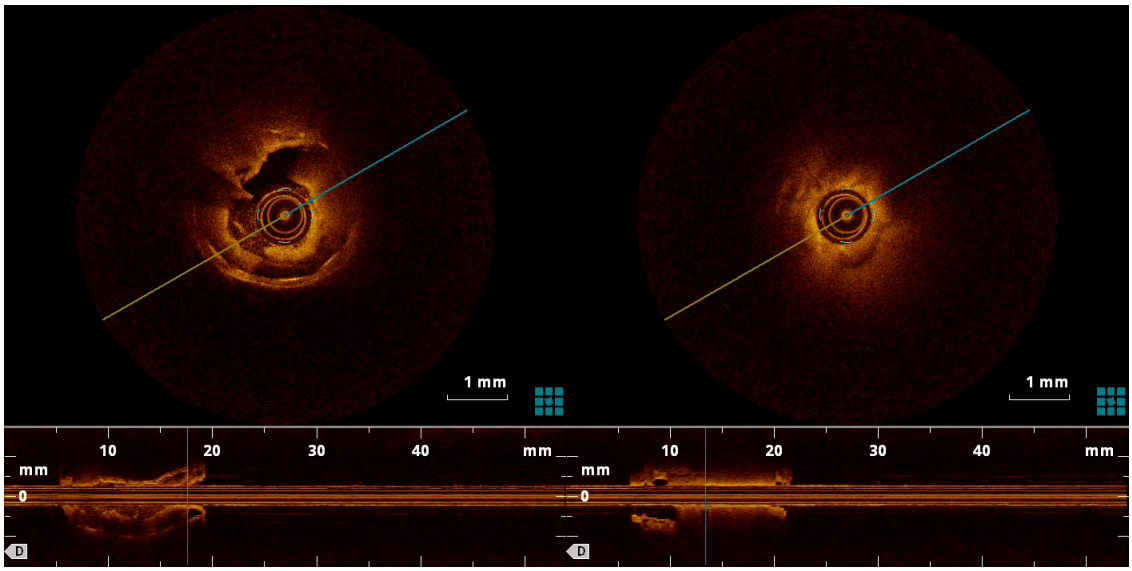


Figure 4.34. Ex vivo OCT frames from rabbit 5 iliac arteries stented with Xience DES, left panel (luminal thrombus) and Absorb BVS, right panel (occlusive thrombus)

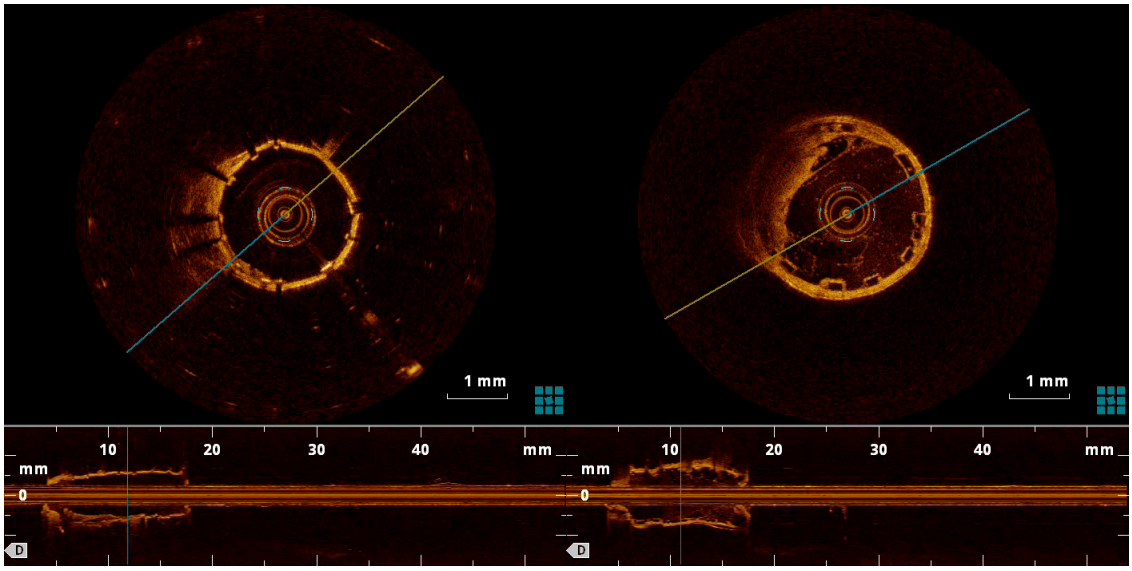


Figure 4.35. Ex vivo OCT frames from rabbit 6 iliac arteries stented with Xience DES, left panel (minimal thrombus) and Absorb BVS, right panel (luminal thrombus)

Overall, there was no difference in the extent of stent thrombosis between the two stent types, as assessed qualitatively using ex vivo OCT (see figure 4.36 below).

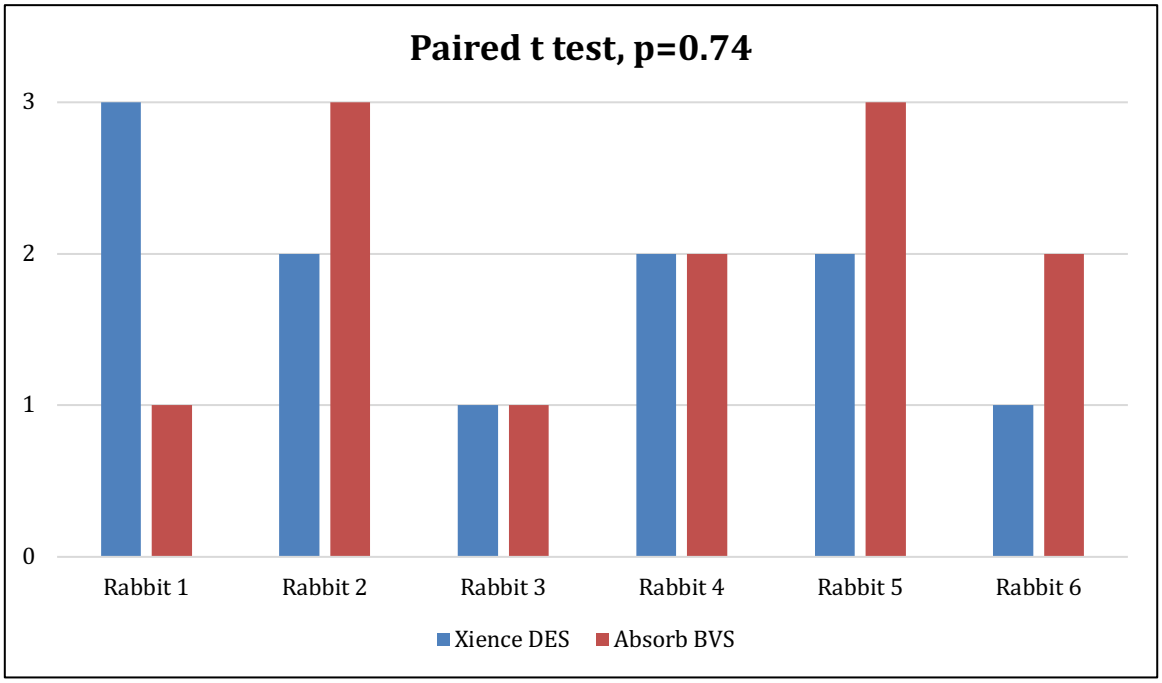


Figure 4.36. Thrombus burden, assessed using ex vivo OCT imaging, in 12 explanted iliac arteries, comparing Xience DES to Absorb BVS (0=none, 1=minimal, 2=luminal, 3=occlusive thrombus)

4.3.2. Recovery model

Following initial pilot studies involving unilateral iliac stenting with BMS in 2 animals with a recovery period of 7 and 14 days, 10 study animals were planned for bilateral iliac stenting with a Biotronik PRO-Kinetic Energy BMS on one side and one of the two bio-engineered RGD-peptidomimetic stents on the contralateral side. 8 out of the 10 animals underwent successful stenting of both iliac arteries. 2 animals only had unilateral iliac stenting due to technical difficulties. The stent came off the balloon in one and in another the stent could not be advanced beyond the inguinal ligament. 8 out of the 10 animals were successfully recovered for 28+/-2 days. One animal was found dead in its cage on the day following surgery and a post-mortem revealed a probable intra-peritoneal haemorrhage possibly from the right iliac stent which was found outside the vessel. The second animal developed bilateral hind limp paralysis on the day following surgery. There were no signs of trauma, Doppler studies confirmed the preservation of bilateral iliac artery blood flow and on surgical exploration, the femoral nerves appeared intact, although the bladder was full.

After discussion with the named veterinary surgeon, the animal was euthanised at that point.

Eight animals received stent implants in a total of 14 iliac arteries, as summarised in tables 4.3 and 4.4 below. In rabbit numbers 1 and 4, no stent was deployed in the left iliac artery, as explained above. In rabbit number 5, the BMS in the left iliac artery appeared to be outside the main vessel, either in a side branch or the surrounding tissue, and so this was excluded from histological analysis.

Table 4.3. Summary of implants in recovery model

	Date of implant	Right iliac stent	Left iliac stent	Date of explant	Recovery period
Rabbit 1	26/09/14	BMS	None	23/10/14	27 days
Rabbit 2	29/09/14	RGD PLA-CS60	BMS	28/10/14	29 days
Rabbit 3	29/09/14	BMS	RGD-CS60	27/10/14	28 days
Rabbit 4	30/09/14	BMS	None	29/10/14	29 days
Rabbit 5	01/10/14	RGD-CS60	BMS*	29/10/14	28 days
Rabbit 6	21/10/14	BMS	RGD-CS60	18/11/14	28 days
Rabbit 7	05/11/14	RGD-CS60	BMS	05/12/14	30 days
Rabbit 8	06/11/14	RGD PLA-CS60	BMS	05/12/14	29 days

*Rabbit 5 left iliac artery was excluded from histological analysis, as the stent was not deployed in the iliac artery

Table 4.4. Summary of total number of vessels with each type of stent

Group	Stent type	Number of stents for analysis
1	RGD PLA-CS60	2
2	RGD-CS60	4
3	BMS	7*

*One stent (rabbit 5 left iliac BMS) was excluded from analysis, as the stent was not deployed in the iliac artery

4.3.2.1. Radiographic evaluation

There were no stent fractures detected by x-ray. The radiographic images of all the stented vessels are shown in figures 4.37 to 4.39 below together with the corresponding photographs.

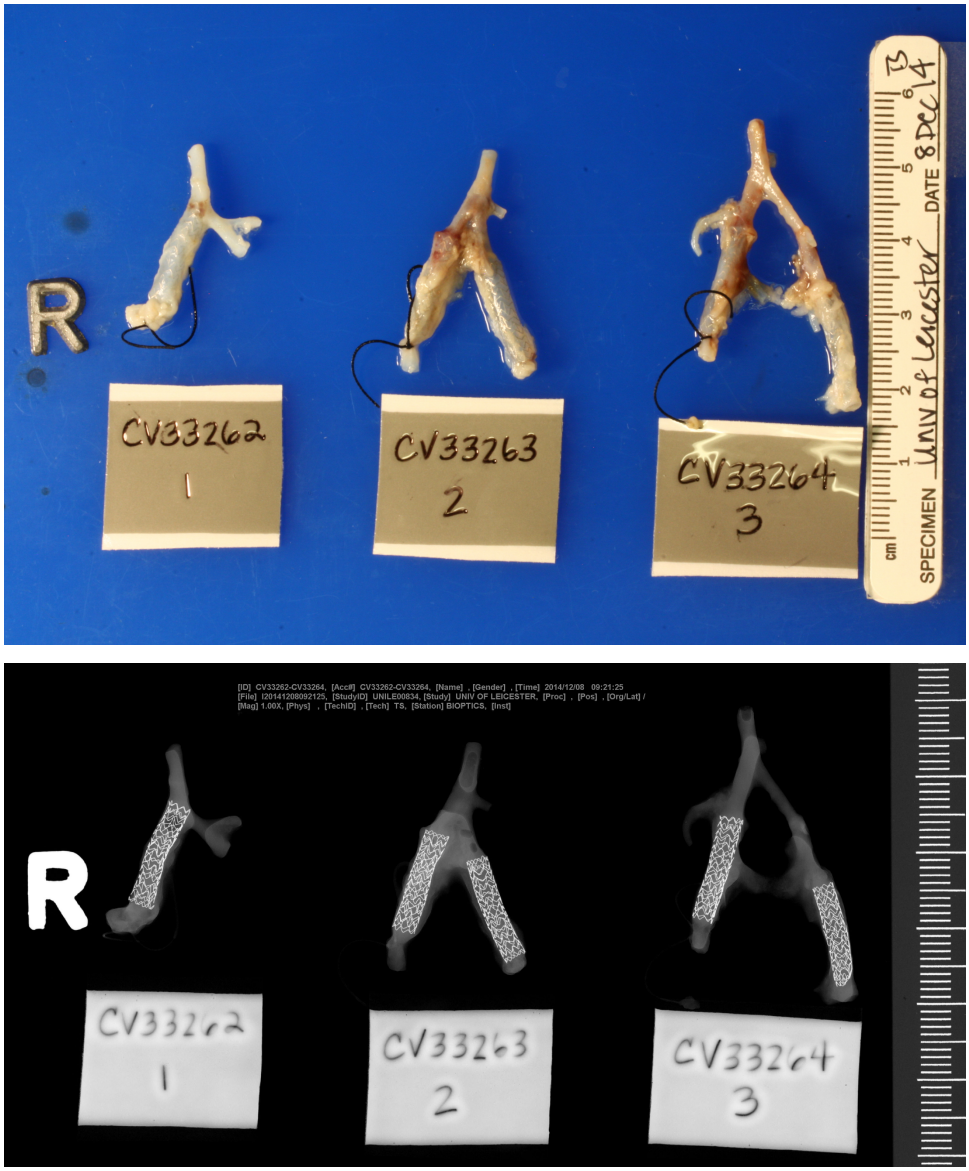


Figure 4.37. Photographs (upper panel) and x-rays (lower panel) of aorto-iliac bifurcations from rabbits 1-3

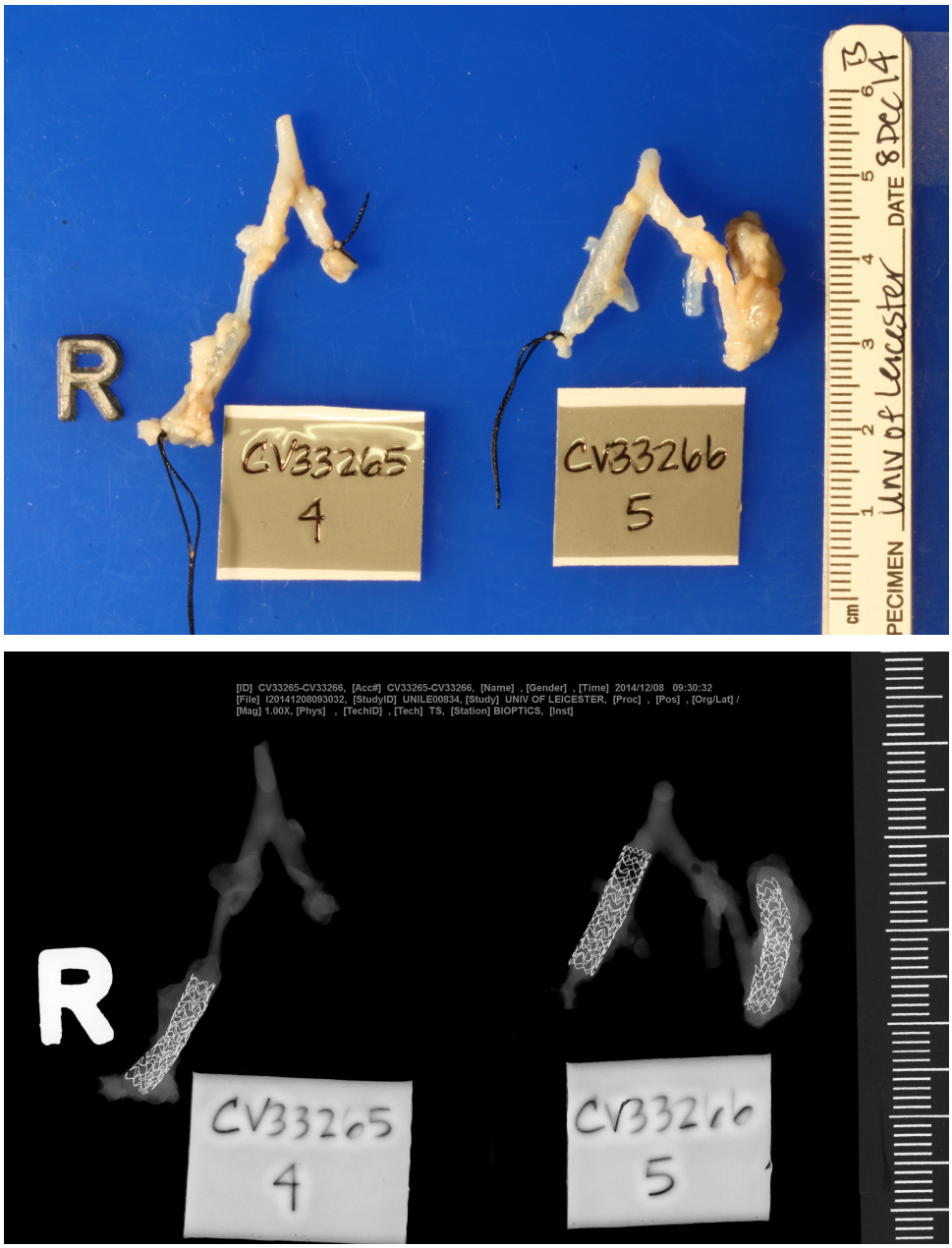


Figure 4.38. Photographs (upper panel) and x-rays (lower panel) of aorto-iliac bifurcations from rabbits 4-5 (rabbit 5 left iliac artery was excluded from analysis as the stent appears outside the iliac artery)

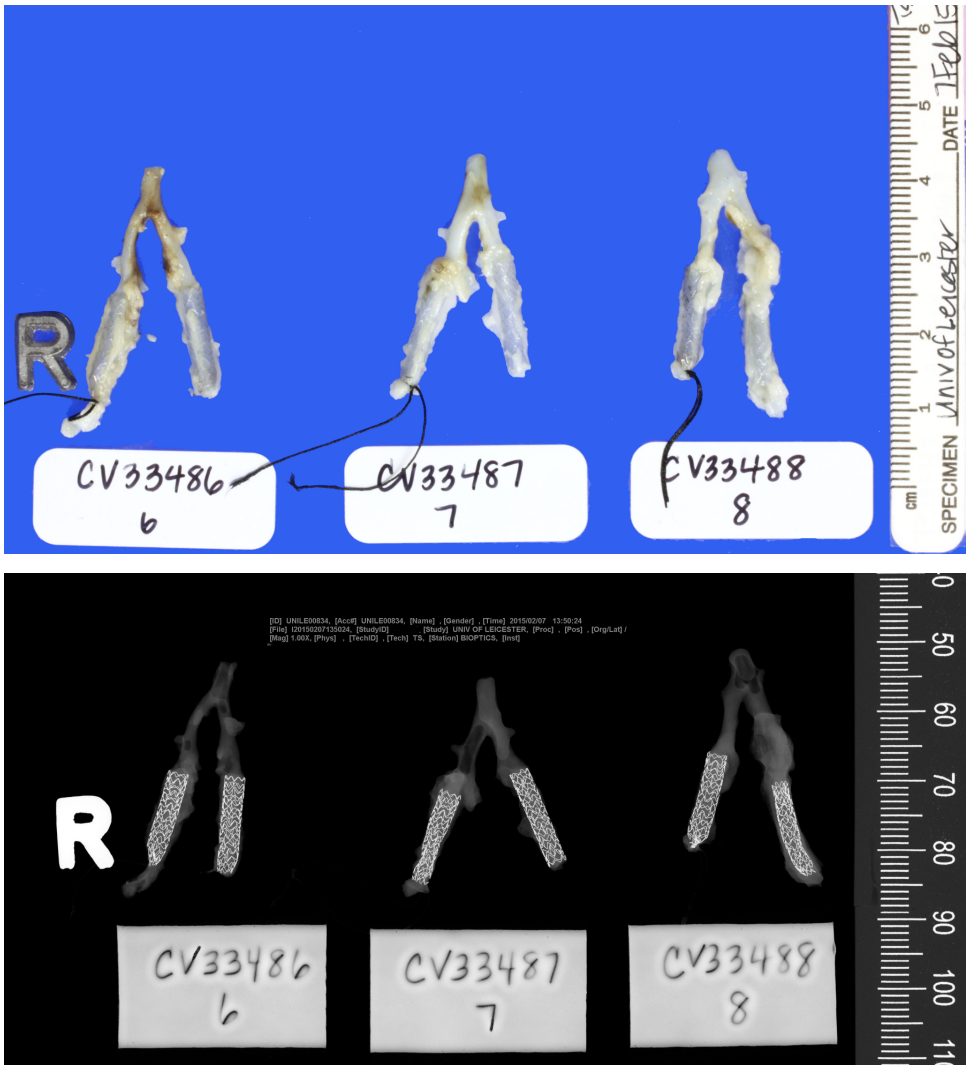


Figure 4.39. Photographs (upper panel) and x-rays (lower panel) of aorto-iliac bifurcations from rabbits 6-8

4.3.2.2. Histological findings

The detailed analyses of all vessels are shown in tables 4.5 to 4.7 and the representative light microscopy images are shown in figures 4.40 to 4.42 in the sections below. Although the statistical analyses are shown, it is acknowledged that the sample size in group 1 (n=2) is insufficient to draw any meaningful conclusions.

Table 4.5. Morphometric comparison of cross-sectional vessel areas and neointimal thickness. Analysis includes vessel means ± standard deviation from proximal, mid and distal sections (group 1=RGD PLA-CS60, group 2=RGD-CS60, group 3=BMS).

Stent group	EEL area (mm^2)	IEL area (mm^2)	Lumen area (mm^2)	Medial area (mm^2)	Neointimal area (mm^2)	Stenosis (%)	Neointimal thickness (mm)
1 (n=2)	6.83 ± 0.42	6.53 ± 0.36	5.45 ± 0.55	0.29 ± 0.11	1.08 ± 0.57	16.52 ± 8.38	0.08 ± 0.06
2 (n=4)	6.57 ± 0.99	6.23 ± 0.87	5.72 ± 0.85	0.34 ± 0.17	0.51 ± 0.14	8.34 ± 2.44	0.02 ± 0.01
3 (n=7)	6.29 ± 1.16	5.92 ± 1.05	5.21 ± 1.18	0.37 ± 0.18	0.72 ± 0.34	12.94 ± 7.64	0.04 ± 0.03
P-value (Kruskal – Wallis test with Dunnett's post hoc correction)							
1 vs 3	1.00	1.00	1.00	1.00	0.42	0.80	0.16
2 vs 3	1.00	1.00	1.00	1.00	0.49	0.38	0.65
1 vs 2	1.00	1.00	1.00	1.00	**0.05**	0.10	**0.02**

Table 4.6. Histological comparison of healing. Analysis includes vessel means ± standard deviation from proximal, mid and distal sections (group 1=RGD PLA-CS60, group 2=RGD-CS60, group 3=BMS).

Stent group	Strut mal-apposition	Struts with fibrin	Struts with granulomas	Struts with giant cells	Struts with RBCs (%)	Struts with calcificat-ion (%)	Endothel-ialisation (%)
1 (n=2)	0 ± 0	0 ± 0	0 ± 0	13.39 ± 9.16	2.39 ± 3.72	5.66 ± 10.92	97.62 ± 6.05
2 (n=4)	0 ± 0	1.88 ± 4.41	0 ± 0	15.07 ± 13.93	0.52 ± 1.80	0.60 ± 2.06	100 ± 0
3 (n=7)	0 ± 0	2.02 ± 4.53	0 ± 0	18.90 ± 9.86	0 ± 0	2.61 ± 6.58	96.67 ± 4.92
P-value (Kruskal – Wallis test with Dunnett's post hoc correction)							
1 vs 3	1.00	0.85	1.00	0.99	**0.02**	0.86	1.00
2 vs 3	1.00	1.00	1.00	0.72	1.00	1.00	0.90
1 vs 2	1.00	1.00	1.00	1.00	0.17	0.52	0.37

Table 4.7. Histological comparison of non-parametric scores. Analysis includes vessel means ± standard deviation from proximal, mid and distal sections (group 1=RGD PLA-CS60, group 2=RGD-CS60, group 3=BMS).

Stent group	Mean injury score	Mean fibrin score	Neointimal inflammation score	Adventitial inflammation score
1 (n=2)	1.03 ± 0.90	0 ± 0	0 ± 0	0 ± 0
2 (n=4)	0.13 ± 0.14	0 ± 0	0 ± 0	0 ± 0
3 (n=7)	0.29 ± 0.47	0 ± 0	0.05 ± 0.22	0 ± 0
P-value (Kruskal – Wallis test with Dunnett's post hoc correction)				
1 vs 3	**0.02**	1.00	1.00	1.00
2 vs 3	1.00	1.00	1.00	1.00
1 vs 2	**0.03**	1.00	1.00	1.00

Group 1 – Biotronik PRO-Kinetic Energy BMS RGD PLA-CS60

All stented lumens were widely patent, with stents widely expanded and struts well apposed to the vessel wall (figure 4.40). Neointimal growth was mild and composed of smooth muscle cells organising towards the luminal surface amid a proteoglycan matrix. Mean luminal stenosis for group 1 was 16.52 ± 8.38%. There was no fibrin deposition observed on any of the struts. Histological sections showed minimal calcification (5.66 ± 10.92 %) around struts. Mean injury (mean score = 1.03 ± 0.90) was significantly higher in the RGD PLA-CS60 group as compared to group 2 and 3 (RGD PLA-CS60 vs. BMS, p=0.02; RGD PLA-CS60 vs. RGD-CS60, p=0.03). Neointimal inflammation and adventitial inflammation were not observed in group 1, but there were minimal strut-associated giant cells (mean= 13.39 ± 9.16%) observed. Luminal surfaces showed nearly complete endothelialisation (mean= 97.62 ± 6.05%). There was no luminal thrombus in any of the stented vessels. Non-stented proximal and distal segments were within normal limits.

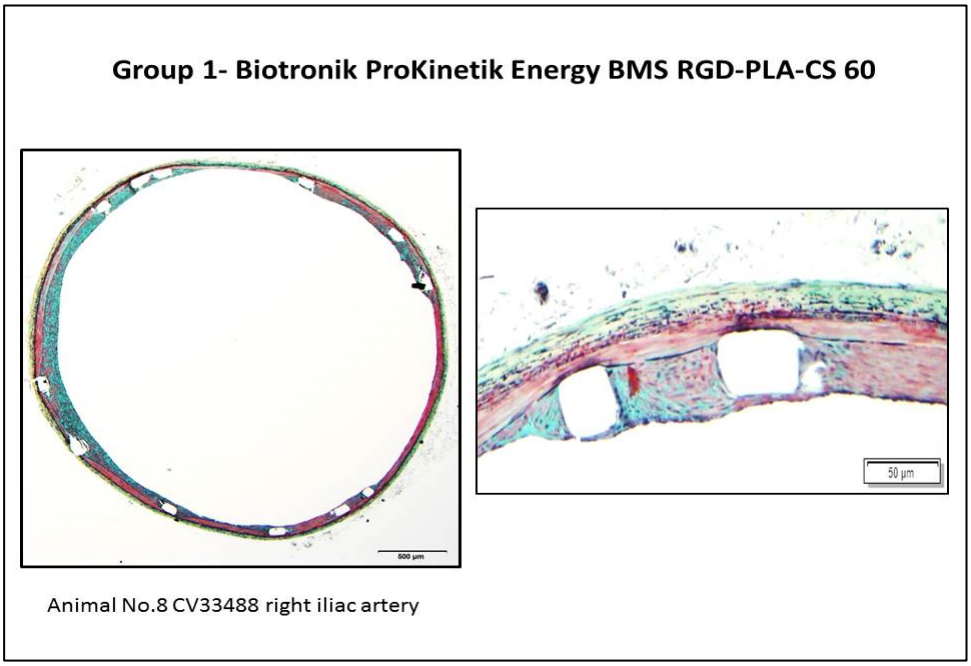


Figure 4.40. Low (2x) and high (20x) power representative light microscopy image of Biotronik PRO-Kinetic Energy BMS RGD PLA-CS60 (group 1) (Movat stains)

Group 2 – Biotronik PRO-Kinetic Energy BMS RGD-CS60

All stented lumens were widely patent, with stents widely expanded and struts well apposed to the vessel walls (figure 4.41). Neointimal growth was mild and composed of smooth muscle cells organising towards the luminal surface amid a proteoglycan matrix. Mean luminal stenosis was 8.34 ± 2.44%. Fibrin deposition was not observed on any of the struts. Histological sections did not show evidence of calcification. Mean injury was minimal (mean score= 0.13 ± 0.14) and was significantly less than in group 1 (RGD PLA-CS60 vs. RGD-CS60, p=0.03), while being similar to group 3 (RGD-CS60 vs. BMS, p=1.00). Neointimal inflammation was largely absent with sparsely observed strut-associated giant cells (mean= 15.07 ± 13.93%). Adventitial inflammation was absent. Luminal surfaces showed complete endothelialisation (mean= 100 ± 0 %). There was no luminal thrombus in any of the stented vessels. Non-stented proximal and distal segments were within normal limits.

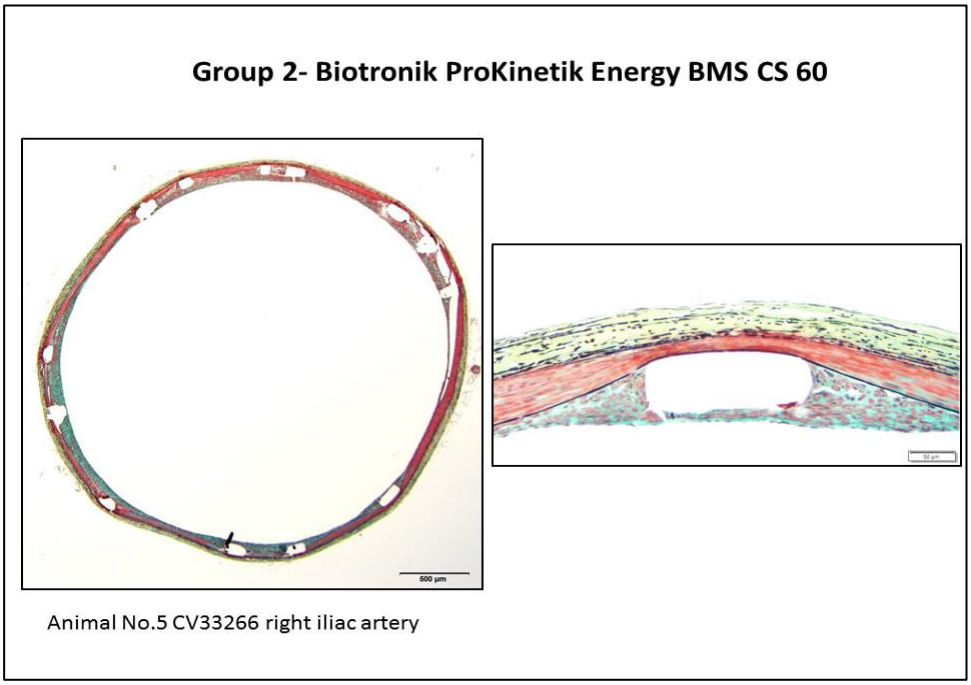


Figure 4.41. Low (2x) and high (20x) power representative light microscopy image of Biotronik PRO-Kinetic Energy BMS RGD-CS60 (group 2) (Movat stains)

Group 3 – Biotronik PRO-Kinetic Energy BMS

All stented lumens were widely patent, with stents widely expanded and struts well apposed to the vessel walls (figure 4.42). Neointimal growth was mild and composed of smooth muscle cells organising towards the luminal surface amid a proteoglycan matrix. Mean luminal stenosis was 12.94 ± 7.64%. Fibrin deposition was not observed on any of the struts. Histological sections did not show evidence of calcification. Mean injury was low (mean score = 0.29 ± 0.47) and was significantly less than in group 1 (RGD PLA-CS60 vs. BMS, p=0.02), while being similar to group 2 (RGD-CS60 vs. BMS, p=1.00). Neointimal inflammation was largely absent with mild to moderate strut-associated giant cells (mean= 18.90 ± 9.86%) observed. Adventitial inflammation was absent. Luminal surfaces showed near complete endothelialisation (mean= 96.67 ± 4.92%). There was no luminal thrombus in any of the stented vessels. Non-stented proximal and distal segments were within normal limits.

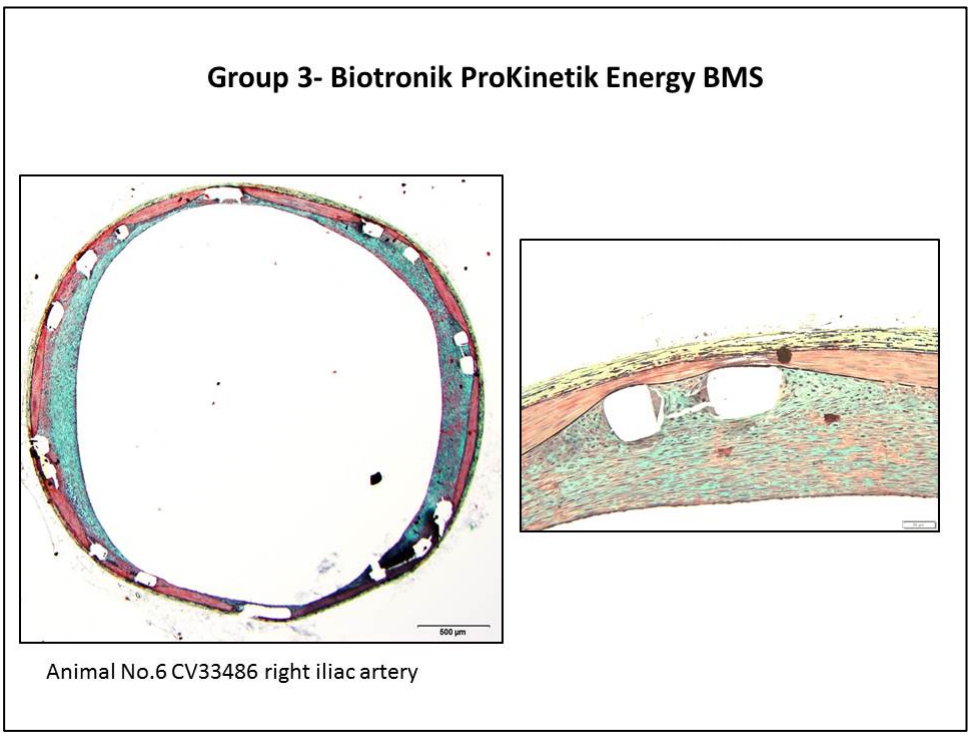


Figure 4.42. Low (2x) and high (20x) power representative light microscopy image of Biotronik PRO-Kinetic Energy BMS (group 3) (Movat stains)

4.4. Conclusions

A reproducible in vivo model using bilateral rabbit iliac stenting has been successfully developed to test both the acute thrombogenicity and more long-term biocompatibility of novel coronary stents. Antiplatelet treatment has been tailored to provide the appropriate level of activity for the duration of each model.

4.4.1. Learning points

Several learning points were identified during the development of both the acute and recovery models.

Firstly, rabbit platelets are very sensitive to activation and desensitisation. The use of a minimum 22G cannula in the central ear artery of a sedated animal and collection of blood directly in to the testing tubes minimised platelet activation during the blood sampling process, as demonstrated by the Multiplate assay. The addition of apyrase between each centrifugation step (except the final one) and

the re-suspension of the washed platelet pellet to plasma as opposed to HBS minimised desensitisation due to released ADP during the platelet washing process, as demonstrated using flow cytometry.

Initially, the process of administering dual antiplatelet therapy pre- and post-operatively proved challenging. The use of a quill to give daily doses of crushed aspirin and clopidogrel orally in water was mostly rejected by the animals. The addition of crushed aspirin and clopidogrel to daily drinking water bottles led to a reduction in fluid intake, most likely due to the unpalatability of the mixture. Eventually, the fluid intake increased sufficiently after the addition of Ribena™ to the mixture.

It proved very difficult to intubate and maintain ventilation with an endotracheal tube despite adequate sedation. However, the animals tolerated spontaneous ventilation with a facemask very well.

The femoral and iliac arteries frequently responded to instrumentation with a degree of spasm that made arteriotomy and insertion of angioplasty equipment challenging. This was significantly reduced by drop-wise application of additional local anaesthesia over the vessels prior to any manipulation.

When incising the peritoneum, the animals benefitted from an increase in the flow of inhaled nitrous oxide via the facemask. This was evident by a cessation of the tachycardia response induced when performing this part of the procedure.

Finally, the signal from the perivascular flow probes was much improved by injecting ultrasound gel in the space between the iliac arteries and the flow probe sensors.

4.4.2. Study limitations

The acute ST model is known to have a high thrombosis rate due to a combination of deep vessel injury and flow reduction. In addition, no heparin was administered in the acute protocol. Although deep vessel injury does occur with

pre-dilation and stent implantation during PCI, use of flow reduction and the lack of heparin administration represents a divergence from normal clinical practice. However, as ST is a relatively rare event in clinical practice in the presence of normal coronary artery flow rates, a model like this with high thrombogenicity is required to evaluate the difference in ST rates between different therapies without needing to sacrifice large numbers of animals.

Another limitation was the inability to accurately size stents for implantation, as angiography was not available. However, any potential bias was limited by implanting both the test and control stents bilaterally in the same animal. Although one would expect both iliac arteries in the same animal to be of a similar size, the side of implantation was varied equally for each type of stent in both the acute and recovery model to limit any possible bias.

In the acute model, although the stented vessels were carefully flushed, explanted and emersion fixed, the OCT examination was carried out ex vivo and the level of stent associated thrombus is likely to have been less representative than if the OCT pull-back had been carried out in vivo, prior to termination of the animal. In addition, the assessment of the degree of ST in the OCT pull-backs was only semi-quantitative and partly subjective in nature.

Another potential limitation of the acute model was the calculation of platelet counts attached to the stents, which was made using the assumption that this has a linear relationship with the average immunofluorescence measured. Also, there was a potential under-estimation of immunofluorescence due to imaging through tissue and stent.

The main limitation of the recovery model was the inability to place stents completely accurately in the common iliac arteries. This was due to a more limited dissection that was needed to avoid potential complications during the recovery period. This could have been overcome if facilities for angiography were available. However, despite this limitation, the majority of stents were found to be located in the correct position in the common iliac arteries on explantation and recovery of stented vessels.

4.4.3. Acute model

Using the acute stent thrombosis model to compare the Xience DES and the Absorb BVS, there were numerically more episodes of cyclical flow variation with the BVS, but this did not reach statistical significance. There was no significant difference in the thrombus burden between the two types of stent, as assessed by ex vivo OCT. There was however significantly more platelet adhesion to the BVS as compared to the DES.

4.4.3.1. Iliac blood flow

CFV is due to platelet adhesion within the vessel and its frequency is reduced in the presence of antiplatelet medication (228, 229), but in the absence of concomitant heparin administration, CFV was seen with both types of stent in the two-hour period following downstream flow reduction. There were numerically more episodes of CFV and one instance of complete occlusion with the BVS group, but the difference between the two groups did not reach statistical significance.

4.4.3.2. Platelet adhesion

There was significantly more platelet adherence to the Absorb BVS compared to the Xience DES in the acute protocol experiments. This may partly be explained by the unfavourable peristrut rheology for acutely implanted BVS as compared to metallic stents. Metallic stents have been shown, using laser Doppler anemometry data, to induce laminar flow disruption close to their struts (230). Similarly, it has been shown using 3D angiographic reconstruction and computational fluid dynamic data that low shear stress regions and altered flow patterns exist in-between BVS struts (231). It is well known that there is a positive correlation between strut thickness and flow disturbance (159, 232) and that this impacts on clinical outcomes, including target vessel revascularisation rates (233). Given the thicker struts of the BVS (150μm) compared to the Xience DES (81μm), as well as the fact that the BVS struts typically protrude more in to the vessel lumen, as opposed to metallic stent struts which are generally embedded

in the vessel well, the resulting flow disturbance is likely to lead to low endothelial shear stress, which favours blood stagnation, thereby predisposing to ST (159). The potential clinical impact of this effect, along with clinical data related to the Absorb BVS is discussed in chapter 5.

4.4.4. Recovery model

Eight out of the ten animals survived the 28-day recovery period. All vessels appeared widely patent with no evidence of stent malapposition and no occlusive thrombus.

Analysis of morphometric data showed that both the monolayer and poly-layer RGD-peptidomimetic experimental stents and the control Biotronik PRO-Kinetik Energy BMS had similar vessel cross-sectional areas (EEL, IEL, lumen and medial area). Neointimal area was different between the groups: RGD PLA-CS60 coated stents showed the highest neointimal area (1.08 ± 0.57 mm^2), while RGD-CS60 coated stents showed the least neointimal area (0.51 ± 0.14 mm^2). Uncoated BMS showed a neointimal area of 0.72 ± 0.34 mm^2. There was a significant difference in neointimal area observed between RGD PLA-CS60 coated stents when compared to RGD-CS60 coated stents (p=0.05). No difference was observed when RGD PLA-CS60 or RGD-CS60 coated stents were compared to BMS.

Neointimal thickness was also significantly greater in the experimental RGD PLA-CS60 coated stents (mean neointimal thickness = 0.08 ± 0.06 mm) compared to RGD-CS60 coated stents (mean neointimal thickness = 0.02 ± 0.01mm) (p = 0.02). No difference was observed when treatment groups were compared to control BMS (mean neointimal thickness = 0.04 ± 0.03 mm).

Mean injury was significantly higher in the RGD PLA-CS60 group (mean score = 1.03 ± 0.90) as compared to groups 2 and 3 (RGD PLA-CS60 vs. BMS, p = 0.02; RGD PLA-CS60 vs. RGD-CS60, p = 0.03) showing frequent eccentric medial destruction with associated red blood cell accumulation in group 1. This finding

may have contributed to higher neointimal growth in RGD PLA-CS60 coated stents as compared to the other groups evaluated.

Neointimal and adventitial inflammation were absent in all groups. Strut-associated giant cells were seen to a similar extent in all groups. Fibrin deposition was absent in all stented arterial segments, suggesting low levels of persistent thrombus on the stented vessels. Red blood cells and calcification were both seen to a greater extent in RGD PLA-CS60 coated stents, but this only reached statistical significance when comparing red blood cells between groups 1 and 3. Endothelialisation of luminal surfaces by light microscopy appeared nearly complete in all stented segments without significant differences between the groups. It is important to point out in this regard that platelets are believed to play an important role in both thrombus formation and the vessel wall response to injury and may contribute to atherogenesis and subsequent restenosis (234).

In conclusion, all 3 stent types, including the BMS, showed good evidence of healing and endothelialisation with no significant neointimal or adventitial inflammation and no stent thrombosis at 28-day follow-up. Although neointimal area and thickness, as well as mean injury scores, red blood cells and calcification, were seen to a greater extent in the poly-layer RGD peptide stents, it is difficult to draw any definitive conclusions as there were only 2 stents in this group. Although the initial plan had been to implant 5 of each of the bio-engineered stents along with contralateral BMS implants, the final number was reduced due to technical difficulty with implanting two of the stents, and 2 out of the 10 animals did not survive the recovery period.

Future work to assess re-endothelialisation of similar types of stents (as discussed in chapter 5) should be assessed at earlier time points when a difference between stent groups may be observed.

Chapter 5: Conclusions and Discussion

5.1. Principle aims of thesis

This thesis set out to better understand the underlying mechanisms and risk factors for coronary stent thrombosis (ST) with the overall aim of developing strategies to reduce its risk.

To this end, the specific aims were to study in depth, patients with definite ST and to compare these findings with a control group who had undergone percutaneous coronary intervention (PCI) without developing ST. In these patients, I performed a detailed analysis, including recording demographics, clinical details and optical coherence tomography (OCT) imaging to try and learn more about why ST patients are different, in order to be able to try and predict their clinical event. I also assessed platelet function and thrombin generation potential of patients with ST and controls.

To understand the basic mechanisms behind ST, I developed an in vivo rabbit iliac model to assess the acute thrombogenicity of the first in a generation of fully bioabsorbable vascular scaffolds and to study the potential clinical use of novel bio-engineered coronary stents, which aim to improve biocompatibility and thereby reduce the ongoing risk of ST in patients with permanent metallic stents.

5.2. Research and clinical skills learned

During my period of research, I learned numerous research and clinical skills, including the following:

1. I successfully applied for both ethical and research and development (R&D) approval for both the clinical and animal studies.

2. I managed the £600,000 Seventh Framework Programme (FP7) European Commission grant for the UK arm of the PRESTIGE study and negotiated the purchase of research equipment and consumables throughout the study.

3. I gained additional funding from the National Institute for Health Research (NIHR) Cardiovascular Biomedical Research Unit (BRU) small grants scheme for the optical coherence tomography (OCT) arm of the PRESTIGE study (£12,500), as well as funding for the perivascular flow probes and flow console used in the Doppler studies for the in vivo work (£20,000).

4. I set up a multi-centre clinical study, which included writing the study protocols, case report forms (CRFs), standard operating procedures (SOPs), patient information leaflets (PILs), consent forms, patient invite letters and GP letters. Having applied for and gained adoption as a NIHR Clinical Research Network (CRN) Portfolio study, I set-up and co-ordinated an additional 11 UK cardiac centres in close liaison with our European PRESTIGE study partners.

5. I also learned a number of laboratory skills and set up the protocols and SOPs for the in vitro and in vivo studies.

6. I learned how to perform and interpret OCT intracoronary imaging to an optimal standard, as well learning fine surgical and angioplasty skills during the animal studies.

7. I presented my work at a number of local, national and international meetings and have published some of my data, with more papers in progress, as summarised in the appendix.

5.3. Summary of findings

5.3.1. The PRESTIGE clinical study

Despite being a relatively rare complication of PCI, 138 patients with definite ST were recruited to the PRESTIGE study in the UK over 29 months, using a multi-centre approach driven by my engagement with the study centres. In addition, 353 control patients were recruited. As described in chapter 2, the ST patients were younger, had a higher incidence of diabetes mellitus, more thromboembolic antecedents (e.g. deep vein thrombosis, pulmonary embolism and transient ischaemic attacks), a higher rate of non-cardiac surgery in the preceding 3 months, were more likely to have received first generation drug eluting stents (DES), be prescribed less aspirin at the time of presentation and had less circumflex artery stents. In addition, although not statistically significant in the multivariate analysis, there were numerically more smokers, patients with previous myocardial infarction (MI), previous stroke, malignancy and more patients with bleeding episodes in the preceding month in the ST group compared with controls. There were numerically more patients in the ST group with calcified, tortuous stented vessels, smaller diameter stents, a higher rate of bifurcation stenting with a two-stent strategy and more vessels with residual distal coronary stenoses. There were also relatively fewer second or third generation DES in the ST group.

Although no definitive conclusions can be made from the UK OCT data, due to relatively small numbers, a potential dominant mechanism for ST was found in over 80% of those that did undergo OCT at the time of presentation with ST. For the early ST time points (within 30 days), stent under-expansion and malapposition were the most frequent dominant factors found on OCT. For the later ST time points (greater than 1 year), uncovered stent struts and neoatherosclerosis were the most frequent dominant factors. Other significant factors identified on OCT were severe in stent restenosis, severe under-expansion, residual proximal and distal disease, bifurcation stenting, overlapping stents and extra-stent cavities.

The complete European PRESTIGE OCT results have been submitted for publication to the European Heart Journal (*Optical Coherence Tomography Findings in Patients with Coronary Stent Thrombosis*). To summarise, a total of 231 patients presenting with ST underwent OCT imaging; 14 (6.1%) had image quality precluding further analysis. Of the remaining patients, 62 (28.6%) and 155 (71.4%) presented with early and late/very late ST respectively. The underlying stent type was DES in two thirds of patients. Mean reference vessel diameter was 2.9±0.6 mm and mean reference vessel area was 6.8±2.6 mm^2. Stent under-expansion (stent expansion index <0.8) was observed in 44.4% of patients. The number of lesions with any frame showing uncovered or malapposed struts decreased according to the time of presentation (acute, subacute, late and very late ST: 100%, 89.1%, 76.2%, 54.1%, p<0.001; and 86.7%, 76.1%, 61.9%, 37.6%, p<0.001, respectively). The most commonly adjudicated dominant findings according to presentation were: acute, uncovered struts (66.7%); subacute, uncovered struts (61.7%) and underexpansion (25.5%); late, uncovered struts (33.3%) and severe restenosis (19.1%); and very late, neoatherosclerosis (31.3%) and uncovered struts (20.1%).

5.3.2. Platelet function testing and thrombin generation studies

In both the ST patients and control group, the rate of “high-on-treatment platelet reactivity” (HPR) on clopidogrel was marked (up to 33%) in contrast to those on the more potent $P2Y_{12}$ receptor antagonists, prasugrel and ticagrelor, which was very low. The HPR rate on aspirin was close to 10% in both the ST group and controls.

Thrombin generation studies were undertaken in those patients recruited at the University Hospitals of Leicester NHS Trust (UHL) site. Although there was no difference found between the ST cases and controls, this study may have been under-powered to show a difference.

5.3.3. In vivo assessment of novel coronary stents

Both the acute ST and recovery rabbit iliac protocols proved to be valuable reproducible models to assess the acute and more long term biocompatibility of intracoronary stents. The Absorb bioabsorbable vascular scaffold (BVS) appeared to be more thrombogenic than the Xience DES acutely. There was a numerically higher number of labelled platelets attached to the Absorb BVS compared to the Xience DES in all 6 animals studied and overall this difference was statistically significant. Although the frequency of cyclical flow variation (CFV) in the Absorb BVS limb was numerically higher than the Xience DES, this was not statistically significant. There was no difference in OCT-determined thrombus burden between stent types.

In the recovery model, both the bio-engineered RGD peptide coated stents and the comparator bare metal stents (BMS) showed good evidence of healing and endothelialisation with little neointimal or adventitial inflammation and no ST at 28-days follow-up. Although neointimal area and thickness, as well as mean injury scores, red blood cells and calcification, were seen to a greater extent in the poly-layer RGD peptide stents, it was difficult to draw any definitive conclusions as there were only 2 stents in this group.

5.4. Study limitations

The PRESTIGE clinical study was limited by the matching process, which led to fewer than the planned 5 controls for the majority of ST cases. It would have been preferable to match a fewer number of controls to each ST case, in order to increase the feasibility of a more complete control group. In addition, the PFTs (especially at the earlier time points), thrombin generation and OCT datasets were incomplete, partly due to the unavailability of dedicated research team members when patients presented out of hours and also due to the lack of PFT and OCT equipment at some satellite sites. This should have been taken in to consideration when setting up the study.

The main limitation of the in vivo rabbit iliac model, was the lack of angiography facilities at the animal clinical research facility. This would have enabled more accurate placement of stents in both the acute and recovery models and allowed in vivo OCT assessment of intra-stent thrombus formation. A similar model used by our PRESTIGE study co-investigators in Munich used an arterial sheath inserted in to the carotid artery, which enabled stents to be advanced more easily and accurately using angiographic guidance. Using a sheath in the femoral artery during my pilot studies proved difficult and caused unacceptable trauma.

5.5. Thesis summary and future considerations

As demonstrated by the number of patients with definite ST recruited to the PRESTIGE study in the UK, ST continues to be an important complication of PCI. According to British Cardiovascular Intervention Society (BCIS) figures for the year 2013-2014 (the period over which the majority of the PRESTIGE ST cases were recruited in the UK), the cumulative incidence of ST over the study period was just under 1% (table 5.1). This is likely to be an underestimate, as some of the ST cases presenting to these centres may not have been captured. In addition, given the high mortality rate associated with ST, some patients may have died in the community or acutely on admission to hospital and these patients were not included in the study.

Table 5.1 Estimated cumulative incidence of stent thrombosis at individual UK sites in the PRESTIGE study

Cardiac centre	Approximate annual PCI numbers (2013 BCIS figures)	PRESTIGE ST recruitment period (months)	Approximate number of PCIs during recruitment period	Number of ST cases recruited	Cumulative incidence of ST over study period
Leicester	1400	24	2800	30	1.1%
Glasgow	2500	18	3750	5	0.1%
Wolverhampton	1300	17	1850	27	1.5%
Kettering	1100	17	1550	16	1.0%
Southampton	600	16	800	15	1.9%
King's	1300	15	1600	5	0.3%
Derby	800	13	850	1	0.1%
Plymouth	700	13	750	9	1.2%
Newcastle	2700	13	2900	23	0.8%
Worcester	900	9	650	3	0.5%
Frimley	1000	7	600	3	0.5%
East Sussex	500	5	200	1	0.5%
TOTAL	**14800**	**167**	**18300**	**138**	**0.8%**

PCI has come a long way and the issues and their resolution are summarised schematically in figure 5.1 below.

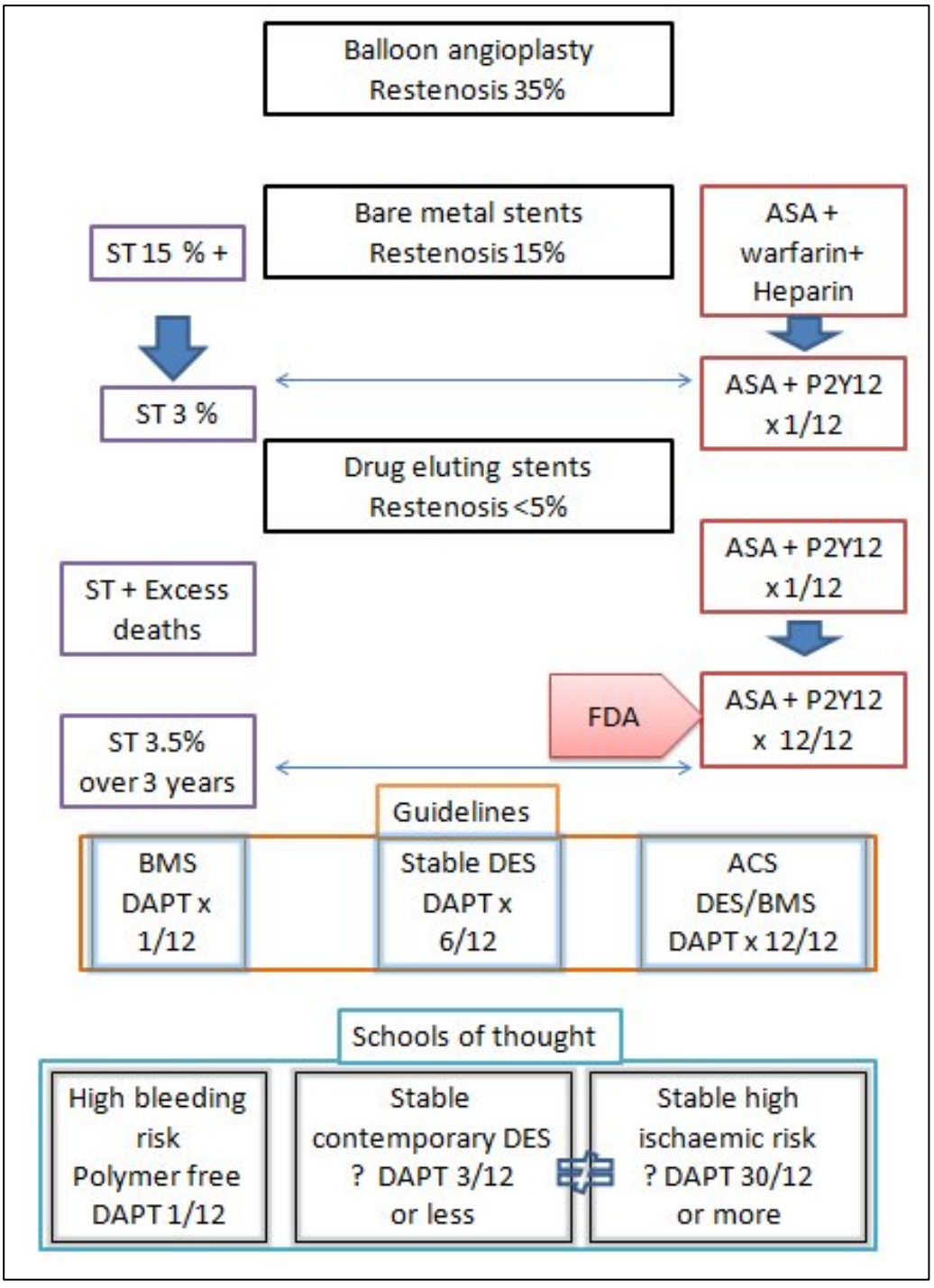


Figure 5.1 The journey of percutaneous coronary intervention and the impact of stent thrombosis; ST, stent thrombosis; BMS, bare metal stent; DES, drug eluting stent; DAPT, dual antiplatelet therapy; ACS, acute coronary syndrome; ASA, aspirin; FDA, Food and Drug Administration

We are now at a time when PCI has become the treatment of choice in most patients presenting with ischaemic heart disease. The main risk attributable to bare-metal stenting was restenosis requiring repeat revascularisation. The introduction of DES has reduced the occurrence of clinical restenosis to <5%. For both types of stents, ST is a serious clinical adverse event after PCI, resulting in abrupt vessel closure with risk of MI and death. Although early ST and late ST occur with similar frequency after BMS or DES and outnumber very late ST by far, very late ST has emerged as a distinct clinical entity more germane to (at least the first-generation) DES than BMS. Decisions regarding percutaneous treatment of obstructive coronary disease have become increasingly challenging for patients and physicians since the observation of delayed ST in DES. Although there is no absolute marker of risk for ST or risk score, several predictors may help identify patients at risk and among those, premature discontinuation of DAPT and poor stenting technique (i.e. stent under-sizing and mal-apposition) require the most attention. Despite multiple studies investigating both shortened and extended duration of DAPT, as summarised in figures 5.2 and 5.3 below, the optimal duration of DAPT remains largely unclear. Current guidelines recommend DAPT for 1 month after BMS and for 6 months after DES implantation in stable patients or in the context of ACS for 1 year (99).

A recent meta-analysis of randomised trials, comparing more than one year of DAPT with aspirin alone in high-risk patients with a history of prior MI, showed a reduction in ischaemic events, including cardiovascular death, recurrent MI and stroke, with prolonged DAPT, but with an increase in major bleeding, although not fatal bleeding or non-cardiovascular death (235).

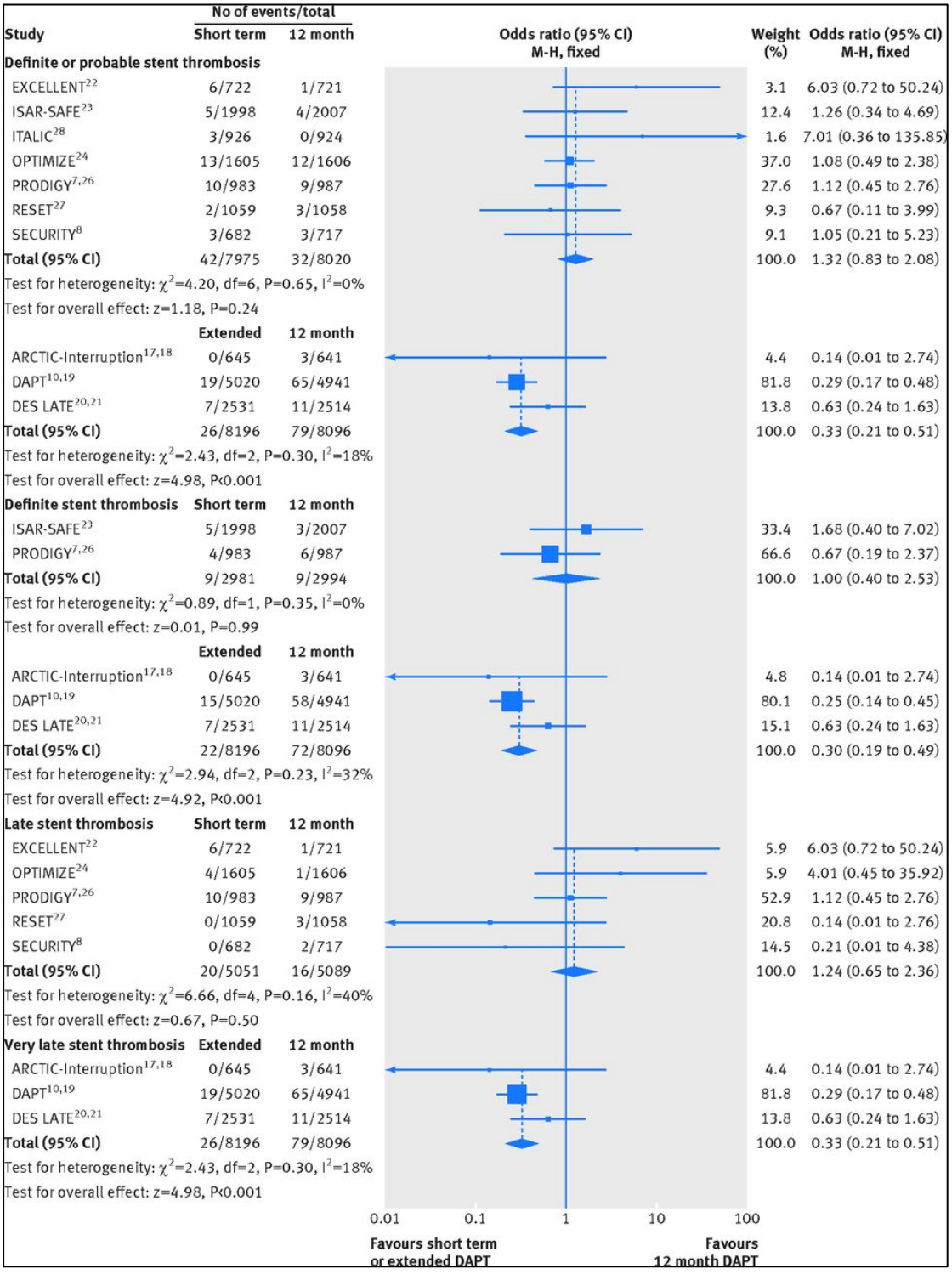


Figure 5.2 Impact of short and long term DAPT duration on stent thrombosis, with benefit of extended duration DAPT for very late stent thrombosis; reproduced from Navarese et al (236).

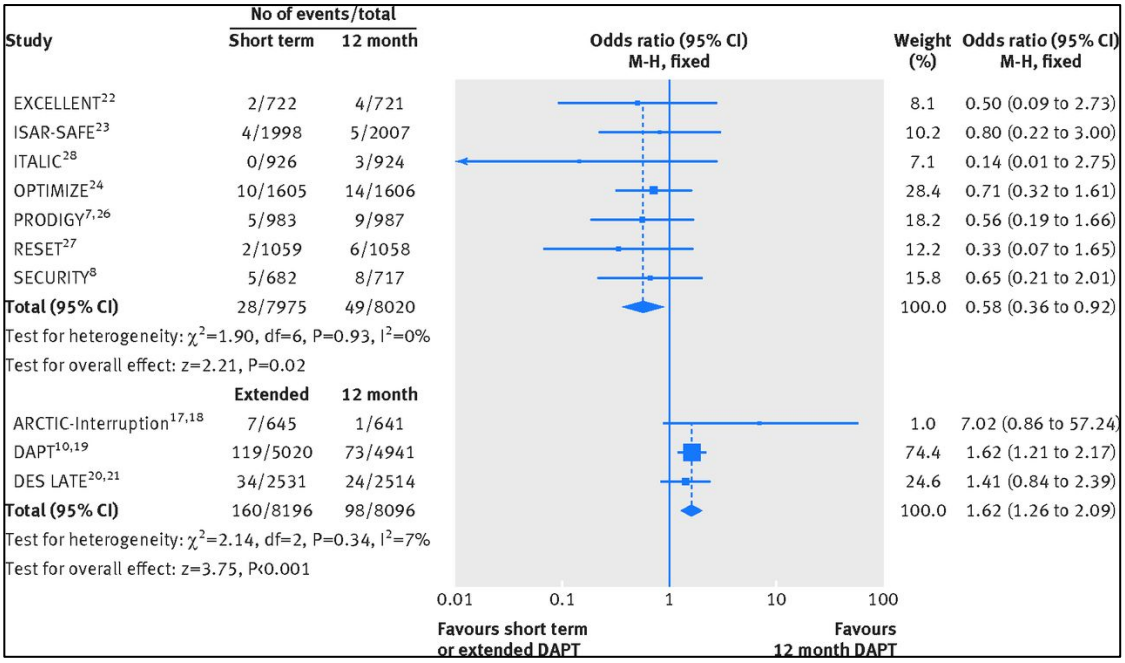


Figure 5.3 Excess major bleeding seen with DAPT duration beyond 12 months, while shorter duration of DAPT results in lower risk of bleeding; reproduced from Navarese et al (236).

Further randomised controlled trials are required to help establish the optimal duration and the benefit: risk ratio, as DAPT may be associated with increased risk of bleeding, especially with newer more potent $P2Y_{12}$ antagonists, and may need to be designed to allow for tailoring of DAPT to different patients with different ischaemic/bleeding risk profiles. Some of the ongoing and forthcoming studies investigating DAPT duration are summarised in table 5.2.

Table 5.2 Ongoing and forthcoming studies related to the duration of dual antiplatelet therapy following PCI; source: clinicaltrials.gov as on 23rd December 2015

Study Name	Trial Number	Study population	Duration of DAPT comparison	Primary outcome measure
ShorT and OPtimal Duration of Dual AntiPlatelet Therapy-2 Study (STOPDAPT-2)	NCT02619760	Patients undergoing PCI with CoCr-EES stents.	1 month DAPT followed by Clopidogrel monotherapy vs. 12 month DAPT followed by Aspirin monotherapy	Composite of CV death, MI, stent thrombosis, CVA and TIMI major or minor bleeding. 12 months for non-inferiority, 5 years for superiority trial.
Optimal Duration of Dual Antiplatelet Therapy After Drug-eluting Stent Implantation (OPTIDUAL)	NCT00822536	Patients who have completed 12 months of DAPT following PCI with DES.	48 months of DAPT vs. 12 months DAPT following index PCI procedure.	Composite of death, non-fatal MI, non-fatal stroke and major bleeding. 3 year follow-up. Stent thrombosis as secondary endpoint.
Safety of 6-month Duration of Dual Antiplatelet Therapy After Acute Coronary Syndromes (SMART-DATE)	NCT01701453	Patients presenting with Acute coronary syndrome.	6 months of DAPT vs 12 months of DAPT using Clopidgrel and Aspirin	Composite of death, spontaneous MI, stent thrombosis, CVA and Type 3-5 BARC bleeding over 6-18-month follow-up
CYPRESS - CYPHER for Evaluating Sustained Safety	NCT00954707	Patients undergoing intervention with PCI using CYPHER stent.	Randomisation following 12 months of DAPT free from death, MI, stroke, repeat coronary revascularization, major bleeding, and ST, to receive either continued Aspirin/Clopidogrel for an additional 18 months, or Aspirin/Placebo.	MACCE (Death, myocardial infarction, emergent bypass surgery, or target lesion revascularization), Stent Thrombosis and bleeding.
Efficacy and Safety of New Generation Drug Eluting Stents Associated With an Ultra Short Duration of Dual Antiplatelet Therapy. Design of the	NCT02099617	patients ≥75 years old, suffering from stable angina, silent ischemia (1 month DAPT) or acute coronary syndromes (6	Randomisation to SYNERGY II stent or BMS, both groups receiving either 1 month DAPT (stable angina or silent ischaemia) or 6	Composite of all-cause death, non-fatal myocardial infarction, non-fatal stroke, ischemia-driven target lesion

Short Duration of Dual antiplatElet Therapy With SyNergy II Stent in Patients Older Than 75 Years Undergoing Percutaneous Coronary Revascularization. (SENIOR)		months DAPT) related to significant coronary artery disease and requiring percutaneous coronary intervention. Use of SYNERGY II stent.	months DAPT (acute coronary syndromes).	revascularization at 12 months.
HOST-IDEA (Harmonized Optimal Strategy to Treat Coronary Artery Disease - Coronary Intervention With Next Generation Drug-Eluting Stent Platforms and Abbreviated Dual Antiplatelet Therapy) Trial	NCT02601157	Patients with stable angina undergoing PCI with DES.	2x2 factorial design RCT with patients randomised to receive ORSIRO stent (biodegradable polymer, sirolimus-eluting stent) or COROFLEX ISAR (polymer-free sirolimus-eluting stent); and also randomised to receive either 3 months or 1 year DAPT.	1-year target lesion failure (TLF) as a composite of cardiac death, target vessel related myocardial infarction and clinically driven target lesion revascularization.
Short-term Dual Anti Platelet Therapy in Patients With ACS Treated With the COMBO Dual-therapy Stent (REDUCE)	NCT02118870	Patients presenting with ACS (NSTEMI, STEMI or UA) undergoing PCI with COMBO stent (sirolimus eluting stent with anti-CD34 antibody coating to capture endothelial progenitor cells).	Randomised to receive either 90 days or 1 year DAPT following PCI, with Prasugrel or Ticagrelor strongly recommended over Clopidogrel as $P2Y_{12}$ inhibitor.	Composite of all-cause mortality, Myocardial Infarction (MI), ST, stroke, bleeding at 12 months
A Randomized Controlled Comparison Between One Versus More Than Six Months of Dual Antiplatelet Therapy After Biolimus A9-eluting Stent Implantation	NCT02513810	Patients undergoing PCI for stable angina, ACS excluded.	Randomised to receive Biofreedom stent (polymer-free Biolimus A9 eluting stent) with 1 month DAPT vs. PCI with Biomatrix Flex (Biodegradable polymer with Biolimus A9 elution) with 6 -12 months DAPT.	Composite of cardiac death, nonfatal myocardial infarction, target vessel revascularization, major bleeding and cerebrovascular accident at 1 year.
Nobori Dual Antiplatelet Therapy as Appropriate Duration	NCT01514227	Patients undergoing PCI using Nobori Biolimus A9 eluting stent platform.	Randomised to receive either 6 or 18 months of DAPT following PCI.	NACCE include all-cause death, non-fatal myocardial infarction, cerebrovascular

				events, and major bleeding at 18 months.
Randomized Trial of COBRA PzF Stenting to Reduce Duration of Triple Therapy (COBRA-REDUCE)	NCT02594501	Patients presenting with ischaemic symptoms, evidence of coronary disease. All patients should also be on Coumadin or NOAC.	Randomised to receive DAPT for 14 days and PCI with COBRA stent (polyzene-F coating to encourage thromboresistance and enhance tissue healing), or PCI with standard DES with 6 months of DAPT. Patient to continue VKA/NOAC during the study.	BARC class >=2 bleeding after hospital discharge at 6 months. MACE (death, myocardial infarction, definite stent thrombosis, ischemia-driven target lesion revascularization or ischemic stroke at 12 months) and stent thrombosis as secondary endpoints.
Twelve vs 24 Months of Dual Antiplatelet Therapy in Patients With Coronary Revascularization for In-stent Restenosis	NCT02402491	Patients undergoing percutaneous intervention with stent deployment for treatment of in-stent restenosis	Randomised to receive either 12 months or 24 months of DAPT following treatment of ISR with DES.	Incidence of a composite end point including all cause deaths, myocardial infarction, the incidence of Academic Research Consortium defined definite or probable stent thrombosis and stroke (MACCE) at 24 months
GLOBAL LEADERS: A Clinical Study Comparing Two Forms of Anti-platelet Therapy After Stent Implantation	NCT01813435	All-comer patients undergoing PCI for stable angina, NSTEMI or STEMI.	Randomised to receive either Aspirin & Ticagrelor for 1 month followed by Ticagrelor monotherapy for 23 months, or standard treatment of Aspirin & $P2Y_{12}$ inhibitor (either Clopidogrel or Ticagrelor) for 12 months followed by Aspirin Monotherapy. All patients to receive Biomatrix stents (biodegradable polymer, Biolimus A9 eluting stent) and Bivalirudin for	The composite of all-cause mortality or non-fatal new Q-wave MI up to 2 years post randomisation. Definite Stent thrombosis at 2 years according to ARC definition measured as a secondary endpoint.

			procedural anticoagulation.	

Outcome in patients who suffer ST is poor, with high mortality figures. Therefore, treatment of ST should be directed to achieving rapid reperfusion, preferably by primary PCI. New antiplatelet agents and new-generation DES are available and initial results appear that we may be at optimal performance, but these have to be further studied in randomised controlled trials with adequate power and sufficient length of follow-up, until final conclusions can be drawn. The ideal would be extremely short duration of DAPT (one month maximum) with low restenosis rates (<5%). Research efforts should continue. Reconciling the desire from most cardiologists to shorten the DAPT period in DES with the trials that suggest prolonged DAPT in those at high ischaemic and low bleeding risk (up to 30 months) suggest that further trials are needed and that DAPT duration may need to be individually tailored.

Whilst mechanistic challenges of permanent metallic stents remain, including delayed healing, in stent restenosis, late acquired mal-apposition and neoatherosclerosis, all of which predispose to the ongoing risk of ST, the potential long-term benefits of a fully bioresorbable scaffold remain very attractive. However, the first generation BVS are significantly bulkier than the 3rd generation metallic DES, predominantly due to the need for thicker polymeric struts to provide comparable mechanical properties. In addition, the implantation of these devices appears to have a learning curve and the use of intracoronary imaging is important for appropriate sizing and to reduce the risk of under-expansion, mal-apposition and geographical miss. Some of these issues may explain the disappointing results recently presented for the Absorb BVS in the ABSORB-II and ABSORB-III trials as described in chapter 1.

These devices should only be used in carefully selected patients and a general consensus amongst UK interventional cardiologists is summarised below (as discussed at the OCT network programme: OCT in stent failure and complex PCI meeting, London, March 2017).

Favourable

- Young patients (<65) at risk of future interventions
- Simple type A lesions
- Proximal/mid LAD
- Multi-vessel disease
- Diabetes mellitus

Less favourable

- STEMI
- Bifurcations, jailing large side branches
- SVG lesions (due to size)
- Long diffuse disease, with potential need for overlapping devices
- Ostial lesions
- Calcification
- Tortuosity
- In-stent restenosis
- Chronic total occlusions

Not favourable

- Diameter <2.5mm and >4mm
- True bifurcations with 2-stent strategy needed
- Heavy calcification
- Requirement of DAPT <1 year
- Left main stem lesions

With all the lessons learned over the years, implantation of these devices should be optimised using the PSP algorithm (prepare, size and post-dilate), adapted from the Abbott Vascular absorb.com website.

Prepare the vessel

- Pre-dilate using a 1:1 balloon-to-artery ratio using a non-compliant balloon
- Confirm full expansion in two orthogonal angiographic views
- Use plaque-modification devices, including rotational atherectomy or cutting balloons for calcified lesions

Size the vessel

- Use adjunctive techniques to size the vessel (QCA, IVUS, OCT)
- Only use BVS for vessel diameters between 2.5mm and 3.75mm

Post-dilate

- Use non-compliant balloon and inflate to high pressure
- Use balloon size up to 0.5mm above nominal scaffold diameter
- Ensure <10% final residual stenosis and full strut apposition

In light of the recent concerns over elevated rates of major adverse cardiac events, specifically myocardial infarction and scaffold thrombosis, as of 31st May 2017, these devices will only be available for implantation in the setting of clinical registries at selected sites. The situation will be reviewed again in the summer of 2018.

Ongoing and future studies will need to investigate the clinical impact of more stringent intravascular imaging in sizing and optimising the scaffold implantation. In addition, the benefit and need for prolonged DAPT after BVS implantation may be necessary. It is likely that future iterations will be needed before widespread adoption of bioabsorbable stent technology can be recommended. It is however important to concentrate on the fact that this is breakthrough technology and it may be that we only see the true benefits of these devices once the bio-absorption process is actually complete.

5.6. My learning points

Despite major advances in stent technology and antiplatelet/antithrombotic therapy, coronary ST continues to be a significant risk of PCI and is associated

with poor clinical outcomes. Patients can be risk stratified according to baseline clinical characteristics and coronary anatomy pre-PCI, as well as angiographic results post PCI. Based on some of results of the PRESTIGE study, I led the development of a risk scoring system to predict ST, which has recently been published (237). These risks should certainly be taken in to consideration when planning interventional strategies in patients with coronary artery disease. Coronary stents should be carefully sized and optimally deployed with adequate post dilatation to avoid mal-apposition. Intracoronary imaging should be used when necessary to assess coronary lesions and to optimise results of stenting, especially in high risk coronary anatomy. OCT should certainly be used in patients presenting with ST and in stent restenosis, in order to understand and potentially correct the underlying mechanisms contributing to stent failure.

Given the high incidence of HPR with clopidogrel, the more potent $P2Y_{12}$ receptor antagonist should be used in the majority of patients with ACS and should also be considered in stable patients undergoing PCI, who are at particularly high risk of ST. During my research period in Leicester, I performed PFTs in patients with stable angina undergoing high risk PCI (including left main stem lesions), in whom the development of ST would be particularly detrimental. Those with HPR on clopidogrel would be switched to the more potent agent, ticagrelor. Although this strategy is currently unproven, most of the PFT studies done thus far have predominantly included patients who have a relatively low risk of ST.

Although current generation DES, including those with absorbable polymers and polymer-free DES, are significantly more biocompatible than previous devices, the permanent presence of a metallic implant within the coronary artery still poses an apparently lifelong risk of ST, especially given the increasingly recognised process of neoatherosclerosis. Therefore, the concept of a fully bioabsorbable intracoronary scaffold is particularly attractive. However, given the issues encountered with the first generation of these devices, future iterations will need to have thinner struts with a significantly shorter bioabsorption period.

Appendix I: Case Report Forms

CRF: Stent Thrombosis Case (1/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

PRESTIGE
STENT THROMBOSIS CASE CRF

All PRESTIGE patient data needs to be entered in the PRESTIGE electronic CRF (eCRF) according to the Data Forms Instructions manual.

- For sites who have access to the eCRF: this paper CRF can be used as a worksheet before entering the data in the eCRF (not obligatory).
- For satellite sites without access to the eCRF: this paper CRF must be used, signed off and forwarded to the site with access to the CRF.

All data entered on this paper CRF or the electronic CRF must be verifiable from a source document. Source documents are those on which patient information is first recorded and includes hospital and laboratory records, consultation files and worksheets (when used to initially record data). To be considered a source document, it must be signed and dated by the individual completing it. Each page of a source document should uniquely identify the patient. Please note, this CRF is **NOT** a source document.

Instructions for completion of the paper CRF:

- Use black ink.
- Do not leave blanks (except when data cannot be obtained).
- Record Site Number (satellite sites need to enter the Site Number of the site with access to the eCRF) and Patient Number on top of every page.
- Complete 'data filled out by' and 'date' in the footer of every page.
- Record 'Date' always as 'dd/mmm/yyyy' (e.g. 05/Jan/2012).
- Record 'Time' always as 'HH:MM-24h clock' (from 00:00 to 23:59, midnight is 00:00, last entry on a given day is 23:59).
- Corrections: Do not use correction fluid. Make corrections on the CRF pages as follows: Draw a single line through the error or incorrect data. Write the correct data above or beside the error. Initial and date the correction.
- Use capital letters for free text fields.
- For decimal numbers, use points, do not use a comma (e.g. 3.5 and not 3,5).

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

STENT THROMBOSIS CASE CRF

Site number: |_4_|_4_|_1_|_0_|_0_|

Satellite Site? ❍ No ❍ Yes: .....

Patient number: |___|___|___|___|___|

Patient initials: |___|___|___|

Date of birth: |___|___| |___|___|___| |___|___|___|___|
dd mmm yyyy

Gender: ❍ Male ❍ Female

Date of initial stent implantation: |___|___| |___|___|___| |___|___|___|___|
dd mmm yyyy

Type of initial stent: ☐ BMS ☐ DES

Date of stent thrombosis: |___|___| |___|___|___| |___|___|___|___|
dd mmm yyyy

Elapsed time since stent implantation:

❍ early (0-30 days post stent implantation)

❍ late (>30 days)

❍ very late (>12 months)

Data/samples collected, examinations performed:

Baseline characteristics	❍ yes	❍ no
Blood platelet testing	❍ yes	❍ no
DNA	❍ yes	❍ no
Thrombus aspiration	❍ yes	❍ no
OCT	❍ yes	❍ no
IVUS	❍ yes	❍ no
Triggering questionnaire	❍ yes	❍ no

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

STENT THROMBOSIS CASE

CRF1: In hospital data

1. Initial stent implantation procedure

1.1. Date and indication initial stent implantation	
Date stent implantation	|___|___| |___|___|___| |___|___|___|___| (dd/mmm/yyyy)
Indication	❍ STEMI ❍ NSTEMI ❍unstable angina ❍stable angina
1.2. Clinical characteristics at initial stent implantation time	
Body weight	..... kg
Smoking status	❍ active ❍ ex-smoker ❍ never smoked ❍ unknown
1.3. Medical history at initial stent implantation time	
Previous MI	□ no □ anterior □ inferior □ lateral □ posterior
Stroke	❍ yes ❍ no ❍ unknown
Heart failure (LVEF < 30%)	❍ yes ❍ no ❍ unknown
CABG	❍ yes ❍ no ❍ unknown
Renal insufficiency (serum creat>175µmol/l, GFR<30 ml/min)	❍ yes ❍ no ❍ unknown
Hemodialysis	❍ yes ❍ no ❍ unknown
Diabetes mellitus	❍ yes ❍ no ❍ unknown
AHT	❍ yes ❍ no ❍ unknown
PAD (AAA, PTA, amputation, carotid disease)	❍ yes ❍ no ❍ unknown
Malignancy	❍ active ❍ non-active ❍ none ❍ unknown
History of AF	❍ yes ❍ no ❍ unknown
Autoimmune disease	❍ yes ❍ no ❍ unknown
Thromboembolic antecedents	□ None □ CVA □ TIA □ DVT □ pulmonary embolism □ unknown

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (4/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

1.4. Procedural and angiographic characteristics at initial stent implantation time

Number of stents implanted in vessel that later suffered stent thrombosis ❍ 1 ❍ 2 ❍ 3 ❍ 4

Type	Name	Length (mm)	Diameter (mm)
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			

Overlapping stents? ❍ yes ❍ no ❍ not applicable

Number of stents in non-ST vessel ❍ 0 ❍ 1 ❍ 2 ❍ 3 ❍ 4

Type	Name	Length (mm)	Diameter (mm)
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			

ACC Lesion classification	❍ A ❍ B1 ❍ B2 ❍ C
Stented segment(s) (CASS site map)	|___| |___| |___| |___|
Lesion length	..... mm
Reference vessel diameter	..... mm
Calcification	❍ none ❍ mild ❍ severe
Tortuosity	❍ yes ❍ no
Visible thrombus	❍ yes ❍ no
PCI of a SVG	❍ yes ❍ no
PCI for ISR	❍ yes ❍ no
PCI for CTO	❍ yes ❍ no
PCI with rotablation	❍ yes ❍ no
Multivessel disease	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

If yes, left main involved	❍ yes ❍ no
Ostial lesion	❍ yes ❍ no
Stent post dilatation	❍ yes ❍ no
Highest pressure NCB	..... atm
IVUS/OCT guidance	❍ yes ❍ no
Number of diseased vessels	❍ 1 ❍ 2 ❍ 3
Bifurcation lesion	❍ yes ❍ no If yes, ☐ provisional ☐ T-stenting ☐ culotte ☐ crush ☐ other: .....
Post procedure TIMI flow	❍ 0 ❍ 1 ❍ 2 ❍ 3
Residual stenosis > 50% <u>proximal</u> from target lesion Residual stenosis > 50% <u>distal</u> from target lesion Residual dissection at the end of the procedure (angio)	❍ yes ❍ no ❍ yes ❍ no ❍ yes ❍ no
Presence of visible thrombus at the end of the procedure	❍ yes ❍ no
Restenotic lesion	❍ yes ❍ no
Use of GP IIb/IIIa	❍ yes ❍ no
Use of Bivalirudin	❍ yes ❍ no
Patient tested for clopidogrel resistance	❍ yes ❍ no
Pre loaded with DAPT If yes, which (generic name)?	❍ yes ❍ no .....

2. Actual information on this patient: stent thrombosis procedure

2.1 Date of stent thrombosis	
Date stent thrombosis	|___|___| |___|___|___| |___|___|___|___|(dd/mmm/yyyy)
2.2 Clinical characteristics	
ST presents as	❍ STEMI ❍ NSTEMI ❍unstable angina
Body weight	..... kg
Length	..... cm
Smoking status	❍ active ❍ never ❍ stopped >12 months ❍ stopped <12 months
2.3 Medical history	
Indicate changes in medical history since initial stent implantation	□ No changes □ MI □ Stroke □ CABG □ Hemodialysis □ Diabetes mellitus □ Malignancy □ History of AF □ Thromboembolic antecedents □ Renal insufficiency (serum creatinin>2 mg/dl, GFR<30 ml/min) □ Heart failure (LVEF < 30%)
Bleeding episode within 1 month prior to ST	❍ yes ❍ no
Non-cardiac surgery within previous 90 days	❍ yes ❍ no
2.4 Medical therapy	
Aspirin	❍ yes ❍ no
Clopidogrel	❍ yes ❍ no
Prasugrel	❍ yes ❍ no
Ticagrelor	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (7/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

Patient on DAPT?	❍ yes ❍ no
if no, time since stop DAPT	..... Months □ not applicable
if no, time since stop aspirin	..... Months □ not applicable
if no, time since stop clopidogrel	..... Months □ not applicable
if no, time since stop prasugrel	..... Months □ not applicable
if no, time since stop ticagrelor	..... Months □ not applicable
Reason for stopping ASA	□ not applicable (did not stop ASA) □ side effects □ bleeding □ surgery/dental procedure □ financial □ medical advice □ other
Reason for stopping ADP blocker	□ not applicable (did not stop ADP blocker) □ side effects □ bleeding □ surgery/dental procedure □ financial □ medical advice □ other
Oral anticoagulant	□ No □ Warfarin □ Coumadin □ Dabigatran □ Rivaroxaban
ACE-I	❍ yes ❍ no
ARB	❍ yes ❍ no
Beta blocker	❍ yes ❍ no
Statin	❍ yes ❍ no
Diuretic	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

PPI	❍ yes ❍ no
Insulin	If yes, generic name: .....
Oral antidiabetics	❍ yes ❍ no
	❍ yes ❍ no
ST treated with	□ thrombolysis □ PCI □ medical □ CABG

2.5 ECG (this admission)

STEMI	□ anterior □ inferior □ lateral posterior □ no STEMI
NSTEMI	❍ yes ❍ no

2.6 Vital signs

Systolic/Diastolic blood pressure	..... / ..... mmHg
Pulse	..... bpm
CPR before procedure	❍ yes ❍ no
CPR at the beginning of procedure	❍ yes ❍ no
Hemodynamic shock at the beginning of procedure (defined as persistent hypotension (<90mm HG) with severe reduction in cardiac index)	❍ yes ❍ no

2.7 Biochemical values

Haemoglobin	..... ❍ g/dL, g% ❍ g/L ❍ mmol/L
White blood cell count	..... 10**9/l
Platelet Count	..... 10**9/l
International normalized ratio	.....
CRP	..... mg/l □ Below detection limit
Serum creatinine	..... µmol/l
Cholesterol	..... mmol/l
HDL	..... mmol/l
LDL	..... mmol/l
Max CK	..... ❍ U/L ❍ IU/L ❍ mIU/mL ❍ nkat/L ❍ ukat/L ❍ ng/mL

Data filled out by: _____ **Date:** ____/_____/_____

	❍ %URL ❍ ug/L
Max CK-MB	..... ❍ U/L ❍ IU/L ❍ ng/mL ❍ ug/L ❍ mU/mL ❍ mIU/mL ❍ ukat/L
Max Troponin	..... ❍ ug/L ❍ ng/L ❍ picogram/mL □ Below detection limit

2.8 Procedural and angiographic characteristics

Date procedure	|___|___| |___|___|___| |___|___|___|___|(dd/mmm/yyyy)
Time procedure	|___|___| |___|___| (hh:mm, 24H clock)
Time ECG at admission	|___|___| |___|___| (hh:mm, 24H clock)
Symptom to wire time	|___|___| |___|___| (hh:mm, 24H clock)
Door to wire time	|___|___| |___|___| (hh:mm, 24H clock)
TIMI flow at the beginning of the procedure	❍ 0 ❍ 1 ❍ 2 ❍ 3
Thrombus aspiration performed	❍ yes ❍ no
If yes, thrombus retrieved	❍ yes ❍ no
TIMI flow after thrombus aspiration	❍ 0 ❍ 1 ❍ 2 ❍ 3
TIMI flow at the end of the procedure	❍ 0 ❍ 1 ❍ 2 ❍ 3
No reflow or distal embolization at the end of procedure	❍ yes ❍ no
OCT performed after thrombus aspiration	❍ yes ❍ no
IVUS performed	❍ yes ❍ no
Specific comments intracoronary imaging	.....

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (10/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

Concomitant antiplatelet therapy:		
Aspirin: (re) loading	❍ yes ❍ no	If yes, dose ..... mg
Clopidogrel: (re) loading	❍ yes ❍ no	If yes, dose ..... mg
Prasugrel : (re) loading	❍ yes ❍ no	If yes, dose ..... mg
Ticagrelor: loading	❍ yes ❍ no	If yes, dose ..... mg
IIb/IIIa	❍ yes ❍ no	If yes, ☐ Abciximab ☐ Tirofiban ☐ Eptifibatide
Concomitant anticoagulant therapy:		
Heparin	❍ yes ❍ no	If yes, dose ..... U
Enoxaparin	❍ yes ❍ no	If yes, dose ..... mg
Fondaparinux	❍ yes ❍ no	If yes, dose ..... mg
Bivalirudin	❍ yes ❍ no	If yes, dose ..... mg
Balloon dilatation:	❍ yes ❍ no	
use of NC balloon	❍ yes ❍ no	
type	.....	
size	..... mm	
length	..... mm	
max pressure	..... atm	

Number of additional stents implanted during PCI for ST ❍ 0 ❍ 1 ❍ 2 ❍ more

Type	Name	Length (mm)	Diameter (mm)
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			

Was a final favorable angiographic result obtained?	❍ yes ❍ no
Final TIMI flow	❍ 0 ❍ 1 ❍ 2 ❍ 3
Was a final OCT examination post procedure performed?	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

2.9 Triggering mechanisms	
Date and time of onset of symptoms	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy) |___|___|:|___|___| (hh:mm, 24H clock) □ time unknown
Vigorous physical exercise during 1 hour prior to the ST	❍ yes ❍ no
Active infection	❍ yes ❍ no
Emotional stress:	
anger in the 2 hours preceding ST	❍ yes ❍ no
anxiety in the 2 hours preceding the ST	❍ yes ❍ no
life events during 1 week prior to ST	❍ yes ❍ no
other emotional stress	.....

Data filled out by: _____ **Date:** ____/_____/_____

3 Blood platelet function testing

3.1 Acute phase (first point of contact testing)

Blood sampling

Blood sampling performed?	❍ yes ❍ no
If yes, Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock)
If no, Reason	☐ Patient presented in the evening or at night
	☐ Patient received Reopro/Tirofiban/Bivalirudin
	☐ Other reason: ……
Patient on chronic therapy (>7 days)	
Aspirin?	❍ yes ❍ no
Clopidogrel?	❍ yes ❍ no
Prasugrel?	❍ yes ❍ no
Ticagrelor	❍ yes ❍ no
Additional loading dose in the last 7 days	
Aspirin?	❍ yes ❍ no
If yes, Dose	……mg
Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock)
Clopidogrel?	❍ yes ❍ no
If yes, Dose	……mg
Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock
Prasugrel?	❍ yes ❍ no
If yes, Dose	……mg
Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock
Ticagrelor?	❍ yes ❍ no
If yes, Dose	……mg
Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (13/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

VerifyNow P2Y12	
Test performed?	❍ yes ❍ no
If yes, Time of testing (10-120 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
P2Y12 cartridge	..... PRU
	..... Baseline
	..... % inhibition
Results printed and enclosed in patient study file?	❍ yes ❍ no
VerifyNow Aspirin	
Test performed?	❍ yes ❍ no
If yes, Time of testing (30-120 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
Aspirin cartridge	..... ARU
Results printed and enclosed in patient study file?	❍ yes ❍ no
Multiplate	
Multiplate testing TRAP performed?	❍ yes ❍ no
Multiplate testing ADP performed?	❍ yes ❍ no
Multiplate testing ASPI performed?	❍ yes ❍ no
If yes, Time of testing (30-180 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
TRAP:	Area under the curve ____ U
	Aggregation: ____ AU
	Velocity: ____ AU/min
ADP:	Area under the curve ____ U
	Aggregation: ____ AU
	Velocity: ____ AU/min
ASPI:	Area under the curve ____ U
	Aggregation: ____ AU
	Velocity: ____ AU/min
Results printed and enclosed in patient study file?	❍ yes ❍ no
Other blood platelet function tests	
Other blood platelet function tests performed?	❍ yes ❍ no
If yes, which tests?	.....

Data filled out by: _____ **Date:** ____/_____/_____

3.2 At 24 hrs

Blood sampling	
Blood sampling performed?	❍ yes ❍ no
If yes, Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock)
If no, Reason	☐ Patient received Reopro/Tirofiban/Bivalirudin ☐ Other reason: .....
VerifyNow P2Y12	
Test performed?	❍ yes ❍ no
If yes, Time of testing (10-120 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
P2Y12 cartridge	..... PRU ..... Baseline ..... % inhibition
Results printed and enclosed in patient study file?	❍ yes ❍ no
VerifyNow Aspirin	
Test performed?	❍ yes ❍ no
If yes, Time of testing (30-120 min after blood sampling):	|___|___| |___|___| (hh:mm, 24H clock)
Aspirin cartridge	..... ARU
Results printed and enclosed in patient study file?	❍ yes ❍ no
Multiplate	
Multiplate testing TRAP performed?	❍ yes ❍ no
Multiplate testing ADP performed?	❍ yes ❍ no
Multiplate testing ASPI performed?	❍ yes ❍ no
If yes, Time of testing: (30-180 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
TRAP	Area under the curve ____ U Aggregation: ____ AU Velocity: ____ AU/min
ADP	Area under the curve ____ U Aggregation: ____ AU Velocity: ____ AU/min
ASPI	Area under the curve ____ U Aggregation: ____ AU Velocity: ____ AU/min

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (15/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

Results printed and enclosed in patient study file?	❍ yes ❍ no
Other blood platelet function tests	
Other blood platelet function tests performed? If yes, which tests?	❍ yes ❍ no ……

I declare that I have reviewed for accuracy all case report form pages submitted for this patient; the information contained on these pages accurately reflects the medical records including the results of tests and evaluations performed.

Name Investigator: _____

Date: _____

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (16/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

CRF2: 30-60 day data

1. Date visit	
Date 30 day visit	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy) □ Patient died within 30 days □ Patient lost to follow-up
2. Outcome (within 30 days)	
Stroke within 30 days	❍ yes ❍ no
Recurrent ST within 30 days (ARC definition)	❍ yes: definite ST ❍ yes: probable ST ❍ no
Recurrent MI within 30 days	❍ yes, ST vessel related ❍ yes, non ST vessel related ❍ no
Ischemia driven ST vessel revascularization within 30 days	❍ yes ❍ no
Death	❍ yes, cardiac death ❍ yes, non-cardiac death ❍ no
If yes, when	❍ during index hospitalization ❍ after index hospitalization and before day 30
date of death	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
If the patient died within 30 days, the rest of this page and the 1 year and 2 year section should be left blank, please go to the last section 'mark complete'.	
3. Examinations	
EF after ST (echocardiography)	…… %
Date EF measured	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
ECG performed	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (17/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

Blood sampling performed?	❍ yes ❍ no
If yes, Date	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
Time	|___|___| |___|___| (hh:mm, 24H clock)
Haemoglobin	____ ❍ g/dL, g% ❍ g/L ❍ mmol/L
Hematocrit	____ %
Platelet count	____ 10**9/l
Antiplatelet therapy	
Aspirin?	❍ yes ❍ no ____ mg ____x/day
Clopidogrel?	❍ yes ❍ no ____ mg ____ x/day
Prasugrel?	❍ yes ❍ no ____ mg ____ x/day
Ticagrelor?	❍ yes ❍ no ____ mg ____ x/day
VerifyNow P2Y12 Test performed?	❍ yes ❍ no
If yes, Time of testing (10-120 min after blood sampling):	|___|___| |___|___| (hh:mm, 24H clock)
P2Y12 cartridge:	..... PRU
	..... Baseline
	..... % inhibition
Results printed and enclosed in patient study file?	❍ yes ❍ no
VerifyNow Aspirin Test performed?	❍ yes ❍ no
If yes, Time of testing (30-120 min after blood sampling):	|___|___| |___|___| (hh:mm, 24H clock)
Aspirin cartridge:	..... ARU
Results printed and enclosed in patient study file?	❍ yes ❍ no
Multiplate	
Multiplate testing TRAP performed?	❍ yes ❍ no
Multiplate testing ADP performed?	❍ yes ❍ no
Multiplate testing ASPI performed?	❍ yes ❍ no
If yes, Time of testing (30-180 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
TRAP	Area under the curve ____ U
	Aggregation: ____ AU
	Velocity: ____ AU/min
ADP	Area under the curve ____ U

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (18/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

ASPI	Aggregation: ____ AU Velocity: ____ AU/min Area under the curve ____ U Aggregation: ____ AU Velocity: ____ AU/min
Results printed and enclosed in patient study file?	❍ yes ❍ no
Other blood platelet function tests	
Other blood platelet function tests performed?	❍ yes ❍ no
If yes, which tests?	.....

I declare that I have reviewed for accuracy all case report form pages submitted for this patient; the information contained on these pages accurately reflects the medical records including the results of tests and evaluations performed.

Name Investigator: _____

Date: _____

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

CRF3: 1 year data

1. Date visit	
Date 1 year visit	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy) □ Patient died since the last follow-up □ Patient lost to follow-up
2. Outcome (since last follow-up)	
Stroke since last follow-up	❍ yes ❍ no
Recurrent ST since last follow-up (ARC definition)	❍ yes: definite ST ❍ yes: probable ST ❍ no
Recurrent MI since last follow-up	❍ yes, ST vessel related ❍ yes, non ST vessel related ❍ no
Ischemia driven ST vessel revascularization since last follow-up	❍ yes ❍ no
Death	❍ yes, cardiac death ❍ yes, non-cardiac death ❍ no
If yes, date of death	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
If the patient died since the last follow-up, the rest of this page and the 2 year section should be left blank, please go to the last section 'mark complete'.	
3. Examinations	
EF after ST (echocardiography)	..... %
Date EF measured	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
ECG performed	❍ yes ❍ no
Antiplatelet therapy	
Aspirin?	❍ yes ❍ no ____ mg ____x/day
Clopidogrel?	❍ yes ❍ no ____ mg ____ x/day
Prasugrel?	❍ yes ❍ no ____ mg ____ x/day
Ticagrelor?	❍ yes ❍ no ____ mg ____ x/day

Data filled out by: _____ **Date:** ____/_____/_____

I declare that I have reviewed for accuracy all case report form pages submitted for this patient; the information contained on these pages accurately reflects the medical records including the results of tests and evaluations performed.

Name Investigator: _____

Date: _____

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (21/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

CRF4: 2 year data

1. Date visit	
Date 2 year visit	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy) □ Patient died since the last follow-up □ Patient lost to follow-up
2. Outcome (since last follow-up)	
Stroke since last follow-up	❍ yes ❍ no
Recurrent ST since last follow-up (ARC definition)	❍ yes: definite ST ❍ yes: probable ST ❍ no
Recurrent MI since last follow-up	❍ yes, ST vessel related ❍ yes, non ST vessel related ❍ no
Ischemia driven ST vessel revascularization since last follow-up	❍ yes ❍ no
Death	❍ yes, cardiac death ❍ yes, non-cardiac death ❍ no
If yes, date of death If the patient died since the last follow-up, the rest of this page should be left blank, please go to the last section 'mark complete'.	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
3. Examinations	
EF after ST (echocardiography)	..... %
Date EF measured	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
ECG performed	❍ yes ❍ no
Antiplatelet therapy	❍ yes ❍ no ____ mg ____x/day
Aspirin?	❍ yes ❍ no ____ mg ____ x/day
Clopidogrel?	❍ yes ❍ no ____ mg ____ x/day
Prasugrel?	❍ yes ❍ no ____ mg ____ x/day
Ticagrelor?	

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

I declare that I have reviewed for accuracy all case report form pages submitted for this patient; the information contained on these pages accurately reflects the medical records including the results of tests and evaluations performed.

Name Investigator: _____

Date: _____

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Stent Thrombosis Case (23/24)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE ST Case

CRF5: 3 year data

4. Date visit	
Date 3 year visit	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy) □ Patient died since the last follow-up □ Patient lost to follow-up
5. Outcome (since last follow-up)	
Stroke since last follow-up	❍ yes ❍ no
Recurrent ST since last follow-up (ARC definition)	❍ yes: definite ST ❍ yes: probable ST ❍ no
Recurrent MI since last follow-up	❍ yes, ST vessel related ❍ yes, non ST vessel related ❍ no
Ischemia driven ST vessel revascularization since last follow-up	❍ yes ❍ no
Death	❍ yes, cardiac death ❍ yes, non-cardiac death ❍ no
If yes, date of death If the patient died since the last follow-up, the rest of this page should be left blank, please go to the last section 'mark complete'.	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
6. Examinations	
EF after ST (echocardiography)	..... %
Date EF measured	|___|___| |___|___|___| |_2_|_0_|___|___| (dd/mmm/yy)
ECG performed	❍ yes ❍ no
Antiplatelet therapy	❍ yes ❍ no ____ mg ____x/day
Aspirin?	❍ yes ❍ no ____ mg ____ x/day
Clopidogrel?	❍ yes ❍ no ____ mg ____ x/day
Prasugrel?	❍ yes ❍ no ____ mg ____ x/day
Ticagrelor?	

Data filled out by: _____ **Date:** ____/_____/_____

I declare that I have reviewed for accuracy all case report form pages submitted for this patient; the information contained on these pages accurately reflects the medical records including the results of tests and evaluations performed.

Name Investigator: _____

Date: _____

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

PRESTIGE

MATCHED CONTROL CASE CRF

All PRESTIGE patient data needs to be entered in the PRESTIGE electronic CRF (eCRF) according to the Data Forms Instructions manual.

- For sites who have access to the eCRF: this paper CRF can be used as a worksheet before entering the data in the eCRF (not obligatory).
- For satellite sites without access to the eCRF: this paper CRF must be used, signed off and forwarded to the site with access to the CRF.

All data entered on this paper CRF or the electronic CRF must be verifiable from a source document. Source documents are those on which patient information is first recorded and includes hospital and laboratory records, consultation files and worksheets (when used to initially record data). To be considered a source document, it must be signed and dated by the individual completing it. Each page of a source document should uniquely identify the patient. Please note, this CRF is **NOT** a source document.

Instructions for completion of the paper CRF:

- Use black ink.
- Do not leave blanks (except when data cannot be obtained).
- Record Site Number (satellite sites need to enter the Site Number of the site with access to the eCRF) and Patient Number on top of every page.
- Complete 'data filled out by' and 'date' in the footer of every page.
- Record 'Date' always as 'dd/mmm/yyyy' (e.g. 05/Jan/2012).
- Record 'Time' always as 'HH:MM-24h clock' (from 00:00 to 23:59, midnight is 00:00, last entry on a given day is 23:59).
- Corrections: Do not use correction fluid. Make corrections on the CRF pages as follows: Draw a single line through the error or incorrect data. Write the correct data above or beside the error. Initial and date the correction.
- Use capital letters for free text fields.
- For decimal numbers, use points, do not use a comma (e.g. 3.5 and not 3,5).

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

MATCHED CONTROL CASE CRF

Site number: |_4_|_4_|_1_|_0_|_0_|

Satellite Site? ❍ No ❍ Yes: .....

Patient number: |___|___|___|___|___|

Patient initials: |___|___|___|

Date of birth: |___|___| |___|___|___| |___|___|___|___|
dd mmm yyyy

Gender: ❍ Male ❍ Female

Date of initial stent implantation: |___|___| |___|___|___| |___|___|___|___|
dd mmm yyyy

Type of initial stent: ☐ BMS ☐ DES

Matches ST case number: |___|___|___|___|___|

Data/samples collected:

Baseline characteristics	❍ yes	❍ no
Blood platelet testing	❍ yes	❍ no
DNA	❍ yes	❍ no

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

MATCHED CONTROL CASE

1. Initial stent implantation procedure

1.1. Date and indication initial stent implantation	
Date stent implantation	|___|___| |___|___|___| |___|___|___|___| (dd/mmm/yyyy)
Indication	❍ STEMI ❍ NSTEMI ❍unstable angina ❍stable angina
1.2. Clinical characteristics at initial stent implantation time	
Body weight	..... kg
Smoking status	❍ active ❍ ex-smoker ❍ never smoked ❍ unknown
1.3. Medical history at initial stent implantation time	
Previous MI	□ no □ anterior □ inferior □ lateral □ posterior
Stroke	❍ yes ❍ no ❍ unknown
Heart failure (LVEF < 30%)	❍ yes ❍ no ❍ unknown
CABG	❍ yes ❍ no ❍ unknown
Renal insufficiency (serum creat>175µmol/l , GFR<30 ml/min)	❍ yes ❍ no ❍ unknown
Hemodialysis	❍ yes ❍ no ❍ unknown
Diabetes mellitus	❍ yes ❍ no ❍ unknown
AHT	❍ yes ❍ no ❍ unknown
PAD (AAA, PTA, amputation, carotid disease)	❍ yes ❍ no ❍ unknown
Malignancy	❍ active ❍ non-active ❍ none ❍ unknown
History of AF	❍ yes ❍ no ❍ unknown
Autoimmune disease	❍ yes ❍ no ❍ unknown
Thromboembolic antecedents	□ None □ CVA □ TIA □ DVT □ pulmonary embolism □ unknown

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Control Case (4/9)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

1.4. Procedural and angiographic characteristics at initial stent implantation time

Number of stents implanted ❍ 1 ❍ 2 ❍ 3 ❍ 4

Type	Name	Length (mm)	Diameter (mm)
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			
❍ BMS ❍ DES			

Overlapping stents? ❍ yes ❍ no ❍ not applicable

ACC Lesion classification	❍ A ❍ B1 ❍ B2 ❍ C
Stented segment(s) (CASS site map)	|___| |___| |___| |___|
Lesion length	..... mm
Reference vessel diameter	..... mm
Calcification	❍ none ❍ mild ❍ severe
Tortuosity	❍ yes ❍ no
Visible thrombus	❍ yes ❍ no
PCI of a SVG	❍ yes ❍ no
PCI for ISR	❍ yes ❍ no
PCI for CTO	❍ yes ❍ no
PCI with rotablation	❍ yes ❍ no
Multivessel disease If yes, left main involved	❍ yes ❍ no ❍ yes ❍ no
Ostial lesion	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Control Case (5/9)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

Stent post dilatation	❍ yes ❍ no
Highest pressure NCB	…… atm
IVUS/OCT guidance	❍ yes ❍ no
Number of diseased vessels	❍ 1 ❍ 2 ❍ 3
Bifurcation lesion	❍ yes ❍ no If yes, ☐ provisional ☐ T-stenting ☐ culotte ☐ crush ☐ other: ……
Post procedure TIMI flow	❍ 0 ❍ 1 ❍ 2 ❍ 3
Residual stenosis > 50% proximal from target lesion Residual stenosis > 50% distal from target lesion Residual dissection at the end of the procedure (angio)	❍ yes ❍ no ❍ yes ❍ no ❍ yes ❍ no
Presence of visible thrombus at the end of the procedure	❍ yes ❍ no
Restenotic lesion	❍ yes ❍ no
Use of GP IIb/IIIa	❍ yes ❍ no
Use of Bivalirudin	❍ yes ❍ no
Patient tested for clopidogrel resistance	❍ yes ❍ no
Pre loaded with DAPT If yes, which (generic name)?	❍ yes ❍ no ……

Data filled out by: _____ **Date:** ____/_____/_____

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

2. Actual information on this patient

2.1. Date when actual data is collected	
Date actual data collected	|___|___| |___|___|___| |___|___|___|___| (dd/mmm/yyyy)
2.2. Clinical characteristics	
Body weight	..... kg
Length	..... cm
Smoking status	❍ active ❍ never ❍ stopped >12 months ❍ stopped <12 months
2.3. Medical history	
Indicate changes in medical history since initial stent implantation	□ No changes □ MI □ Stroke □ CABG □ Hemodialysis □ Diabetes mellitus □ Malignancy □ History of AF □ Thromboembolic antecedents □ Renal insufficiency (serum creatinin>2 mg/dl, GFR<30 ml/min) □ Heart failure (LVEF < 30%)
Bleeding episode within 1 month prior to selection as matched control case	❍ yes ❍ no
Non-cardiac surgery within previous 90 days	❍ yes ❍ no
2.4. Medical therapy	
Aspirin	❍ yes ❍ no
Clopidogrel	❍ yes ❍ no
Prasugrel	❍ yes ❍ no
Ticagrelor	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Control Case (7/9)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

Patient on DAPT?	❍ yes ❍ no
if no, time since stop DAPT	..... Months □ not applicable
if no, time since stop aspirin	..... Months □ not applicable
if no, time since stop clopidogrel	..... Months □ not applicable
if no, time since stop prasugrel	..... Months □ not applicable
if no, time since stop ticagrelor	..... Months □ not applicable
Reason for stopping ASA	□ not applicable (did not stop ASA) □ side effects □ bleeding □ surgery/dental procedure □ financial □ medical advice □ other
Reason for stopping ADP blocker	□ not applicable (did not stop ADP blocker) □ side effects □ bleeding □ surgery/dental procedure □ financial □ medical advice □ other
Oral anticoagulant	□ No □ Warfarin □ Coumadin □ Dabigatran □ Rivaroxaban
ACE-I	❍ yes ❍ no
ARB	❍ yes ❍ no
Beta blocker	❍ yes ❍ no
Statin	❍ yes ❍ no
Diuretic	❍ yes ❍ no
PPI	❍ yes ❍ no If yes, generic name: .....
Insulin	❍ yes ❍ no
Oral antidiabetics	❍ yes ❍ no

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Control Case (8/9)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

2.5. Blood sampling

Blood sampling performed?	❍ yes ❍ no
If yes, Date	|___|___| |___|___|___| |_2_|_0_|___|___|
Time	|___|___| |___|___| (hh:mm, 24H clock)
Haemoglobin	____ ❍ g/dL, g% ❍ g/L ❍ mmol/L
Hematocrit	____ %
Platelet count	____ 10**9/l
Antiplatelet therapy	
Aspirin?	❍ yes ❍ no ____ mg ____x/day
Clopidogrel?	❍ yes ❍ no ____ mg ____x/day
Prasugrel?	❍ yes ❍ no ____ mg ____x/day
Ticagrelor?	❍ yes ❍ no ____ mg ____x/day
VerifyNow P2Y12 Test performed?	❍ yes ❍ no
If yes, Time of testing (10-120 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
P2Y12 cartridge	..... PRU
	..... Baseline
	..... % inhibition
Results printed and enclosed in patient study file?	❍ yes ❍ no
VerifyNow Aspirin Test performed?	❍ yes ❍ no
If yes, Time of testing (30-120 min after blood sampling):	|___|___| |___|___| (hh:mm, 24H clock)
Aspirin cartridge	..... ARU
Results printed and enclosed in patient study file?	❍ yes ❍ no
Multiplate	
Multiplate testing TRAP performed?	❍ yes ❍ no
Multiplate testing ADP performed?	❍ yes ❍ no
Multiplate testing ASPI performed?	❍ yes ❍ no
If yes, Time of testing: (30-180 min after blood sampling)	|___|___| |___|___| (hh:mm, 24H clock)
TRAP	Area under the curve ____ U
	Aggregation: ____ AU
	Velocity: ____ AU/min
ADP	Area under the curve ____ U
	Aggregation: ____ AU

Data filled out by: _____ **Date:** ____/_____/_____

CRF: Control Case (9/9)

SITE NUMBER	PATIENT NUMBER	CRF
|_4_|_4_|_1_|_0_|_0_|	|___|___|___|___|___|	PRESTIGE MC Case

ASPI	Velocity: ____ AU/min Area under the curve ____ U Aggregation: ____ AU Velocity: ____ AU/min
Results printed and enclosed in patient study file?	❍ yes ❍ no
Other blood platelet function tests	
Other blood platelet function tests performed?	❍ yes ❍ no
If yes, which tests?	.....

I declare that I have reviewed for accuracy all case report form pages submitted for this patient; the information contained on these pages accurately reflects the medical records including the results of tests and evaluations performed.

Name Investigator: _____

Date: _____

Data filled out by: _____ **Date:** ____/_____/_____

Appendix II: Prizes and publications during MD (res)

Prizes

- **British Cardiovascular Interventional Society Advanced Cardiovascular Intervention Young Investigator Award runner-up 2016**. Title: Understanding factors in the development of stent thrombosis: results from a large UK cohort study

Publications

- **Malik N**, Gershlick AH. The clinical and economic impact of bivalirudin for percutaneous coronary intervention, ***Expert Review of Pharmacoeconomics & Outcomes Research****,* 2013; 13(6): 699-706

- Riegger J, Byrne R, Joner M, Chandraratne S, Gershlick AH, ten Berg JM, Adriaenssens T, Guagliumi G, Godschalk TC, Neumann FJ, Trenk D, Feldman LJ, Steg PG, Desmet W, Alfonso F, Goodall AH, Wojdyla R, Dudek D, Philippi V, Opinaldo S, Titova A, **Malik N**, Cotton J, Jhagroe DA, Heestermans AACM, Sinnaeve P, Vermeersch P, Valina C, Schulz C, Kastrati A and Massberg S on behalf of the PRESTIGE investigators. Histopathological evaluation of thrombus in patients presenting with stent thrombosis. A multicenter European study. A report of the PRESTIGE consortium. ***European Heart Journal***, 2016; 37(19); 1538-1549

- **Malik N**, Banning AS, Belger M, Fakhouri W, Graham-Clarke PL, Banning A, Baumbach A, Blackman DJ, deBelder A, Lefevre T, Stables R, Zaman A, Gershlick AH. A risk scoring system to predict coronary stent thrombosis, ***Current Medical Research & Opinion***, 2017; 33(5); 859-867

- Adriaenssens T, Joner M, Godschalk TC, **Malik N**, Alfonso F, Xhepa E, De Cock D, Komukai K, Tada T, Cuesta J, Sirbu V, Feldman LJ, Neumann FJ, Goodall AH, Heestermans T, Buysschaert I, Hlinomaz O, Belmans A, Desmet W, ten Berg JM, Gershlick AH, Massberg S, Kastrati A, Guagliumi G, Byrne RA, on behalf of the PREvention of late Stent Thrombosis by an Interdisciplinary Global European effort (PRESTIGE) investigators. Optical Coherence Tomography Findings in Patients with Coronary Stent Thrombosis: A report of the PREvention of late Stent Thrombosis by an Interdisciplinary Global European effort (PRESTIGE) consortium, ***Circulation***, 2017; 136(11); 1007-1021

- Godschalk TC, Byrne RA, Adriaenssens T, **Malik N**, Feldman LJ, Guagliumi G, Alfonso F, Neumann FJ, Trenk D, Joner M, Schulz C, Steg PG, Goodall AH, Wojdyla R, Dudek D, Wykrzykowska JJ, Hlinomaz O, Zaman AG, Curzen N, Dens J, Sinnaeve P, Desmet W, Gershlick AH, Kastrati A, Massberg S, Ten Berg JM on behalf of the PREvention of late Stent Thrombosis by an Interdisciplinary Global European effort (PRESTIGE) investigators. Observational Study of Platelet Reactivity in Patients Presenting With ST-Segment Elevation Myocardial Infarction Due to Coronary Stent Thrombosis Undergoing Primary Percutaneous Coronary Intervention: Results from the European PREvention of Stent Thrombosis by an Interdisciplinary Global European Effort Registry, ***JACC Cardiovascular Interventions***, 2017; 10(24); 2548-2556

- Joner M, Koppara T, Byrne RA, Castellanos MI, Lewerich J, Novotny J, Guagliumi G, Xhepa E, Adriaenssens T, Godschalk TC, **Malik N**, Alfonso F, Tada T, Neumann FJ, Desmet W, Tenberg JM, Gershlick AH, Feldman LJ, Massberg S, Kastrati A, PRESTIGE Investigators. Neoatherosclerosis in Patients With Coronary Stent Thrombosis: Findings From Optical Coherence Tomography Imaging (A Report of the PRESTIGE Consortium), ***JACC Cardiovascular Interventions***, 2018; 11(14); 1340-1350

Abstracts

Oral presentations

- Risk factors for the development of coronary stent thrombosis, ***EuroPCR conference***, Paris, May 2012

- Age related mortality of primary PCI patients at a high-volume UK cardiac centre, ***EuroPCR conference***, May 2012

- Need for Guideliner in left main stem percutaneous coronary intervention – game changer: Challenging case presentation, ***Transcatheter Cardiovascular Therapeutics conference (TCT)***, October 2012

- Recurrent stent thrombosis (PRESTIGE study*)*: Angio review breakout session panel member, ***Advanced Cardiovascular Intervention Meeting (ACI)***, January 2014

Moderated posters

- Age related mortality of primary PCI patients at a high-volume UK cardiac centre, ***British Cardiovascular Society Annual Conference***, May 2012

- Contrast-induced nephropathy during coronary procedures, ***Acute Cardiac Care conference***, October 2012

- The initial experience of a high-volume UK primary PCI centre beginning with an overnight 'big-bang' approach, ***Acute Cardiac Care conference***, October 2012

- Development and validation of a stent thrombosis risk scoring system, ***British Cardiovascular Society Annual Conference***, June 2014

- OCT findings from the European multi-centre PRESTIGE stent thrombosis study, ***British Cardiovascular Society Annual Conference***, June 2014

- Clinical profile of UK stent thrombosis patients in the PRESTIGE study, ***British Cardiovascular Society Annual Conference***, June 2015

- Understanding factors in the development of stent thrombosis: results from a large UK cohort study, ***Advanced Cardiovascular Intervention Conference***, January 2016

Posters

- Pre-hospital telemetry for primary PCI, life-saver or time-waster? ***EuroPCR conference***, May 2012

- Development of a side branch stent for bifurcation disease – from napkin scribble to prototype, ***EuroPCR conference***, May 2012

- Risk factors for the development of coronary stent thrombosis, ***European Society of Cardiology congress***, August 2012

- Age related mortality of primary PCI patients at a high-volume UK cardiac centre, ***European Society of Cardiology congress***, August 2011, update August 2012

- A United Kingdom perspective on the appropriateness of percutaneous coronary intervention in stable angina, ***Transcatheter Cardiovascular Therapeutics conference (TCT)***, October 2012

- Development of a coronary stent thrombosis risk score, ***EuroPCR conference***, May 2014

- Development and validation of a scoring system to predict the risk of stent thrombosis, ***European Society of Cardiology congress***, August 2014

- A rabbit iliac model for testing the acute thrombogenicity and longterm biocompatibility of novel coronary stents and scaffolds, ***European Society of Cardiology congress***, August 2015

- Platelet function in the acute phase of stent thrombosis - the PRESTIGE registry, ***European Society of Cardiology congress***, August 2015

Book Chapters

- Complications of PCI: Chapter 17, **Coronary Dissection**, 2016
- Oxford Textbook of Interventional Cardiology, 2nd edition: Chapter 31, **Stent Thrombosis**, 2018
- Challenging Concepts in Cardiovascular Medicine: **Complex PCI, Chronic Total Occlusion PCI** (awaiting publication)

References

1. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117-71.
2. Bhatnagar P, Wickramasinghe K, Williams J, Rayner M, Townsend N. The epidemiology of cardiovascular disease in the UK 2014. Heart. 2015;101(15):1182-9.
3. Lippi G, Franchini M, Targher G. Arterial thrombus formation in cardiovascular disease. Nat Rev Cardiol. 2011;8(9):502-12.
4. Gruntzig A. Transluminal dilatation of coronary-artery stenosis. Lancet. 1978;1(8058):263.
5. Meier B. The first patient to undergo coronary angioplasty--23-year follow-up. The New England journal of medicine. 2001;344(2):144-5.
6. Sigwart U, Puel J, Mirkovitch V, Joffre F, Kappenberger L. Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. The New England journal of medicine. 1987;316(12):701-6.
7. Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, et al. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. Stent Restenosis Study Investigators. N Engl J Med. 1994;331(8):496-501.
8. Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz RS, et al. Pathology of acute and chronic coronary stenting in humans. Circulation. 1999;99(1):44-52.
9. Serruys PW, Strauss BH, Beatt KJ, Bertrand ME, Puel J, Rickards AF, et al. Angiographic follow-up after placement of a self-expanding coronary-artery stent. The New England journal of medicine. 1991;324(1):13-7.
10. Mehran R, Dangas G, Abizaid AS, Mintz GS, Lansky AJ, Satler LF, et al. Angiographic patterns of in-stent restenosis: classification and implications for long-term outcome. Circulation. 1999;100(18):1872-8.
11. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. The New England journal of medicine. 2002;346(23):1773-80.
12. Schofer J, Schluter M, Gershlick AH, Wijns W, Garcia E, Schampaert E, et al. Sirolimus-eluting stents for treatment of patients with long atherosclerotic lesions in small coronary arteries: double-blind, randomised controlled trial (E-SIRIUS). Lancet. 2003;362(9390):1093-9.
13. Grube E, Silber S, Hauptmann KE, Mueller R, Buellesfeld L, Gerckens U, et al. TAXUS I: six- and twelve-month results from a randomized, double-blind trial on a slow-release paclitaxel-eluting stent for de novo coronary lesions. Circulation. 2003;107(1):38-42.
14. Colombo A, Drzewiecki J, Banning A, Grube E, Hauptmann K, Silber S, et al. Randomized study to assess the effectiveness of slow- and moderate-release polymer-based paclitaxel-eluting stents for coronary artery lesions. Circulation. 2003;108(7):788-94.

15. Tanabe K, Serruys PW, Grube E, Smits PC, Selbach G, van der Giessen WJ, et al. TAXUS III Trial: in-stent restenosis treated with stent-based delivery of paclitaxel incorporated in a slow-release polymer formulation. Circulation. 2003;107(4):559-64.
16. Camenzind E, Steg PG, Wijns W. Stent thrombosis late after implantation of first-generation drug-eluting stents: a cause for concern. Circulation. 2007;115(11):1440-55; discussion 55.
17. Nordmann AJ, Briel M, Bucher HC. Mortality in randomized controlled trials comparing drug-eluting vs. bare metal stents in coronary artery disease: a meta-analysis. European heart journal. 2006;27(23):2784-814.
18. Pfisterer M, Brunner-La Rocca HP, Buser PT, Rickenbacher P, Hunziker P, Mueller C, et al. Late clinical events after clopidogrel discontinuation may limit the benefit of drug-eluting stents: an observational study of drug-eluting versus bare-metal stents. Journal of the American College of Cardiology. 2006;48(12):2584-91.
19. Spaulding C, Daemen J, Boersma E, Cutlip DE, Serruys PW. A pooled analysis of data comparing sirolimus-eluting stents with bare-metal stents. The New England journal of medicine. 2007;356(10):989-97.
20. Stone GW, Moses JW, Ellis SG, Schofer J, Dawkins KD, Morice MC, et al. Safety and efficacy of sirolimus- and paclitaxel-eluting coronary stents. The New England journal of medicine. 2007;356(10):998-1008.
21. Kastrati A, Mehilli J, Pache J, Kaiser C, Valgimigli M, Kelbaek H, et al. Analysis of 14 trials comparing sirolimus-eluting stents with bare-metal stents. The New England journal of medicine. 2007;356(10):1030-9.
22. Byrne RA, Joner M, Kastrati A. Stent thrombosis and restenosis: what have we learned and where are we going? The Andreas Gruntzig Lecture ESC 2014. European heart journal. 2015.
23. Palmerini T, Kirtane AJ, Serruys PW, Smits PC, Kedhi E, Kereiakes D, et al. Stent thrombosis with everolimus-eluting stents: meta-analysis of comparative randomized controlled trials. Circulation Cardiovascular interventions. 2012;5(3):357-64.
24. Palmerini T, Biondi-Zoccai G, Della Riva D, Stettler C, Sangiorgi D, D'Ascenzo F, et al. Stent thrombosis with drug-eluting and bare-metal stents: evidence from a comprehensive network meta-analysis. Lancet. 2012;379(9824):1393-402.
25. Sarno G, Lagerqvist B, Nilsson J, Frobert O, Hambraeus K, Varenhorst C, et al. Stent thrombosis in new-generation drug-eluting stents in patients with STEMI undergoing primary PCI: a report from SCAAR. Journal of the American College of Cardiology. 2014;64(1):16-24.
26. Bangalore S, Kumar S, Fusaro M, Amoroso N, Kirtane AJ, Byrne RA, et al. Outcomes with various drug eluting or bare metal stents in patients with diabetes mellitus: mixed treatment comparison analysis of 22,844 patient years of follow-up from randomised trials. BMJ. 2012;345:e5170.
27. Cutlip DE, Windecker S, Mehran R, Boam A, Cohen DJ, van Es GA, et al. Clinical end points in coronary stent trials: a case for standardized definitions. Circulation. 2007;115(17):2344-51.
28. Iakovou I, Schmidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G, et al. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. JAMA : the journal of the American Medical Association. 2005;293(17):2126-30.

29. Kuchulakanti PK, Chu WW, Torguson R, Ohlmann P, Rha SW, Clavijo LC, et al. Correlates and long-term outcomes of angiographically proven stent thrombosis with sirolimus- and paclitaxel-eluting stents. Circulation. 2006;113(8):1108-13.
30. Daemen J, Wenaweser P, Tsuchida K, Abrecht L, Vaina S, Morger C, et al. Early and late coronary stent thrombosis of sirolimus-eluting and paclitaxel-eluting stents in routine clinical practice: data from a large two-institutional cohort study. Lancet. 2007;369(9562):667-78.
31. Smit JJ, van 't Hof AW, de Boer MJ, Hoorntje JC, Dambrink JH, Gosselink AT, et al. Incidence and predictors of subacute thrombosis in patients undergoing primary angioplasty for an acute myocardial infarction. Thrombosis and haemostasis. 2006;96(2):190-5.
32. Rinaldi MJ, Kirtane AJ, Piana RN, Caputo RP, Gordon PC, Lopez JJ, et al. Clinical, procedural, and pharmacologic correlates of acute and subacute stent thrombosis: results of a multicenter case-control study with 145 thrombosis events. American heart journal. 2008;155(4):654-60.
33. van Werkum JW, Heestermans AA, Zomer AC, Kelder JC, Suttorp MJ, Rensing BJ, et al. Predictors of coronary stent thrombosis: the Dutch Stent Thrombosis Registry. Journal of the American College of Cardiology. 2009;53(16):1399-409.
34. Kirtane AJ, Stone GW. How to minimize stent thrombosis. Circulation. 2011;124(11):1283-7.
35. Byrne RA, Serruys PW, Baumbach A, Escaned J, Fajadet J, James S, et al. Report of a European Society of Cardiology-European Association of Percutaneous Cardiovascular Interventions task force on the evaluation of coronary stents in Europe: executive summary. European heart journal. 2015;36(38):2608-20.
36. Tada T, Byrne RA, Simunovic I, King LA, Cassese S, Joner M, et al. Risk of stent thrombosis among bare-metal stents, first-generation drug-eluting stents, and second-generation drug-eluting stents: results from a registry of 18,334 patients. JACC Cardiovascular interventions. 2013;6(12):1267-74.
37. Raber L, Magro M, Stefanini GG, Kalesan B, van Domburg RT, Onuma Y, et al. Very late coronary stent thrombosis of a newer-generation everolimus-eluting stent compared with early-generation drug-eluting stents: a prospective cohort study. Circulation. 2012;125(9):1110-21.
38. Kimura T, Morimoto T, Kozuma K, Honda Y, Kume T, Aizawa T, et al. Comparisons of baseline demographics, clinical presentation, and long-term outcome among patients with early, late, and very late stent thrombosis of sirolimus-eluting stents: Observations from the Registry of Stent Thrombosis for Review and Reevaluation (RESTART). Circulation. 2010;122(1):52-61.
39. Armstrong EJ, Feldman DN, Wang TY, Kaltenbach LA, Yeo KK, Wong SC, et al. Clinical presentation, management, and outcomes of angiographically documented early, late, and very late stent thrombosis. JACC Cardiovasc Interv. 2012;5(2):131-40.
40. Schulz S, Schuster T, Mehilli J, Byrne RA, Ellert J, Massberg S, et al. Stent thrombosis after drug-eluting stent implantation: incidence, timing, and relation to discontinuation of clopidogrel therapy over a 4-year period. European heart journal. 2009;30(22):2714-21.
41. Kumar DR, Hanlin E, Glurich I, Mazza JJ, Yale SH. Virchow's contribution to the understanding of thrombosis and cellular biology. Clinical medicine & research. 2010;8(3-4):168-72.

42. Conde ID, Kleiman NS. Arterial thrombosis for the interventional cardiologist: from adhesion molecules and coagulation factors to clinical therapeutics. Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions. 2003;60(2):236-46.
43. D'Ascenzo F, Bollati M, Clementi F, Castagno D, Lagerqvist B, de la Torre Hernandez JM, et al. Incidence and predictors of coronary stent thrombosis: evidence from an international collaborative meta-analysis including 30 studies, 221,066 patients, and 4276 thromboses. International journal of cardiology. 2013;167(2):575-84.
44. Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, Gold HK, et al. Vascular responses to drug eluting stents: importance of delayed healing. Arteriosclerosis, thrombosis, and vascular biology. 2007;27(7):1500-10.
45. Kotani J, Awata M, Nanto S, Uematsu M, Oshima F, Minamiguchi H, et al. Incomplete neointimal coverage of sirolimus-eluting stents: angioscopic findings. Journal of the American College of Cardiology. 2006;47(10):2108-11.
46. Joner M, Nakazawa G, Finn AV, Quee SC, Coleman L, Acampado E, et al. Endothelial cell recovery between comparator polymer-based drug-eluting stents. Journal of the American College of Cardiology. 2008;52(5):333-42.
47. Kolandaivelu K, Swaminathan R, Gibson WJ, Kolachalama VB, Nguyen-Ehrenreich KL, Giddings VL, et al. Stent thrombogenicity early in high-risk interventional settings is driven by stent design and deployment and protected by polymer-drug coatings. Circulation. 2011;123(13):1400-9.
48. Sabate M, Cequier A, Iniguez A, Serra A, Hernandez-Antolin R, Mainar V, et al. Everolimus-eluting stent versus bare-metal stent in ST-segment elevation myocardial infarction (EXAMINATION): 1 year results of a randomised controlled trial. Lancet. 2012;380(9852):1482-90.
49. Sabate M, Brugaletta S, Cequier A, Iniguez A, Serra A, Hernadez-Antolin R, et al. The EXAMINATION trial (Everolimus-Eluting Stents Versus Bare-Metal Stents in ST-Segment Elevation Myocardial Infarction): 2-year results from a multicenter randomized controlled trial. JACC Cardiovascular interventions. 2014;7(1):64-71.
50. Romero ME, Yahagi K, Kolodgie FD, Virmani R. Neoatherosclerosis From a Pathologist's Point of View. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(10):e43-9.
51. Nakazawa G, Vorpahl M, Finn AV, Narula J, Virmani R. One step forward and two steps back with drug-eluting-stents: from preventing restenosis to causing late thrombosis and nouveau atherosclerosis. JACC Cardiovascular imaging. 2009;2(5):625-8.
52. Armstrong EJ, Sab S, Singh GD, Lim W, Yeo KK, Waldo SW, et al. Predictors and outcomes of recurrent stent thrombosis: results from a multicenter registry. JACC Cardiovascular interventions. 2014;7(10):1105-13.
53. Yonetsu T, Kato K, Kim SJ, Xing L, Jia H, McNulty I, et al. Predictors for neoatherosclerosis: a retrospective observational study from the optical coherence tomography registry. Circulation Cardiovascular imaging. 2012;5(5):660-6.
54. Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, et al. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. Stent Anticoagulation Restenosis Study Investigators. The New England journal of medicine. 1998;339(23):1665-71.
55. Schomig A, Neumann FJ, Kastrati A, Schuhlen H, Blasini R, Hadamitzky M, et al. A randomized comparison of antiplatelet and anticoagulant therapy after the

placement of coronary-artery stents. The New England journal of medicine. 1996;334(17):1084-9.
56. Mehran R, Baber U, Steg PG, Ariti C, Weisz G, Witzenbichler B, et al. Cessation of dual antiplatelet treatment and cardiac events after percutaneous coronary intervention (PARIS): 2 year results from a prospective observational study. Lancet. 2013;382(9906):1714-22.
57. Stone GW, Witzenbichler B, Weisz G, Rinaldi MJ, Neumann FJ, Metzger DC, et al. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. Lancet. 2013;382(9892):614-23.
58. Sibbing D, Braun S, Morath T, Mehilli J, Vogt W, Schomig A, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. Journal of the American College of Cardiology. 2009;53(10):849-56.
59. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. Journal of the American College of Cardiology. 2005;45(2):246-51.
60. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Huisman MV. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. Arch Intern Med. 2007;167(15):1593-9.
61. Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ. 2008;336(7637):195-8.
62. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Jukema JW, Huisman MV. Clopidogrel nonresponsiveness in patients undergoing percutaneous coronary intervention with stenting: a systematic review and meta-analysis. American heart journal. 2007;154(2):221-31.
63. Tentzeris I, Siller-Matula J, Farhan S, Jarai R, Wojta J, Huber K. Platelet function variability and non-genetic causes. Thrombosis and haemostasis. 2011;105 Suppl 1:S60-6.
64. Harmsze AM, van Werkum JW, Taubert D, Hackeng CM, Deneer VH. Esomeprazole but not pantoprazole is associated with lower plasma concentrations of clopidogrel's active metabolite. The Annals of pharmacotherapy. 2011;45(4):542-3.
65. Niu Q, Wang Z, Zhang Y, Wang J, Zhang P, Wang C, et al. Combination Use of Clopidogrel and Proton Pump Inhibitors Increases Major Adverse Cardiovascular Events in Patients With Coronary Artery Disease: A Meta-Analysis. Journal of cardiovascular pharmacology and therapeutics. 2016.
66. Gargiulo G, Costa F, Ariotti S, Biscaglia S, Campo G, Esposito G, et al. Impact of proton pump inhibitors on clinical outcomes in patients treated with a 6- or 24-month dual-antiplatelet therapy duration: Insights from the PROlonging Dual-antiplatelet treatment after Grading stent-induced Intimal hyperplasia studY trial. American heart journal. 2016;174:95-102.
67. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, et al. Genetic determinants of response to clopidogrel and cardiovascular events. The New England journal of medicine. 2009;360(4):363-75.
68. Gurbel PA, Shuldiner AR, Bliden KP, Ryan K, Pakyz RE, Tantry US. The relation between CYP2C19 genotype and phenotype in stented patients on

maintenance dual antiplatelet therapy. American heart journal. 2011;161(3):598-604.
69. Yukhanyan L, Freynhofer MK, Siller-Matula J, Schror K, Huber K. Genetic variability in response to clopidogrel therapy and its clinical implications. Thrombosis and haemostasis. 2011;105 Suppl 1:S55-9.
70. Price MJ, Berger PB, Teirstein PS, Tanguay JF, Angiolillo DJ, Spriggs D, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA : the journal of the American Medical Association. 2011;305(11):1097-105.
71. Trenk D, Stone GW, Gawaz M, Kastrati A, Angiolillo DJ, Muller U, et al. A randomized trial of prasugrel versus clopidogrel in patients with high platelet reactivity on clopidogrel after elective percutaneous coronary intervention with implantation of drug-eluting stents: results of the TRIGGER-PCI (Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel) study. Journal of the American College of Cardiology. 2012;59(24):2159-64.
72. Collet JP, Cuisset T, Range G, Cayla G, Elhadad S, Pouillot C, et al. Bedside monitoring to adjust antiplatelet therapy for coronary stenting. The New England journal of medicine. 2012;367(22):2100-9.
73. Steigen TK, Maeng M, Wiseth R, Erglis A, Kumsars I, Narbute I, et al. Randomized study on simple versus complex stenting of coronary artery bifurcation lesions: the Nordic bifurcation study. Circulation. 2006;114(18):1955-61.
74. Hoye A, Iakovou I, Ge L, van Mieghem CA, Ong AT, Cosgrave J, et al. Long-term outcomes after stenting of bifurcation lesions with the "crush" technique: predictors of an adverse outcome. Journal of the American College of Cardiology. 2006;47(10):1949-58.
75. Attizzani GF, Capodanno D, Ohno Y, Tamburino C. Mechanisms, pathophysiology, and clinical aspects of incomplete stent apposition. Journal of the American College of Cardiology. 2014;63(14):1355-67.
76. Foin N, Gutierrez-Chico JL, Nakatani S, Torii R, Bourantas CV, Sen S, et al. Incomplete stent apposition causes high shear flow disturbances and delay in neointimal coverage as a function of strut to wall detachment distance: implications for the management of incomplete stent apposition. Circulation Cardiovascular interventions. 2014;7(2):180-9.
77. Alfonso F, Suarez A, Angiolillo DJ, Sabate M, Escaned J, Moreno R, et al. Findings of intravascular ultrasound during acute stent thrombosis. Heart. 2004;90(12):1455-9.
78. Cayla G, Hulot JS, O'Connor SA, Pathak A, Scott SA, Gruel Y, et al. Clinical, angiographic, and genetic factors associated with early coronary stent thrombosis. JAMA : the journal of the American Medical Association. 2011;306(16):1765-74.
79. Chechi T, Vecchio S, Vittori G, Giuliani G, Lilli A, Spaziani G, et al. ST-segment elevation myocardial infarction due to early and late stent thrombosis a new group of high-risk patients. Journal of the American College of Cardiology. 2008;51(25):2396-402.
80. Frobert O, Lagerqvist B, Olivecrona GK, Omerovic E, Gudnason T, Maeng M, et al. Thrombus aspiration during ST-segment elevation myocardial infarction. The New England journal of medicine. 2013;369(17):1587-97.

81. Reeves RR, Patel M, Armstrong EJ, Sab S, Waldo SW, Yeo KK, et al. Angiographic characteristics of definite stent thrombosis: role of thrombus grade, collaterals, epicardial coronary flow, and myocardial perfusion. Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions. 2015;85(1):13-22.
82. Riegger J, Byrne RA, Joner M, Chandraratne S, Gershlick AH, Ten Berg JM, et al. Histopathological evaluation of thrombus in patients presenting with stent thrombosis. A multicenter European study: a report of the prevention of late stent thrombosis by an interdisciplinary global European effort consortium. Eur Heart J. 2016;37(19):1538-49.
83. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. The EPIC Investigation. N Engl J Med. 1994;330(14):956-61.
84. Randomised placebo-controlled trial of effect of eptifibatide on complications of percutaneous coronary intervention: IMPACT-II. Integrilin to Minimise Platelet Aggregation and Coronary Thrombosis-II. Lancet. 1997;349(9063):1422-8.
85. Effects of platelet glycoprotein IIb/IIIa blockade with tirofiban on adverse cardiac events in patients with unstable angina or acute myocardial infarction undergoing coronary angioplasty. The RESTORE Investigators. Randomized Efficacy Study of Tirofiban for Outcomes and REstenosis. Circulation. 1997;96(5):1445-53.
86. Investigators E. Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization. N Engl J Med. 1997;336(24):1689-96.
87. Investigators E. Randomised placebo-controlled and balloon-angioplasty-controlled trial to assess safety of coronary stenting with use of platelet glycoprotein-IIb/IIIa blockade. Lancet. 1998;352(9122):87-92.
88. Lincoff AM, Califf RM, Moliterno DJ, Ellis SG, Ducas J, Kramer JH, et al. Complementary clinical benefits of coronary-artery stenting and blockade of platelet glycoprotein IIb/IIIa receptors. Evaluation of Platelet IIb/IIIa Inhibition in Stenting Investigators. N Engl J Med. 1999;341(5):319-27.
89. O'Shea JC, Hafley GE, Greenberg S, Hasselblad V, Lorenz TJ, Kitt MM, et al. Platelet glycoprotein IIb/IIIa integrin blockade with eptifibatide in coronary stent intervention: the ESPRIT trial: a randomized controlled trial. JAMA. 2001;285(19):2468-73.
90. Therapy EIESotPIIRwI. Novel dosing regimen of eptifibatide in planned coronary stent implantation (ESPRIT): a randomised, placebo-controlled trial. Lancet. 2000;356(9247):2037-44.
91. Mehran R, Lansky AJ, Witzenbichler B, Guagliumi G, Peruga JZ, Brodie BR, et al. Bivalirudin in patients undergoing primary angioplasty for acute myocardial infarction (HORIZONS-AMI): 1-year results of a randomised controlled trial. Lancet. 2009;374(9696):1149-59.
92. Steg PG, van 't Hof A, Hamm CW, Clemmensen P, Lapostolle F, Coste P, et al. Bivalirudin started during emergency transport for primary PCI. The New England journal of medicine. 2013;369(23):2207-17.
93. Clemmensen P, Wiberg S, Van't Hof A, Deliargyris EN, Coste P, Ten Berg J, et al. Acute stent thrombosis after primary percutaneous coronary intervention:

insights from the EUROMAX trial (European Ambulance Acute Coronary Syndrome Angiography). JACC Cardiovascular interventions. 2015;8(1 Pt B):214-20.
94. Shahzad A, Kemp I, Mars C, Wilson K, Roome C, Cooper R, et al. Unfractionated heparin versus bivalirudin in primary percutaneous coronary intervention (HEAT-PPCI): an open-label, single centre, randomised controlled trial. Lancet. 2014;384(9957):1849-58.
95. Kubo T, Imanishi T, Takarada S, Kuroi A, Ueno S, Yamano T, et al. Assessment of culprit lesion morphology in acute myocardial infarction: ability of optical coherence tomography compared with intravascular ultrasound and coronary angioscopy. Journal of the American College of Cardiology. 2007;50(10):933-9.
96. Kobayashi Y, Teirstein P, Linnemeier T, Stone G, Leon M, Moses J. Rotational atherectomy (stentablation) in a lesion with stent underexpansion due to heavily calcified plaque. Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions. 2001;52(2):208-11.
97. Latib A, Takagi K, Chizzola G, Tobis J, Ambrosini V, Niccoli G, et al. Excimer Laser LEsion modification to expand non-dilatable stents: the ELLEMENT registry. Cardiovascular revascularization medicine : including molecular interventions. 2014;15(1):8-12.
98. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Jr., Ganiats TG, Holmes DR, Jr., et al. 2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology. 2014;64(24):e139-228.
99. Authors/Task Force m, Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, et al. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). Eur Heart J. 2014;35(37):2541-619.
100. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, et al. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. Lancet. 2009;373(9665):723-31.
101. Wiviott SD, Braunwald E, McCabe CH, Horvath I, Keltai M, Herrman JP, et al. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. Lancet. 2008;371(9621):1353-63.
102. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. The New England journal of medicine. 2009;361(11):1045-57.
103. Steg PG, Harrington RA, Emanuelsson H, Katus HA, Mahaffey KW, Meier B, et al. Stent thrombosis with ticagrelor versus clopidogrel in patients with acute coronary syndromes: an analysis from the prospective, randomized PLATO trial. Circulation. 2013;128(10):1055-65.
104. Schomig A. Ticagrelor--is there need for a new player in the antiplatelet-therapy field? N Engl J Med. 2009;361(11):1108-11.

105. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009;360(4):354-62.
106. Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. J Am Coll Cardiol. 2008;51(3):256-60.
107. Wallentin L, Varenhorst C, James S, Erlinge D, Braun OO, Jakubowski JA, et al. Prasugrel achieves greater and faster P2Y12receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease. Eur Heart J. 2008;29(1):21-30.
108. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, et al. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. Circulation. 2007;116(25):2923-32.
109. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2007;357(20):2001-15.
110. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2009;361(11):1045-57.
111. Gwon HC, Hahn JY, Park KW, Song YB, Chae IH, Lim DS, et al. Six-month versus 12-month dual antiplatelet therapy after implantation of drug-eluting stents: the Efficacy of Xience/Promus Versus Cypher to Reduce Late Loss After Stenting (EXCELLENT) randomized, multicenter study. Circulation. 2012;125(3):505-13.
112. Schulz-Schupke S, Byrne RA, Ten Berg JM, Neumann FJ, Han Y, Adriaenssens T, et al. ISAR-SAFE: a randomized, double-blind, placebo-controlled trial of 6 vs. 12 months of clopidogrel therapy after drug-eluting stenting. European heart journal. 2015;36(20):1252-63.
113. Feres F, Costa RA, Abizaid A, Leon MB, Marin-Neto JA, Botelho RV, et al. Three vs twelve months of dual antiplatelet therapy after zotarolimus-eluting stents: the OPTIMIZE randomized trial. JAMA : the journal of the American Medical Association. 2013;310(23):2510-22.
114. Kim BK, Hong MK, Shin DH, Nam CM, Kim JS, Ko YG, et al. A new strategy for discontinuation of dual antiplatelet therapy: the RESET Trial (REal Safety and Efficacy of 3-month dual antiplatelet Therapy following Endeavor zotarolimus-eluting stent implantation). Journal of the American College of Cardiology. 2012;60(15):1340-8.
115. Colombo A, Chieffo A, Frasheri A, Garbo R, Masotti-Centol M, Salvatella N, et al. Second-generation drug-eluting stent implantation followed by 6- versus 12-month dual antiplatelet therapy: the SECURITY randomized clinical trial. Journal of the American College of Cardiology. 2014;64(20):2086-97.
116. Collet JP, Silvain J, Barthelemy O, Range G, Cayla G, Van Belle E, et al. Dual-antiplatelet treatment beyond 1 year after drug-eluting stent implantation (ARCTIC-Interruption): a randomised trial. Lancet. 2014;384(9954):1577-85.

117. Mauri L, Kereiakes DJ, Yeh RW, Driscoll-Shempp P, Cutlip DE, Steg PG, et al. Twelve or 30 months of dual antiplatelet therapy after drug-eluting stents. The New England journal of medicine. 2014;371(23):2155-66.
118. Lee CW, Ahn JM, Park DW, Kang SJ, Lee SW, Kim YH, et al. Optimal duration of dual antiplatelet therapy after drug-eluting stent implantation: a randomized, controlled trial. Circulation. 2014;129(3):304-12.
119. Gilard M, Barragan P, Noryani AA, Noor HA, Majwal T, Hovasse T, et al. 6- versus 24-month dual antiplatelet therapy after implantation of drug-eluting stents in patients nonresistant to aspirin: the randomized, multicenter ITALIC trial. Journal of the American College of Cardiology. 2015;65(8):777-86.
120. Valgimigli M, Campo G, Percoco G, Monti M, Ferrari F, Tumscitz C, et al. Randomized comparison of 6- versus 24-month clopidogrel therapy after balancing anti-intimal hyperplasia stent potency in all-comer patients undergoing percutaneous coronary intervention Design and rationale for the PROlonging Dual-antiplatelet treatment after Grading stent-induced Intimal hyperplasia study (PRODIGY). American heart journal. 2010;160(5):804-11.
121. Yeh RW, Kereiakes DJ, Steg PG, Windecker S, Rinaldi MJ, Gershlick AH, et al. Benefits and Risks of Extended Duration Dual Antiplatelet Therapy After PCI in Patients With and Without Acute Myocardial Infarction. Journal of the American College of Cardiology. 2015;65(20):2211-21.
122. Bonaca MP, Bhatt DL, Cohen M, Steg PG, Storey RF, Jensen EC, et al. Long-term use of ticagrelor in patients with prior myocardial infarction. N Engl J Med. 2015;372(19):1791-800.
123. Kereiakes DJ, Yeh RW, Massaro JM, Cutlip DE, Steg PG, Wiviott SD, et al. DAPT Score Utility for Risk Prediction in Patients With or Without Previous Myocardial Infarction. J Am Coll Cardiol. 2016;67(21):2492-502.
124. Yeh RW, Secemsky EA, Kereiakes DJ, Normand SL, Gershlick AH, Cohen DJ, et al. Development and Validation of a Prediction Rule for Benefit and Harm of Dual Antiplatelet Therapy Beyond 1 Year After Percutaneous Coronary Intervention. JAMA. 2016;315(16):1735-49.
125. Gibson CM, Chakrabarti AK, Mega J, Bode C, Bassand JP, Verheugt FW, et al. Reduction of stent thrombosis in patients with acute coronary syndromes treated with rivaroxaban in ATLAS-ACS 2 TIMI 51. Journal of the American College of Cardiology. 2013;62(4):286-90.
126. Bonaca MP, Scirica BM, Braunwald E, Wiviott SD, O'Donoghue ML, Murphy SA, et al. Coronary stent thrombosis with vorapaxar versus placebo: results from the TRA 2 degrees P-TIMI 50 trial. Journal of the American College of Cardiology. 2014;64(22):2309-17.
127. Witzenbichler B, Maehara A, Weisz G, Neumann FJ, Rinaldi MJ, Metzger DC, et al. Relationship between intravascular ultrasound guidance and clinical outcomes after drug-eluting stents: the assessment of dual antiplatelet therapy with drug-eluting stents (ADAPT-DES) study. Circulation. 2014;129(4):463-70.
128. Wiebe J, Nef HM, Hamm CW. Current status of bioresorbable scaffolds in the treatment of coronary artery disease. Journal of the American College of Cardiology. 2014;64(23):2541-51.
129. Serruys PW, Chevalier B, Dudek D, Cequier A, Carrie D, Iniguez A, et al. A bioresorbable everolimus-eluting scaffold versus a metallic everolimus-eluting stent for ischaemic heart disease caused by de-novo native coronary artery lesions

(ABSORB II): an interim 1-year analysis of clinical and procedural secondary outcomes from a randomised controlled trial. Lancet. 2015;385(9962):43-54.
130. Capodanno D, Gori T, Nef H, Latib A, Mehilli J, Lesiak M, et al. Percutaneous coronary intervention with everolimus-eluting bioresorbable vascular scaffolds in routine clinical practice: early and midterm outcomes from the European multicentre GHOST-EU registry. EuroIntervention : journal of EuroPCR in collaboration with the Working Group on Interventional Cardiology of the European Society of Cardiology. 2015;10(10):1144-53.
131. Ellis SG, Kereiakes DJ, Metzger DC, Caputo RP, Rizik DG, Teirstein PS, et al. Everolimus-Eluting Bioresorbable Scaffolds for Coronary Artery Disease. The New England journal of medicine. 2015.
132. Serruys PW, Chevalier B, Sotomi Y, Cequier A, Carrie D, Piek JJ, et al. Comparison of an everolimus-eluting bioresorbable scaffold with an everolimus-eluting metallic stent for the treatment of coronary artery stenosis (ABSORB II): a 3 year, randomised, controlled, single-blind, multicentre clinical trial. Lancet. 2016;388(10059):2479-91.
133. Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. Journal of the American College of Cardiology. 2006;48(1):193-202.
134. Nakazawa G, Finn AV, Joner M, Ladich E, Kutys R, Mont EK, et al. Delayed arterial healing and increased late stent thrombosis at culprit sites after drug-eluting stent placement for acute myocardial infarction patients: an autopsy study. Circulation. 2008;118(11):1138-45.
135. Virmani R, Guagliumi G, Farb A, Musumeci G, Grieco N, Motta T, et al. Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: should we be cautious? Circulation. 2004;109(6):701-5.
136. Cook S, Ladich E, Nakazawa G, Eshtehardi P, Neidhart M, Vogel R, et al. Correlation of intravascular ultrasound findings with histopathological analysis of thrombus aspirates in patients with very late drug-eluting stent thrombosis. Circulation. 2009;120(5):391-9.
137. Yabushita H, Bouma BE, Houser SL, Aretz HT, Jang IK, Schlendorf KH, et al. Characterization of human atherosclerosis by optical coherence tomography. Circulation. 2002;106(13):1640-5.
138. Jang IK, Bouma BE, Kang DH, Park SJ, Park SW, Seung KB, et al. Visualization of coronary atherosclerotic plaques in patients using optical coherence tomography: comparison with intravascular ultrasound. Journal of the American College of Cardiology. 2002;39(4):604-9.
139. Kume T, Akasaka T, Kawamoto T, Watanabe N, Toyota E, Neishi Y, et al. Assessment of coronary arterial plaque by optical coherence tomography. The American journal of cardiology. 2006;97(8):1172-5.
140. Kume T, Akasaka T, Kawamoto T, Ogasawara Y, Watanabe N, Toyota E, et al. Assessment of coronary arterial thrombus by optical coherence tomography. The American journal of cardiology. 2006;97(12):1713-7.
141. Tearney GJ, Yabushita H, Houser SL, Aretz HT, Jang IK, Schlendorf KH, et al. Quantification of macrophage content in atherosclerotic plaques by optical coherence tomography. Circulation. 2003;107(1):113-9.
142. Kume T, Akasaka T, Kawamoto T, Okura H, Watanabe N, Toyota E, et al. Measurement of the thickness of the fibrous cap by optical coherence tomography. American heart journal. 2006;152(4):755 e1-4.

143. Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. Journal of the American College of Cardiology. 2008;51(20):1925-34.
144. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA : the journal of the American Medical Association. 2009;302(8):849-57.
145. Varenhorst C, James S, Erlinge D, Brandt JT, Braun OO, Man M, et al. Genetic variation of CYP2C19 affects both pharmacokinetic and pharmacodynamic responses to clopidogrel but not prasugrel in aspirin-treated patients with coronary artery disease. European heart journal. 2009;30(14):1744-52.
146. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet. 2009;373(9660):309-17.
147. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dorrler K, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. European heart journal. 2009;30(8):916-22.
148. Hochholzer W, Trenk D, Fromm MF, Valina CM, Stratz C, Bestehorn HP, et al. Impact of cytochrome P450 2C19 loss-of-function polymorphism and of major demographic characteristics on residual platelet function after loading and maintenance treatment with clopidogrel in patients undergoing elective coronary stent placement. Journal of the American College of Cardiology. 2010;55(22):2427-34.
149. Airoldi F, Colombo A, Morici N, Latib A, Cosgrave J, Buellesfeld L, et al. Incidence and predictors of drug-eluting stent thrombosis during and after discontinuation of thienopyridine treatment. Circulation. 2007;116(7):745-54.
150. Park DW, Park SW, Park KH, Lee BK, Kim YH, Lee CW, et al. Frequency of and risk factors for stent thrombosis after drug-eluting stent implantation during long-term follow-up. The American journal of cardiology. 2006;98(3):352-6.
151. Guagliumi G, Sirbu V, Musumeci G, Gerber R, Biondi-Zoccai G, Ikejima H, et al. Examination of the in vivo mechanisms of late drug-eluting stent thrombosis: findings from optical coherence tomography and intravascular ultrasound imaging. JACC Cardiovascular interventions. 2012;5(1):12-20.
152. Soeda T, Uemura S, Park SJ, Jang Y, Lee S, Cho JM, et al. Incidence and Clinical Significance of Poststent Optical Coherence Tomography Findings: One-Year Follow-Up Study From a Multicenter Registry. Circulation. 2015;132(11):1020-9.
153. Prati F, Kodama T, Romagnoli E, Gatto L, Di Vito L, Ramazzotti V, et al. Suboptimal stent deployment is associated with subacute stent thrombosis: optical coherence tomography insights from a multicenter matched study. From the CLI Foundation investigators: the CLI-THRO study. American heart journal. 2015;169(2):249-56.
154. Souteyrand G, Amabile N, Mangin L, Chabin X, Meneveau N, Cayla G, et al. Mechanisms of stent thrombosis analysed by optical coherence tomography: insights from the national PESTO French registry. European heart journal. 2016;37(15):1208-16.

155. Taniwaki M, Radu MD, Zaugg S, Amabile N, Garcia-Garcia HM, Yamaji K, et al. Mechanisms of Very Late Drug-Eluting Stent Thrombosis Assessed by Optical Coherence Tomography. Circulation. 2016;133(7):650-60.
156. Koppara T, Cheng Q, Yahagi K, Mori H, Sanchez OD, Feygin J, et al. Thrombogenicity and early vascular healing response in metallic biodegradable polymer-based and fully bioabsorbable drug-eluting stents. Circulation Cardiovascular interventions. 2015;8(6):e002427.
157. Finn AV, Joner M, Nakazawa G, Kolodgie F, Newell J, John MC, et al. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. Circulation. 2007;115(18):2435-41.
158. Alfonso F, Fernandez-Vina F, Medina M, Hernandez R. Neoatherosclerosis: the missing link between very late stent thrombosis and very late in-stent restenosis. Journal of the American College of Cardiology. 2013;61(12):e155.
159. Koskinas KC, Chatzizisis YS, Antoniadis AP, Giannoglou GD. Role of endothelial shear stress in stent restenosis and thrombosis: pathophysiologic mechanisms and implications for clinical translation. Journal of the American College of Cardiology. 2012;59(15):1337-49.
160. Otsuka F, Byrne RA, Yahagi K, Mori H, Ladich E, Fowler DR, et al. Neoatherosclerosis: overview of histopathologic findings and implications for intravascular imaging assessment. European heart journal. 2015;36(32):2147-59.
161. Taniwaki M, Windecker S, Zaugg S, Stefanini GG, Baumgartner S, Zanchin T, et al. The association between in-stent neoatherosclerosis and native coronary artery disease progression: a long-term angiographic and optical coherence tomography cohort study. European heart journal. 2015;36(32):2167-76.
162. Claessen BE, Henriques JP, Jaffer FA, Mehran R, Piek JJ, Dangas GD. Stent thrombosis: a clinical perspective. JACC Cardiovascular interventions. 2014;7(10):1081-92.
163. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. The New England journal of medicine. 2001;345(7):494-502.
164. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet. 2001;358(9281):527-33.
165. Steinhubl SR, Berger PB, Mann JT, 3rd, Fry ET, DeLago A, Wilmer C, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA : the journal of the American Medical Association. 2002;288(19):2411-20.
166. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. Lancet. 2005;366(9497):1607-21.
167. Sabatine MS, Cannon CP, Gibson CM, Lopez-Sendon JL, Montalescot G, Theroux P, et al. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. The New England journal of medicine. 2005;352(12):1179-89.
168. Sabatine MS, Cannon CP, Gibson CM, Lopez-Sendon JL, Montalescot G, Theroux P, et al. Effect of clopidogrel pretreatment before percutaneous coronary intervention in patients with ST-elevation myocardial infarction treated with

fibrinolytics: the PCI-CLARITY study. JAMA : the journal of the American Medical Association. 2005;294(10):1224-32.
169. Bonello L, Tantry US, Marcucci R, Blindt R, Angiolillo DJ, Becker R, et al. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. Journal of the American College of Cardiology. 2010;56(12):919-33.
170. Combescure C, Fontana P, Mallouk N, Berdague P, Labruyere C, Barazer I, et al. Clinical implications of clopidogrel non-response in cardiovascular patients: a systematic review and meta-analysis. Journal of thrombosis and haemostasis : JTH. 2010;8(5):923-33.
171. Brar SS, ten Berg J, Marcucci R, Price MJ, Valgimigli M, Kim HS, et al. Impact of platelet reactivity on clinical outcomes after percutaneous coronary intervention. A collaborative meta-analysis of individual participant data. Journal of the American College of Cardiology. 2011;58(19):1945-54.
172. Hochholzer W, Trenk D, Frundi D, Blanke P, Fischer B, Andris K, et al. Time dependence of platelet inhibition after a 600-mg loading dose of clopidogrel in a large, unselected cohort of candidates for percutaneous coronary intervention. Circulation. 2005;111(20):2560-4.
173. Gurbel PA, Bliden KP, Butler K, Tantry US, Gesheff T, Wei C, et al. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. Circulation. 2009;120(25):2577-85.
174. Gurbel PA, Bliden KP, Butler K, Antonino MJ, Wei C, Teng R, et al. Response to ticagrelor in clopidogrel nonresponders and responders and effect of switching therapies: the RESPOND study. Circulation. 2010;121(10):1188-99.
175. Dichiara J, Bliden KP, Tantry US, Chaganti SK, Kreutz RP, Gesheff TB, et al. Platelet function measured by VerifyNow identifies generalized high platelet reactivity in aspirin treated patients. Platelets. 2007;18(6):414-23.
176. Wheeler GL, Braden GA, Steinhubl SR, Kereiakes DJ, Kottke-Marchant K, Michelson AD, et al. The Ultegra rapid platelet-function assay: comparison to standard platelet function assays in patients undergoing percutaneous coronary intervention with abciximab therapy. American heart journal. 2002;143(4):602-11.
177. van Werkum JW, van der Stelt CA, Seesing TH, Hackeng CM, ten Berg JM. A head-to-head comparison between the VerifyNow P2Y12 assay and light transmittance aggregometry for monitoring the individual platelet response to clopidogrel in patients undergoing elective percutaneous coronary intervention. Journal of thrombosis and haemostasis : JTH. 2006;4(11):2516-8.
178. von Beckerath N, Pogatsa-Murray G, Wieczorek A, Sibbing D, Schomig A, Kastrati A. Correlation of a new point-of-care test with conventional optical aggregometry for the assessment of clopidogrel responsiveness. Thrombosis and haemostasis. 2006;95(5):910-1.
179. Jakubowski JA, Payne CD, Li YG, Brandt JT, Small DS, Farid NA, et al. The use of the VerifyNow P2Y12 point-of-care device to monitor platelet function across a range of P2Y12 inhibition levels following prasugrel and clopidogrel administration. Thrombosis and haemostasis. 2008;99(2):409-15.
180. Sibbing D, Braun S, Jawansky S, Vogt W, Mehilli J, Schomig A, et al. Assessment of ADP-induced platelet aggregation with light transmission

aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. Thrombosis and haemostasis. 2008;99(1):121-6.
181. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. The New England journal of medicine. 2011;364(18):1746-60.
182. Loeffen R, Spronk HM, ten Cate H. The impact of blood coagulability on atherosclerosis and cardiovascular disease. Journal of thrombosis and haemostasis : JTH. 2012;10(7):1207-16.
183. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000;407(6801):258-64.
184. Borissoff JI, Spronk HM, Heeneman S, ten Cate H. Is thrombin a key player in the 'coagulation-atherogenesis' maze? Cardiovascular research. 2009;82(3):392-403.
185. Dangas GD, Caixeta A, Mehran R, Parise H, Lansky AJ, Cristea E, et al. Frequency and predictors of stent thrombosis after percutaneous coronary intervention in acute myocardial infarction. Circulation. 2011;123(16):1745-56.
186. de la Torre-Hernandez JM, Alfonso F, Hernandez F, Elizaga J, Sanmartin M, Pinar E, et al. Drug-eluting stent thrombosis: results from the multicenter Spanish registry ESTROFA (Estudio ESpanol sobre TROmbosis de stents FArmacoactivos). Journal of the American College of Cardiology. 2008;51(10):986-90.
187. van Werkum JW, Heestermans AA, de Korte FI, Kelder JC, Suttorp MJ, Rensing BJ, et al. Long-term clinical outcome after a first angiographically confirmed coronary stent thrombosis: an analysis of 431 cases. Circulation. 2009;119(6):828-34.
188. Hemker HC, Giesen P, AlDieri R, Regnault V, de Smed E, Wagenvoord R, et al. The calibrated automated thrombogram (CAT): a universal routine test for hyper- and hypocoagulability. Pathophysiol Haemost Thromb. 2002;32(5-6):249-53.
189. Oldgren J, Budaj A, Granger CB, Khder Y, Roberts J, Siegbahn A, et al. Dabigatran vs. placebo in patients with acute coronary syndromes on dual antiplatelet therapy: a randomized, double-blind, phase II trial. European heart journal. 2011;32(22):2781-9.
190. Steg PG, Mehta SR, Jukema JW, Lip GY, Gibson CM, Kovar F, et al. RUBY-1: a randomized, double-blind, placebo-controlled trial of the safety and tolerability of the novel oral factor Xa inhibitor darexaban (YM150) following acute coronary syndrome. European heart journal. 2011;32(20):2541-54.
191. Goldstein S, Bates ER, Bhatt DL, Cao C, Holmes D, Kupfer S, et al. Phase 2 study of TAK-442, an oral factor Xa inhibitor, in patients following acute coronary syndrome. Thrombosis and haemostasis. 2014;111(6):1141-52.
192. Alexander JH, Becker RC, Bhatt DL, Cools F, Crea F, Dellborg M, et al. Apixaban, an oral, direct, selective factor Xa inhibitor, in combination with antiplatelet therapy after acute coronary syndrome: results of the Apixaban for Prevention of Acute Ischemic and Safety Events (APPRAISE) trial. Circulation. 2009;119(22):2877-85.
193. Alexander JH, Lopes RD, James S, Kilaru R, He Y, Mohan P, et al. Apixaban with antiplatelet therapy after acute coronary syndrome. The New England journal of medicine. 2011;365(8):699-708.
194. Mega JL, Braunwald E, Mohanavelu S, Burton P, Poulter R, Misselwitz F, et al. Rivaroxaban versus placebo in patients with acute coronary syndromes (ATLAS ACS-TIMI 46): a randomised, double-blind, phase II trial. Lancet. 2009;374(9683):29-38.

195. Gibson CM, Mega JL, Burton P, Goto S, Verheugt F, Bode C, et al. Rationale and design of the Anti-Xa therapy to lower cardiovascular events in addition to standard therapy in subjects with acute coronary syndrome-thrombolysis in myocardial infarction 51 (ATLAS-ACS 2 TIMI 51) trial: a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of rivaroxaban in subjects with acute coronary syndrome. American heart journal. 2011;161(5):815-21 e6.
196. Mega JL, Braunwald E, Wiviott SD, Bassand JP, Bhatt DL, Bode C, et al. Rivaroxaban in patients with a recent acute coronary syndrome. The New England journal of medicine. 2012;366(1):9-19.
197. Mega JL, Braunwald E, Wiviott SD, Murphy SA, Plotnikov A, Gotcheva N, et al. Comparison of the efficacy and safety of two rivaroxaban doses in acute coronary syndrome (from ATLAS ACS 2-TIMI 51). The American journal of cardiology. 2013;112(4):472-8.
198. Sardella G, Calcagno S, Mancone M, Lucisano L, Pennacchi M, Stio RE, et al. Comparison of therapy with Ticagrelor, Prasugrel or high Clopidogrel dose in PCI patients with high on treatment platelet reactivity and genotype variation. TRIPLETE RESET trial. International journal of cardiology. 2015;194:60-2.
199. Lemesle G, Schurtz G, Bauters C, Hamon M. High on-treatment platelet reactivity with ticagrelor versus prasugrel: a systematic review and meta-analysis. Journal of thrombosis and haemostasis : JTH. 2015;13(6):931-42.
200. Lhermusier T, Lipinski MJ, Tantry US, Escarcega RO, Baker N, Bliden KP, et al. Meta-analysis of direct and indirect comparison of ticagrelor and prasugrel effects on platelet reactivity. The American journal of cardiology. 2015;115(6):716-23.
201. Perl L, Zemer-Wassercug N, Rechavia E, Vaduganathan M, Orvin K, Weissler-Snir A, et al. Comparison of platelet inhibition by prasugrel versus ticagrelor over time in patients with acute myocardial infarction. Journal of thrombosis and thrombolysis. 2015;39(1):1-7.
202. Gurbel PA, Tantry US. Clopidogrel resistance? Thrombosis research. 2007;120(3):311-21.
203. Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. The American journal of cardiology. 2001;88(3):230-5.
204. Mansour K, Taher AT, Musallam KM, Alam S. Aspirin resistance. Advances in hematology. 2009;2009:937352.
205. Wegert W, Graff J, Kaiser D, Breddin HK, Klinkhardt U, Harder S. Effects of antiplatelet agents on platelet-induced thrombin generation. Int J Clin Pharmacol Ther. 2002;40(4):135-41.
206. Smid M, Dielis AW, Spronk HM, Rumley A, van Oerle R, Woodward M, et al. Thrombin generation in the Glasgow Myocardial Infarction Study. PloS one. 2013;8(6):e66977.
207. Tobin WO, Kinsella JA, Kavanagh GF, O'Donnell JS, McGrath RA, Collins DR, et al. Longitudinal assessment of thrombin generation potential in response to alteration of antiplatelet therapy after TIA or ischaemic stroke. Journal of neurology. 2013;260(2):590-6.
208. Smid M, Dielis AW, Winkens M, Spronk HM, van Oerle R, Hamulyak K, et al. Thrombin generation in patients with a first acute myocardial infarction. Journal of thrombosis and haemostasis : JTH. 2011;9(3):450-6.

209. Aggarwal RK, Ireland DC, Azrin MA, Ezekowitz MD, de Bono DP, Gershlick AH. Antithrombotic potential of polymer-coated stents eluting platelet glycoprotein IIb/IIIa receptor antibody. Circulation. 1996;94(12):3311-7.
210. Wang S, Garner DJ, Romano M, Peng SK, Goldberg SL, French WJ. A Simple, Reproducible Animal Model of Arterial Occlusion with Mixed Thrombus. Journal of thrombosis and thrombolysis. 1996;3(4):337-41.
211. More RS, Rutty G, Underwood MJ, Brack MJ, Gershlick AH. A time sequence of vessel wall changes in an experimental model of angioplasty. The Journal of pathology. 1994;172(3):287-92.
212. Stefanini GG, Holmes DR, Jr. Drug-eluting coronary-artery stents. The New England journal of medicine. 2013;368(3):254-65.
213. Onuma Y, Dudek D, Thuesen L, Webster M, Nieman K, Garcia-Garcia HM, et al. Five-year clinical and functional multislice computed tomography angiographic results after coronary implantation of the fully resorbable polymeric everolimus-eluting scaffold in patients with de novo coronary artery disease: the ABSORB cohort A trial. JACC Cardiovascular interventions. 2013;6(10):999-1009.
214. Diletti R, Farooq V, Girasis C, Bourantas C, Onuma Y, Heo JH, et al. Clinical and intravascular imaging outcomes at 1 and 2 years after implantation of absorb everolimus eluting bioresorbable vascular scaffolds in small vessels. Late lumen enlargement: does bioresorption matter with small vessel size? Insight from the ABSORB cohort B trial. Heart. 2013;99(2):98-105.
215. Stefanini GG, Byrne RA, Serruys PW, de Waha A, Meier B, Massberg S, et al. Biodegradable polymer drug-eluting stents reduce the risk of stent thrombosis at 4 years in patients undergoing percutaneous coronary intervention: a pooled analysis of individual patient data from the ISAR-TEST 3, ISAR-TEST 4, and LEADERS randomized trials. European heart journal. 2012;33(10):1214-22.
216. Abizaid A, Ribamar Costa J, Jr., Bartorelli AL, Whitbourn R, van Geuns RJ, Chevalier B, et al. The ABSORB EXTEND study: preliminary report of the twelve-month clinical outcomes in the first 512 patients enrolled. EuroIntervention : journal of EuroPCR in collaboration with the Working Group on Interventional Cardiology of the European Society of Cardiology. 2015;10(12):1396-401.
217. Brugaletta S, Gori T, Low AF, Tousek P, Pinar E, Gomez-Lara J, et al. Absorb bioresorbable vascular scaffold versus everolimus-eluting metallic stent in ST-segment elevation myocardial infarction: 1-year results of a propensity score matching comparison: the BVS-EXAMINATION Study (bioresorbable vascular scaffold-a clinical evaluation of everolimus eluting coronary stents in the treatment of patients with ST-segment elevation myocardial infarction). JACC Cardiovascular interventions. 2015;8(1 Pt B):189-97.
218. Ormiston JA, Serruys PW, Regar E, Dudek D, Thuesen L, Webster MW, et al. A bioabsorbable everolimus-eluting coronary stent system for patients with single de-novo coronary artery lesions (ABSORB): a prospective open-label trial. Lancet. 2008;371(9616):899-907.
219. Diletti R, Karanasos A, Muramatsu T, Nakatani S, Van Mieghem NM, Onuma Y, et al. Everolimus-eluting bioresorbable vascular scaffolds for treatment of patients presenting with ST-segment elevation myocardial infarction: BVS STEMI first study. European heart journal. 2014;35(12):777-86.
220. Kraak RP, Hassell ME, Grundeken MJ, Koch KT, Henriques JP, Piek JJ, et al. Initial experience and clinical evaluation of the Absorb bioresorbable vascular scaffold (BVS) in real-world practice: the AMC Single Centre Real World PCI

Registry. EuroIntervention : journal of EuroPCR in collaboration with the Working Group on Interventional Cardiology of the European Society of Cardiology. 2015;10(10):1160-8.
221. Kocka V, Maly M, Tousek P, Budesinsky T, Lisa L, Prodanov P, et al. Bioresorbable vascular scaffolds in acute ST-segment elevation myocardial infarction: a prospective multicentre study 'Prague 19'. European heart journal. 2014;35(12):787-94.
222. Gori T, Schulz E, Hink U, Wenzel P, Post F, Jabs A, et al. Early outcome after implantation of Absorb bioresorbable drug-eluting scaffolds in patients with acute coronary syndromes. EuroIntervention : journal of EuroPCR in collaboration with the Working Group on Interventional Cardiology of the European Society of Cardiology. 2014;9(9):1036-41.
223. Swanson N, Hogrefe K, Javed Q, Malik N, Gershlick AH. Vascular endothelial growth factor (VEGF)-eluting stents: in vivo effects on thrombosis, endothelialization and intimal hyperplasia. J Invasive Cardiol. 2003;15(12):688-92.
224. Kinlough-Rathbone RL, Rand ML, Packham MA. Rabbit and rat platelets do not respond to thrombin receptor peptides that activate human platelets. Blood. 1993;82(1):103-6.
225. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR journal / National Research Council, Institute of Laboratory Animal Resources. 2002;43(4):244-58.
226. Chandler AB. In vitro thrombotic coagulation of the blood; a method for producing a thrombus. Laboratory investigation; a journal of technical methods and pathology. 1958;7(2):110-4.
227. Sunamura M, di Mario C, Piek JJ, Schroeder E, Vrints C, Probst P, et al. Cyclic flow variations after angioplasty: a rare phenomenon predictive of immediate complications. DEBATE Investigator's Group. American heart journal. 1996;131(5):843-8.
228. Yao SK, McNatt J, Cui K, Anderson HV, Maffrand JP, Buja LM, et al. Combined ADP and thromboxane A2 antagonism prevents cyclic flow variations in stenosed and endothelium-injured arteries in nonhuman primates. Circulation. 1993;88(6):2888-93.
229. Folts JD, Crowell EB, Jr., Rowe GG. Platelet aggregation in partially obstructed vessels and its elimination with aspirin. Circulation. 1976;54(3):365-70.
230. Dorler J, Frick M, Hilber M, Breitfuss H, Abdel-Hadi MN, Pachinger O, et al. Coronary stents cause high velocity fluctuation with a flow acceleration and flow reduction in jailed branches: an in vitro study using laser-Doppler anemometry. Biorheology. 2012;49(5-6):329-40.
231. Gogas BD, King SB, 3rd, Timmins LH, Passerini T, Piccinelli M, Veneziani A, et al. Biomechanical assessment of fully bioresorbable devices. JACC Cardiovascular interventions. 2013;6(7):760-1.
232. LaDisa JF, Jr., Olson LE, Guler I, Hettrick DA, Audi SH, Kersten JR, et al. Stent design properties and deployment ratio influence indexes of wall shear stress: a three-dimensional computational fluid dynamics investigation within a normal artery. J Appl Physiol (1985). 2004;97(1):424-30; discussion 16.
233. Kastrati A, Mehilli J, Dirschinger J, Dotzer F, Schuhlen H, Neumann FJ, et al. Intracoronary stenting and angiographic results: strut thickness effect on restenosis outcome (ISAR-STEREO) trial. Circulation. 2001;103(23):2816-21.

234. Evans DJ, Jackman LE, Chamberlain J, Crosdale DJ, Judge HM, Jetha K, et al. Platelet P2Y(12) receptor influences the vessel wall response to arterial injury and thrombosis. Circulation. 2009;119(1):116-22.
235. Udell JA, Bonaca MP, Collet JP, Lincoff AM, Kereiakes DJ, Costa F, et al. Long-term dual antiplatelet therapy for secondary prevention of cardiovascular events in the subgroup of patients with previous myocardial infarction: a collaborative meta-analysis of randomized trials. Eur Heart J. 2016;37(4):390-9.
236. Navarese EP, Andreotti F, Schulze V, Kolodziejczak M, Buffon A, Brouwer M, et al. Optimal duration of dual antiplatelet therapy after percutaneous coronary intervention with drug eluting stents: meta-analysis of randomised controlled trials. BMJ. 2015;350:h1618.
237. Malik N, Banning AS, Belger M, Fakhouri W, Graham-Clarke PL, Banning A, et al. A risk scoring system to predict coronary stent thrombosis. Curr Med Res Opin. 2017;33(5):859-67.