# Markers of risk in patients with acute coronary syndrome treated by percutaneous coronary intervention

Ian Russell Pearson MB ChB MRCP

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**Declaration of Originality**

**"I confirm that the work submitted is my own and that appropriate credit has been given where reference has been made to the work of others."**



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**Ian Pearson**

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## ABSTRACT

**Background -** The clinical diagnosis and categorisation of Acute Coronary Syndrome (ACS) has changed repeatedly over the last decade as have routine treatment strategies.

**Hypothesis** - that adverse clinical events following PCI, may be predicted from the identification of markers of risk at the time of PCI.

**Methods -** Informed consent was obtained from 968 patients fulfilling detailed inclusion and exclusion criteria surrounding a diagnosis of ACS requiring PCI. Standard medical ACS care was provided. PCI operators, techniques, methods and any decision to treat followed usual practice. Data collection took place at the time of intervention and during active follow-up. Blood sample were collected at baseline and 4 and 12 hours after PCI, being processed and refrigerated. Platelet function was assessed at baseline using the VerifyNow test method.

**Results –** Data collection was over a median follow-up time of 3.56 years. Patients were aged 27 to 90 years and a majority were male (75%). Angiographic complications occurred in 13.2% and total complications in 17.1%. A majority (844; 86%) had neither restenosis nor subsequent unplanned revascularization. Recurrent ACS was 6.7% for year 1 and 1.8% additionally for each year thereafter. Stent thrombosis was observed in 18 (1.8%) cases. Bleeding occurred in 9% across the entire follow-up period, being greatest in the first 12 months. Platelet reactivity was highly variable and optimal with regard to outcome in the range of 179 to 243 (Platelet Reactivity Units PRU). Cardiac biomarkers were commonly elevated after PCI but procedural MI was very rare. H-FABP at baseline was strongly predictive of outcome.

**Conclusion –** Adverse clinical events following PCI, such as stent thrombosis, bleeding and in-stent restenosis, may be predicted from the identification of markers of risk at the time of PCI, particularly by the use of risk scores, platelet function testing and measuring biomarker levels.

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# GLOSSARY OF TERMS, ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| **ACS** | Acute Coronary Syndrome: a clinical syndrome characterised by chest pain and other symptoms due to myocardial ischaemia; includes unstable angina, non ST segment-elevation myocardial infarction and ST segment-elevation myocardial infraction |
| **ADP** | Adenosine diphosphate: an organic compound involved in many metabolic pathways; has an important role in the platelet activation/propagation cascade required for arterial thrombosis, essential for normal haemostasis following tissue injury; stored in platelet 'dense granules'; binds to platelet membrane via receptors including $P2Y_{12}$ and $P2Y_1$ to induce activation |
| **Angiogram** | An invasive diagnostic procedure in which an X-ray image of an arterial lumen is obtained by injecting radio-opaque contrast medium, usually iodine-based, into an artery via a long catheter. Coronary angiography is used to diagnose coronary artery disease, with coronary catheterization achieved percutaneously via the femoral or radial arteries |
| **ARC** | Academic Research Consortium: a group of international experts and industry leaders who have published criteria to define cardiovascular adverse endpoints such as cardiac death, MI and stent thrombosis for the purpose of clarity and reproducibility among clinical trials |
| **Atherosclerosis** | A range of pathological degenerative changes which develop in arteries throughout the body with advancing age; characterised by the combination of fatty deposits and sclerosis (hardening) due to fibrosis and calcification; the cause of end-organ ischaemic damage due to arterial stenosis (narrowing) and/or thrombosis. Coronary atherosclerosis is the commonest cause of myocardial infarction |
| **BARC** | Bleeding Academic Research Consortium: a group of international experts and industry leaders who have published criteria to define the clinical severity of bleeding for the purpose of clarity and reproducibility among clinical trials; graded from BARC 1 (minor) to BARC 5 (fatal) |
| **BMS** | Bare Metal Stent |
| **CABG** | Coronary Artery Bypass Surgery: coronary revascularization in which a coronary stenosis is bypassed by the surgical formation of a conduit (graft) between the aorta and the coronary artery distal to the stenosis; usually achieved via a median sternotomy with the patient on cardiac bypass with cross-clamping of the aorta; grafts are fashioned from the patients' superficial leg veins (saphenous veins), internal mammary arteries or radial arteries |
| **CA III** | Carbonic Anhydrase III; an enzyme found only in skeletal muscle |
| **CK-MB** | Creatine Kinase BM isoenzyme; an enzyme found almost exclusively in cardiac muscle; a 'cardio specific' cardiac biomarker |
| **Convalescent PCI** | Non-emergent PCI following presentation with STEMI following chemical revascularisation by antiplatelet and thrombolytic therapy |

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| **CoV** | Coefficient of Variation: the ratio of the standard deviation and the mean of a population; a measure of dispersion and the accepted method for measuring the precision of a test; good biochemical assays should have a CoV of < 10% at the 99$^{th}$ percentile of a healthy population |
| **CTO** | Chronic Total Occlusion: a coronary artery which has been blocked for more than 3 months |
| **DES** | Drug-eluting stent |
| **EuroSCORE** | A risk scoring system to estimate the pre-treatment probability of surviving cardiac surgery generated from the historical results of thousands of patients at many European centres; calculated from the sum of points allocated for adverse baseline characteristics |
| **ECG** | Electrocardiograph: a recording of the electrical activity of the heart produced using electrodes to measure changes in the electrical potential of the skin caused by myocardial depolarization and repolarisation with each heart beat. Characteristic dynamic ECG changes occur during myocardial ischaemia and infarction, such as ST segment depression or elevation |
| **GPBB** | Glycogen phosphorylase isoenzyme BB; a cardiac biomarker |
| **H-FABP** | Human Fatty Acid Binding Protein: a cardiac biomarker |
| **MI** | Myocardial Infarction: death of cardiac myocytes, usually due to ischaemia resulting from coronary atherosclerotic disease; the commonest cause of death worldwide |
| **NSTEMI** | Non ST elevation myocardial infarction: cardiomyocyte necrosis due to ischaemia without ECG ST segment elevation; usually caused by a severely stenotic and/or thrombotic atherosclerotic coronary artery lesion; investigated by early inpatient coronary angiography |
| **MACE, MACCE** | Major Adverse Cardiac Event; Major Adverse Cardiac and Cerebrovascular Event: composite adverse endpoint commonly reported in clinical trials of revascularisation; endpoint usually includes the combination of MI, death (usually cardiac) stent thrombosis and unplanned further revascularisation, sometimes include 'procedural MI' based on cardiac enzyme levels, with the addition of stroke in MACCE; composite endpoints are slightly different between studies and therefore should not be directly compared |
| **Myoglobin** | A non-cardio specific cardiac biomarker |
| **PCI** | Percutaneous Coronary Intervention: any procedure involving the instrumentation of the coronary arteries by means of a cardiac catheter which is introduced by cannulation of a peripheral artery |
| **PES** | Paclitaxel-Eluting Stent; usually refers to Taxus 'lst generation' DES |
| **PPCI** | Primary Percutaneous Coronary Intervention: emergency PCI for STEMI |
| **PRU** | $P2Y_{12}$ Reactivity Units: a measure of the degree of platelet reactivity in response to ADP produced by the VerifyNow device; a low PRU level |

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| | implies a high level of pharmacological $P2Y_{12}$ receptor inhibition i.e. good response to clopidogrel, prasugrel or ticagrelor |
| **$P2Y_{12}$ receptor** | A large molecule found on the surface of platelets with a binding site for ADP; ADP binding leads to platelet activation and arterial thrombus formation; the target of several common drugs used for prevention of coronary thrombosis including clopidogrel, prasugrel and ticagrelor |
| **Rescue PCI** | Emergent PCI for STEMI when pharmacological thrombolysis has been attempted but has been unsuccessful i.e. failure of resolution of ECG ST segment elevation |
| **Revascularisation** | Restoration of an adequate arterial blood supply to an ischaemic organ |
| **SES** | Sirolimus-Eluting Stent; usually refers to the Cypher '1$^{st}$ generation' DES |
| **ST** | Stent Thrombosis: sudden thrombotic occlusion of a stent associated with a high rate of MI and death |
| **Staged PCI** | PCI to multiple lesions undertaken at more than 1 sitting |
| **STEMI** | ST elevation myocardial infarction: cardiomyocyte necrosis due to ischaemia with ECG ST segment elevation; usually caused thrombotic occlusion of a large epicardial coronary artery at the site of a ruptured atherosclerotic plaque; requires emergency coronary revascularisation by thrombolysis and/or PPCI |
| **SYNTAX score** | Angiographic grading system published in 2005 to objectively measure the extent and severity of coronary artery disease; developed by investigators at the Thoraxcenter, Erasmus Medical Centre, the Netherlands, headed by senior investigator Prof. Patrick Serruys. Points are assigned for each stenosis of > 50% in arteries of ≥ 1.5 mm, weighted more heavily for proximal disease in major vessels, with additional points for adverse features such as chronic total occlusion and diffuse disease. The score has been validated in patients undergoing PCI to show that higher scores are associated with higher rates of adverse cardiovascular events. |
| **TnI** | Troponin I; an enzyme found exclusively in cardiac muscle; a 'cardio-specific' cardiac biomarker |
| **ULN** | Upper Limit of Normal: cut-off level for the normal range of a substance measured in blood plasma, such as a cardiac biomarker; usually defined as the 99$^{th}$ percentile of a healthy population |
| **VerifyNow test** | Rapid bedside platelet function test based on light transmission |

# Chapter 1 Introduction and literature review

This introductory chapter is an overview of the aetiology and contemporary diagnosis and management of coronary artery disease and the acute coronary syndromes. 'Acute coronary syndrome' (ACS) is a clinical diagnosis given to patients who present with rapidly progressive or at-rest symptoms suggestive of myocardial ischaemia which is supported by positive results from a range of clinical tests. The most common symptom is a discomfort felt in the upper body, particularly the chest, known as 'angina pectoris'. The diagnosis of ACS constitutes a medical emergency as patients are at risk of myocardial infarction and sudden death. Prompt treatment both reduces the risk of death and limits the extent of myocardial necrosis and permanent impairment of cardiac function in survivors. The commonest underlying disorder found in people with ACS is atherosclerotic coronary artery disease, which remains the commonest cause of death worldwide, with 7.4 million deaths in 2012 (WHO online fact sheet number 310, 2014).

## 1.1. Coronary atherosclerosis

Atherosclerosis is a systemic disorder of arteries characterised by the presence of fibro-fatty plaques and calcification of the arterial wall. This can lead to arterial thrombosis, compromise of blood flow and ischaemic dysfunction or infarction of the downstream organ or tissue. Lloyd-Jones et al (1999) found that the lifetime risk of developing clinical coronary heart disease (angina, acute myocardial infarction or cardiac death), measured from the age of 40 years in 7733 healthy participants in the landmark Framingham Heart Study, was 48.5% for men and 31.7% for women. This population was almost exclusively white and from the same town in North America. At this time a number of risk factors for the condition were identified, known as 'major' or 'classical' cardiovascular risk factors. Non-modifiable risk factors include positive family history and increasing age; modifiable factors include tobacco smoking, hypertension, hyperlipidaemia, diabetes mellitus and a sedentary lifestyle.

### 1.1.1. Prevalence of coronary atherosclerosis

Coronary atherosclerosis develops during an initial asymptomatic (subclinical) phase and may remain clinically silent during an individual's entire lifetime, so the true prevalence of anatomical disease cannot be quantified merely by observing the rate of clinical events. However, there are several studies involving very large numbers of asymptomatic patients who have self-referred for screening for coronary artery calcium, which is deposited in atherosclerotic plaque, using the sensitive technique of computed

tomography calcium scoring. These studies were performed in the USA in the early 1990s, mainly on white individuals.

The coronary calcium score was found to vary widely with age, between the 2 sexes and between subjects with and without the conventional cardiovascular risk factors. In a study by Janowitz et al, 1993, the prevalence of calcium was seen to gradually increase with age. The prevalence in women was about half that found in men until the age of 60 years after which this difference diminished. In male and female subjects between the ages of 0 and 29 years the prevalence was 11% and 6%; in male and female subjects between the ages of 50 and 59 years the prevalence was 72% and 35%; and in subjects between the ages of 80 and 89 years the prevalence was 100% in both sexes.

In other words, if human beings live long enough they will all eventually develop at least some degree of coronary atherosclerosis. This only becomes clinically apparent, however, in about half of men and a third of women.

### 1.1.2. Pathogenesis of atherosclerotic coronary disease

While there are well-established risk factors for the development of coronary disease, no single common aetiological agent of atherogenesis has been identified. Extensive study over decades at the cellular and now molecular level has lead to the current hypothesis of the atherosclerotic plaque as largely the result of a chronic inflammatory response to repeated or sustained injury to the vascular endothelium, associated with lipid accumulation in the arterial wall. The pathophysiology of coronary atherosclerosis is explored in an extensive review by Libby et al, 2005.

#### 1.1.2.1. A disorder of lipid storage?

Lipid accumulation in the arterial wall was recognised early on as a crucial component of atherogenesis and is known to start very early in life. Although the clinical manifestations of coronary atherosclerosis are rare before the age of around 30 years, histological pre-atherosclerotic changes can be identified even in infants. The earliest manifestation of atheromatous disease is the presence in the arterial wall of lipid-filled ‘foam cells’. As demonstrated by Gerrity et al in 1981, foam cells are macrophages, thought to be derived from blood monocytes, which have scavenged and stored lipid, particularly low density lipoprotein (LDL) particles. However, it is still not clear whether lipid accumulation in the early stages of life is a passive process of diffusion from the bloodstream, or whether there is a pathological mechanism of active lipid transportation. Both of these mechanisms of lipid deposition are known to occur.

By puberty, most children have increasing numbers of foam cells in their arteries accompanied by accumulations of extracellular lipid. These are visible macroscopically and known as fatty streaks. Some individuals at this age have already developed atheromata, characterised by a single confluent fatty core. Smooth muscle cells proliferate in these lesions and secrete an extracellular matrix to create a fibrotic cap, which can be identified in individuals from the age of 20 years onwards. *[Notes: **macrophages** are white blood cells which colonise injured/inflamed tissue, induce connective tissue proliferation and have a central role in wound healing. **Lipoproteins** are particles of cholesterol and triglycerides enclosed by a thin layer of phospholipid and an apolipoprotein molecule. **Phospholipids** are amphipathic molecules, consisting of a hydrophobic tail binding with the lipid, and a hydrophilic head which faces outwards, making lipoproteins water-soluble for transportation in the bloodstream]*.

For some years, therefore, atherosclerosis was considered primarily a disease of aberrant lipid storage. However, additional pathological processes were noted and it was realised that the disorder had a more complex pathophysiology. These discoveries were outlined by Ross in a review article of 1986, with a further update in 2010, in which evidence is presented for **vascular injury** as an early step in atherogenesis, resulting in an inflammatory response in the vessel wall, endothelial dysfunction and endothelial cell death.

### 1.1.2.2. Endothelial injury and dysfunction

Vascular endothelial cells line arteries in a delicate monolayer and perform a pivotal role in vascular homeostasis. The endothelium is involved in the regulation of vascular tone, inflammation, coagulation, platelet activation and the activities of vascular smooth muscle cells, co-ordinated by multiple signalling pathways. With all these paracrine and autocrine functions, a cell count of $6\times10^{13}$, a surface area of >1000 $m^2$ and a weight of 1.5 kg, the endothelium may be regarded as an autonomous organ.

During normal life, the vascular endothelium is subject to damage inflicted by the combination of chronic mechanical stress in the pressurised arterial system over time and exposure to substances carried in the blood which are toxic to endothelial cells. The interaction of injured endothelial cells with the inflammatory, immune and anticoagulation systems interacting with accumulated lipid within the arterial wall can, over a period of decades, develop into a variety of different types of atherosclerotic plaque. (Anderson et al, 1995; Kinlay and Ganz, 1997).

Endothelial dysfunction has been demonstrated in numerous studies to be associated with the presence of atherosclerotic plaque. Dysfunction can be detected

clinically by observing an abnormal vasomotor response to different stimuli. The smooth muscle in coronary arteries enables dilatation and constriction, allowing for autoregulation of myocardial blood flow in response to changes in metabolic demand. Hasdai et al (1997) showed that patients with angiographically minor coronary disease develop myocardial perfusion defects on nuclear perfusion scanning following the infusion of acetylcholine (ACh) into the left anterior descending artery (LAD) due to coronary vasospasm; this procedure usually induces vasodilatation in healthy arteries. Schachinger et al (2000), using various tests of vasoreactivity, found that impaired coronary endothelial vasodilatation was an independent predictor of cardiovascular events during long-term follow up in 147 patients, albeit with a small number of events (16 over 7.7 years).

Endothelial injury leads to the 'activation' of endothelial cells: the expression of vascular cell adhesion molecules (VCAMs) capable of interacting with circulating leucocytes and platelets. VCAMs are up-regulated in the presence of inflammatory cytokines including IL-1, TNF-α and IFN-γ, both in vitro and in vivo. Activated endothelial cells also bind to, ingest and modify circulating plasma lipoproteins to enable ingestion by subendothelial monocytes. Oxidisation of subendothelial LDL by oxygen free radicals initiates a complex cascade of pro-inflammatory effects including further accumulation of cells (monocytes, CD-4 and CD-8 lymphocytes and smooth muscle cells) in the intima, apoptosis of endothelial cells and platelet activation. VCAM-1 in particular has been shown to be strongly linked to atherosclerosis, demonstrated by Cybulsky et al in 2001 in their work on gene knock-out mice.

### 1.1.3. Causes of vascular injury

Although advancing age is the most powerful risk factor for coronary atherosclerosis, people with atherosclerotic syndromes are more likely to have a family history of the disorder, and recent advances in gene sequencing have allowed the identification of genotypes associated with the disease. Environmental factors interact with genetic factors to determine each individual's risk of developing atherosclerosis.

Environmental factors found to cause vascular endothelial injury in animals include classical vascular risk factors such as raised serum LDL, tobacco toxins and hyperglycaemia, which were identified in studies during the 1950s and 60s such as the Framingham Heart Study (Dawber et al, 1957). However, it is recognised that a proportion of patients in fact have no identifiable classical risk factors. Further markers of atherosclerosis and coronary events were identified subsequently, including hyperhomocystinaemia, Lp(a) lipoprotein, the serum inflammatory markers CRP and serum amyloid protein A, the prothrombotic factors PAI-I, D-dimer, fibrinogen, and

von Willebrand factor, and microalbumineamia (Folsom, 2006). Infectious agents have also been shown to cause endothelial dysfunction, as discussed by Danesh et al in 1997, although their exact role in the clinical manifestations of coronary disease is unclear.

Chronic platelet activation, platelet adherence and thrombosis appear to have a role in endothelial injury and subsequent atheromatous plaque formation. Platelets do not normally adhere to healthy endothelial cells, which secrete platelet inhibiting agents. However, platelets will adhere to activated endothelium, undergoing activation and degranulation. Degranulating platelets release many inflammatory substances required for wound healing including platelet derived growth factor (PDGF), a chemotactic agent, and TGF beta, which stimulates the creation of extracellular matrix. Platelets are discussed further in subsequent sections. The evidence for an aetiological role of platelets in atherosclerotic disease is discussed by Rondina et al, 2013.

Work to identify a common pathophysiological mechanism by which these diverse factors provoke endothelial injury has highlighted the importance of nitric oxide in normal endothelial function. Nitric Oxide (NO) is a humoral signalling agent produced by endothelial cells from the action of nitric oxide synthase (NOS) on L-arginine. NO production is highly regulated in response to haemodynamic and humoral factors and has several vasoprotective effects. It causes arterial vasodilatation (nitrovasodilating drugs such as GTN work by releasing NO within the vasculature), inhibits platelet activation and aggregation and promotes platelet disaggregation, inhibits smooth muscle proliferation and inhibits leukocyte adhesion. It is thought that impaired NO production may be responsible for many of the processes leading to plaque formation and disruption. A review of the evidence for this was written by Napoli et al, 2006.

### 1.1.4. Neovascular vasa vasorum in atheromatous plaque

‘Vasa vasorum’ is the term given to a network of small blood vessel providing the vascular supply to a larger blood vessel. The human aorta has a vasa vasorum extending distally as far as the level of the renal arteries; distal to this, the metabolic requirements of the aorta are met by diffusion of oxygen and nutrients (interestingly the incidence of aortic aneurysm formation is greater below this level). The coronary arteries also have a vasa vasorum. Pathological neovascularisation – the growth of abnormal micro vessels within the adventitia - has been well documented in atherosclerotic coronary arteries and is thought to have an important role in both plaque growth and rupture, first proposed by Barger et al in 1984.

### 1.1.5. Mechanical factors

Mechanical factors have a crucial role in atherogenesis. Arterial hypertension is a well established classical vascular risk factor, strongly associated with atherosclerosis, as demonstrated in the Framingham studies. Blood pressure is regulated by the renin-angiotensin-aldosterone system. Elevated aldosterone levels have been shown to correlate with adverse clinical outcomes in ACS patients, independent of other risk factors, as shown in studies such as the one by Tomaschitz et al, 2010. Treatment to lower blood pressure by the use of antihypertensive drugs which act on the rennin-angiotensin-aldosterone system results in decreased frequency of vascular events. This was shown by Hall et al in 1993 with the AIREX trial, which studied the effects of ramipril on mortality after ACS compared to placebo over 3 years.

In addition, variations in endothelial shear stress within the vessel appear to have a role in the location of plaque formation; for example, stenoses occur more commonly at branch points where there are changes in blood velocity and direction. Furthermore, the coronary vasculature is subject to external mechanical forces unique to the heart – the heart beat itself produces in the coronary arteries marked and sustained torsion and stretching forces, again particularly at branch points. This was explored in detail in a review by Chatzizisis et al, 2007. Furthermore, haemodynamic instability from a range of factors is often observed in the days leading up to an ACS. It is thought that changes in coronary endothelial shear stress during these trigger events can mechanically disrupt a pre-existing inflammatory plaque and give rise to ACS. For example, Tanaka et al (2008) demonstrated that this depends on atherosclerotic plaque morphology and is particularly likely if the plaque has a thin fibrous cap. This leads us on to a more detailed discussion of the pathophysiological causes of ACS.

## 1.2. The Acute Coronary Syndromes

Coronary atherosclerosis may be asymptomatic throughout an individual's lifetime, or may give rise to several different clinical syndromes including chronic stable angina (CSA), acute coronary syndrome (ACS), acute or chronic cardiac failure, the development of arrhythmias and sudden cardiac death. Chest pain is a common but not universal feature of these coronary syndromes. The type of syndrome experienced by a patient depends to a large extent on differences in morphology of atherosclerotic plaque. The clinical syndromes of ACS may be caused by one or more of a wide range of pathological mechanisms, with atherosclerosis being central or at least contributory to most cases.

### 1.2.1. Coronary thrombosis and plaque rupture.

The pathological mechanism responsible for most cases of ACS is thought to be the formation of thrombosis within the arterial lumen adjacent to an atheromatous plaque (which may have been silently present for many years), often in response to a haemodynamic or inflammatory trigger. Coronary atherosclerotic plaques tend to grow outwards into the peri-arterial tissue, an effect termed 'arterial positive remodelling'; and often cause no obstruction to the flow of blood along the artery. Sometimes, particularly at certain points of haemodynamic stress along the artery, a plaque will gradually grow inwards to encroach on the arterial lumen causing a narrowing (stenosis) sufficient to provoke myocardial ischaemia on physical exertion: the clinical syndrome of chronic stable angina. However, certain types of plaque, often non-stenotic, may undergo rapid alterations in structure ('unstable' plaques) which can induce intra-arterial thrombus formation. Coronary thrombosis can lead to unstable, rapidly progressing or rest symptoms referred to as ACS; or sudden cardiac death. This process is discussed in detail by Libby in his 2001 review article.

Coronary thrombosis causing sudden occlusion of a coronary artery can cause death of cardiac myocytes, myocardial infarction (MI). Total thrombotic occlusion of a large epicardial artery by thrombus produces the syndrome of ST segment elevation MI (STEMI), characterised by sudden ischaemia of a large portion of the myocardium. The volume of myocardium under threat depends on the extent of the territory supplied by the artery, the relative size and patency of the other major coronary vessels (both of which factors are highly variable) and the presence of collateral arterial supply. All other types of myocardial infarction are known as non ST elevation MIs (NSTEMI). The occurrence of unstable symptoms of myocardial ischaemia in the absence of myocardial necrosis is known as unstable angina (UA).

Coronary thrombosis is a common cause of sudden cardiac death. Burke et al in 1997 performed post mortem studies of the coronary arteries of 113 men who had a sudden unexplained death, all with known stable coronary disease, mean age 50±10 years. Fresh coronary thrombus was seen in 59 cases. 41 had 'plaque ruptures': luminal thrombus continuous with underlying lipid core; and 18 had 'plaque erosions': thrombus in direct contact with a plaque but without rupture of a lipid pool on serial sections. The remaining 54 cases had a coronary artery stenosis of greater than 75% but without identifiable thrombus. Unstable plaques are characterised by particular microscopic features: there is often a large pool of necrotic, lipid-rich material; there is often a thin fibrous cap; and there is often 'neovascularisation' within the plaque associated with plaque haemorrhage.

### 1.2.2. Triggers of onset of ACS

Coronary atherosclerosis is very widespread in the population, but myocardial infarction occurs only in certain individuals and usually infrequently. This has led to a search for triggers of rupture of a previously stable coronary artery plaque. In a review article on triggers of ACS, Servoss et al wrote in 2002:

"The association between external triggers and MI onset (relative risk of 2-fold to over 20-fold) is well beyond what is to be expected by chance alone, and its magnitude is comparable with that of the known long-term risk factors of cardiac disease. In aggregate, the known triggers of onset appear to be responsible for at least 20% of acute coronary syndromes."

In 2011 Nawrot et al performed an analysis of the published data on documented triggers of non fatal MI to create a 'population-attributable risk' for each factor. This value was calculated from both the magnitude of the effect of exposure to the factor on an individual and its prevalence within the population, and indicates the proportion of cases which would be avoided if the risk was no longer present. In order of decreasing magnitude of effect, these factors (population-attributable risk) included traffic exposure/air pollution (7.4%), physical exertion (6.2%), alcohol (5%), coffee (5%), anger (3.1%), heavy meal (2.7%), cocaine use (0.9%) and respiratory infections (0.6%). Cocaine was the factor most likely to trigger an ACS within an individual (probably by triggering coronary artery spasm), but traffic exposure has a much greater effect at a population level. The included studies were mostly of case-crossover design, which has the ability to control for confounding, increasing the strength of the analysis. Limitations of the study include the fact that the data for several triggers, for example alcohol consumption, were from a single study; and the trigger with the greatest effect, traffic exposure, could be considered a composite effect of air pollution and emotional stress.

External triggers of MI are not the same as the risk factors for atherosclerosis, and the same factor can have opposite effects on the two processes. For example, sudden physical exercise can trigger an MI, yet it has been well established that regular moderately strenuous exercise can impede the progression of cardiovascular disease.

### 1.2.3. Arterial versus venous thrombosis

Arterial thrombosis is an essential survival mechanism to prevent exsanguination and enable repair of tissue following injury. Coronary thrombosis is an undesirable consequence of this mechanism. It has been recognized for over 150 years that the risk of thrombosis within any blood vessel is dependent on the three-way interaction

between the coagulability of blood, the blood vessel wall and changes in blood flow. An ACS may be precipitated by any combination of factors causing any or all of these processes. This concept is commonly known as Virchow's triad (although not exactly described in this way in Rudolph Virchow's 1850s work on venous thrombosis and pulmonary embolism). His contribution to the understanding of thrombosis is described by Kumar et al, 2010. In contrast to venous thrombosis, stasis of blood is not the most important factor in arterial thrombosis; nevertheless the concept can still provide a framework to outline this complex area.

#### 1.2.3.1. Coagulability and inflammation

The coagulability (propensity to clot) of blood is determined by the balance of multiple pro- and anticoagulant factors. Platelets are crucial to the formation of arterial thrombus, in contrast to venous thrombosis, and bind to each other and to fibrin to initiate the formation of an arterial clot. Systemic inflammation has a central role in coronary thrombosis: it is associated with platelet activation and with increased blood concentration of numerous pro-coagulant factors. Elevation of blood C-reactive protein (CRP), a general inflammatory marker, has been well documented to be a marker of increased risk in ACS, as discussed by Armstrong et al in 2006. The platelet is central to both haemostasis and inflammation.

Davi and Patrono provide a good summary of the role of platelets in inflammation in their 2007 review. The inflammation and coagulation systems are extremely interconnected and co-dependent. In experimental models, tissue factor (produced in abundance by macrophages in atherosclerotic plaque) is the pivotal initiator of thrombin generation in inflammation. On exposure to blood, tissue factor binds to factor VIIa creating a structure with an expanded 3-D conformation (Banner et al, 1996) with the properties of an enzyme which generates thrombin. Thrombin causes conversion of fibrinogen to fibrin but is also the most powerful known trigger of platelet activation. Platelet activation causes a positive feedback loop of further tissue factor-mediated activation of coagulation, via the expression of P-selectin on platelet membranes. Blood P-selectin levels are increased both during acute coronary syndromes and in systemic inflammation (Polgar et al, 2005). Platelets are discussed further in Section 1.6.

Coagulation induces inflammation in other ways. Coagulation proteases (thrombin, tissue factor-VIIa complex, factor Xa) bind to protease activated receptors (PARs). PARs are G-protein coupled receptors on endothelial cells, mononuclear cells, platelets, fibroblasts and smooth muscle cells, which have a variety of pro-inflammatory affects. For example, in 2003 de Jonge et al demonstrated that administration of recombinant factor VIIa to healthy humans induces a 4-fold rise in plasma IL-6 and IL-

8, important mediators of inflammation. There are many causes of systemic inflammation in addition to acute infection including surgery, trauma, malignancy and autoimmune illnesses, all of which have been shown to be associated with ACS. It is possible that some of the triggers of ACS discussed above induce MI by increases in inflammation and coagulability, such as tobacco and air pollution. However, the most common cause of a systemic inflammatory response is acute infection.

Acute influenza infection can induce inflammatory changes in atherosclerotic plaques in coronary arteries in apolipoprotein E knockout mice which are similar to those seen in coronary plaques following fatal MI (Naghavi et al, 2003). Multiple prothrombotic changes during infection in humans have been observed, even in previously healthy individuals with clinically mild infections. Acute respiratory symptoms precede almost one third of ACS cases; there is a 5-7% incidence of ACS in patients with pneumococcal pneumonia; and MI is 30% more likely during influenza season. As Corrales-Medina et al postulated in their 2010 review, the strength of the evidence supports a causal relationship between acute respiratory infection and ACS.

#### 1.2.3.2. The blood vessel wall in ACS

Early changes which occur in the vascular endothelium, already described, and the interaction of endothelial cells, platelets, smooth muscle cells and macrophages give rise to atheromatous plaque formation. Thin-capped atheromata are prone to rupture, exposing a highly thrombogenic lipid core which causes platelet adhesion and activation (even in fast-flowing arterial blood) and the formation of thrombus and ACS. However, there are several types of systemic vasculitis for which there are reports of coronary artery involvement and which can cause ACS, including Churg-Strauss syndrome (Heleman's et al, 1997) and Wegener's granulomatosis (Goodfield et al, 1995). Systemic vasculitis rarely affects the coronary arteries, with the exception of Kawasaki disease (mucocutaneous lymph node syndrome), a disease of young children of unknown aetiology with frequent coronary artery involvement leading to myocardial infarction and coronary aneurysm formation (Kawasaki et al, 1974; Tsuda 2011).

An often very aggressive coronary vasculitis is seen after cardiac transplantation, termed cardiac allograft vasculopathy, and is the main reason for loss of the graft beyond the acute rejection stage. The immune system response has been thought to be the principle mechanism behind this – a form of chronic rejection – although transplant patients are exposed to many additional sources of potential vascular injury including immunosuppressive drugs, hypertension, hypercholesterolaemia and infections. This is discussed in a review article by Schmauss and Weis, 2008.

An additional plaque-mediated cause of ACS is haemorrhage within the plaque due to rupture of micro vessels, a consequence of pathological neovascularisation, causing rapid reduction in luminal area of the coronary artery and unstable angina. Destabilisation of a plaque due to internal haemorrhage from these abnormal vessels may be the trigger for some cases of plaque rupture, particularly when neovascularisation occurs close to the arterial lumen as observed by Kato et al, 2012, in a study using optical coherence tomography (OCT). It is thought that recurrent micro ruptures of fatty plaques cause progressive thickening (sclerosis) of the arterial wall resulting in the formation of a stenosis (narrowing), resulting in angina or ACS.

Another cause of ACS is coronary artery dissection. This can occur spontaneously or after trauma but also includes iatrogenic dissection from cardiac catheterisation. Haemodynamic shear forces weaken the connection between the different layers of the arterial wall, allowing the formation of a false lumen between two of the layers (intima and media, or media and adventitia). Arterial blood entering the false lumen causes rapid expansion, with consequent rapid contraction of the true coronary lumen, provoking an ACS. Spontaneous coronary artery dissection was diagnosed by OCT in 4% of patients with ACS by Nishiguchi et al, 2013, and was seen more in women, often in the absence of traditional coronary risk factors. In addition, ACS can arise if aortic dissection extends proximally to involve the coronary arteries.

#### 1.2.3.3. Changes in blood flow and coronary vasospasm

Many cases of ACS are triggered by sudden changes in blood pressure and pulse rate, such as emotional stress and unusually intense physical exertion. Arteries are highly specialised biological conduits for blood transportation, but the very factors which make arteries efficient enough to allow animals to function at a high metabolic level (high pressure and rapid blood flow) are a relentless source of injury to the delicate, single-layer vascular endothelial cells. The coronary arteries in particular experience uniquely powerful haemodynamic forces. For example, the left coronary artery is completely compressed during systole and only transmits blood during diastole. The heart undergoes rapid changes in morphology during the cardiac cycle, including torsion, all of which forces are transmitted to the coronaries as shear and stress in a pulsatile fashion. Davies et al (2006) have described in detail coronary blood flow (in normal coronary arteries) by pressure wave analysis, describing at least 6 different pressure waves during the cardiac cycle. Furthermore, the heart has a high but very variable oxygen demand and the coronaries have capacity to autoregulate blood flow by dilating and contracting. In 1964 Mosher et al showed that there is a large range of perfusion pressures in canine hearts over which coronary flow is relatively independent of pressure, although this ability becomes impaired in the presence of some

of the risk factors associated with coronary disease such as hypertension (Panza et al, 1990).

There is a circadian rhythm in the incidence of MI: a morning peak at 9am and a second smaller peak at 8pm. A significant proportion of cardiac events occur during sleep: in a literature review of nocturnal acute cardiac events, Lavery et al (1997) found the peak incidence of MI occurred from midnight to 1 am, and the peak incidence of sudden cardiac death between 1 and 2 am. Furthermore, there is also a weekly (peak incidence Monday) and a seasonal (peak in the winter months) variation. These rhythms are thought to be due to changes in autonomic nervous system activity during sleep or physical or emotional stress which affect blood pressure and coronary tone.

As described above, endothelial dysfunction with non-physiological vasoconstriction is often seen in atherosclerotic coronary arteries. There are also endothelium-independent mechanisms for vasoconstriction. Yasue et al in 1986 demonstrated severe coronary spasm angiographically after administration of subcutaneous adrenaline in patients with variant (atypical, Printzmetal's or non-exertional) angina. These attacks could be aborted by phenoxybenzamine (an alpha blocker) but not propranolol (a beta blocker); leading to the conclusion that severe spasm of coronary arteries mediated by alpha adrenoreceptors was the cause of these patients' symptoms. Vasospasm can provoke an ACS even in the absence of plaque rupture or an obstructive coronary lesion, leading to MI or even sudden death. Printzmetal's angina, cocaine and several chemotherapeutic agents used in cancer treatment (5-fluorouracil and derivatives) can induce severe coronary spasm leading to an ACS. Yasue et al also provide a general overview of the condition (1997)

## 1.3. Clinical diagnosis and categorisation of ACS

The term ACS incorporates patients with unstable angina (UA), non ST segment elevation (subendocardial) myocardial infarction (NSTEMI) and ST segment elevation myocardial infarction (STEMI). The diagnosis is first suspected from the clinical history in the presence of typical symptoms supported by the presence of traditional cardiovascular risk factors. Ischaemic changes on the electrocardiograph (ECG) support the diagnosis, but a normal initial ECG does not exclude it. Over subsequent hours, blood is drawn for measurement of cardiac biomarkers. Biomarker negative ACS is known as UA; a rise in biomarkers is required to diagnose acute myocardial infarction (AMI).

### 1.3.1. The definition of acute myocardial infarction

The universal definition of MI document (Thygesen et al, 2012) defines AMI based largely on the detection of elevation in cardiac biomarkers in conjunction with one or more clinical features:

"Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99$^{th}$ percentile of the upper reference limit (URL) together with evidence of myocardial ischaemia with at least one of the following:

- Symptoms of ischaemia;
- ECG changes indicative of new ischaemia (new ST-T changes or new left bundle branch block [LBBB]);
- Development of pathological Q waves in the ECG;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality."

The document also provides definitions for 5 different categories of AMI:

- Type 1: spontaneous MI due to a primary coronary event e.g. plaque rupture;
- Type 2: MI due to increased demand or decreased supply of oxygen; this includes "coronary artery spasm, coronary embolism, anaemia, arrhythmias, hypertension, or hypotension";
- Type 3: sudden cardiac death;
- Type 4: MI due to PCI. 4a: procedural MI; 4b: stent thrombosis;
- Type 5: MI associated with CABG.

### 1.3.2. Differential diagnosis of ACS; diagnostic uncertainty

Chest pain is a common complaint with several potential causes. The likelihood of the diagnosis being ACS is estimated from the clinical history, ECG and coronary angiogram; although even after these diagnostic procedures, the diagnosis can remain unclear. Elevation in cardiac biomarkers, with or without cardiac symptoms, is very common with the haemodynamic stress induced by acute illness. However, the universal definition of MI document makes it clear that these episodes should often not be classified as MI. Conversely, we may misclassify chest pain as ACS, particularly if incidental coronary disease has been demonstrated by angiography.

### 1.3.3. Clinical history

Cardiac ischaemia may be asymptomatic (silent) or induce a range of symptoms which often includes discomfort in the upper body, known as angina pectoris. This pain

may originate from the local release of adenosine in response to myocardial hypoxia (Crea et al, 1990). Chest pain which is located retrosternally, is crushing in nature and has a distinct pattern of radiation (arms, jaw) is known as classical cardiac chest pain; pain of a possible cardiac origin which does not share these features is known as atypical. In a study of 903 patients admitted with suspected AMI, Everts et al (1996) showed that pain can be distributed anywhere over the chest, back, neck and arms; the distribution of cardiac pain tends to be wide rather than localised; and the most sensitive discriminator for MI was pain in the *right* arm. Silent ischaemia is common in diabetes, possibly due to diabetic neuropathy of afferent nerve fibres, but is also common in non-diabetic patients with coronary disease (Deedwania et al, 1991).

Patients may also present with other symptoms such as dyspnoea, nausea and syncope, with or without anginal pain.

### 1.3.4. The Electrocardiogram

Electrocardiography was invented by Willem Einthoven who published a report of his discovery in 1902. The story of the development of electrocardiography is told in detail (and very well) by Fye, 1994; today the electrocardiogram (ECG) is central to the initial assessment of any patient with chest pain. All emergency medical personnel are trained to obtain and interpret an ECG trace. ST segment elevation on the ECG occurs due to acute proximal occlusion of a major epicardial coronary artery and is an indication for emergency revascularisation therapy. Dynamic ST segment depression implies ischaemia or subendocardial infarction; these ECG changes often resolve rapidly following initial medical therapy, in which case immediate revascularisation may be deferred (ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation, 2011, Hamm et al; ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation, 2012, Steg et al).

### 1.3.5. Cardiac biomarkers

Cardiac biomarkers are molecules whose presence (above a prespecified normal reference range) in the bloodstream indicates myocardial injury. Numerous different blood tests to detect a range of cardiac biomarkers have been developed, from aspartate aminotransferase reported by Karmen et al in 1954, to cardio-specific creatine kinase (CK-MB), to modern highly sensitive troponin assays. Troponins (Adams, 1993) are large protein fragments released after cell membrane breakdown of necrotic cardiomyocytes, a process taking several hours; a delay of 12 hours from onset of chest pain to drawing of the blood sample is required to definitely ‘rule out’ MI, as per the ESC guidelines. Blood levels can take weeks to return to baseline following an MI.

A novel cardiac biomarker, human fatty acid binding protein (H-FABP), is a small molecule which is rapidly detectable in blood after myocardial ischaemia, even in the absence of cell necrosis (Glatz et al, 1998). It is released into the bloodstream more rapidly and in greater quantity than other cardiac biomarkers, being detectable within minutes rather than hours.

The effective use of a biomarker requires a reliable assay. Assay precision may be measured using a value known as the coefficient of variation (the ratio of the standard deviation to the mean value) for a molecular concentration of biomarker at the level of 99$^{th}$ centile of the normal range of a healthy population. The Universal Definition of MI document recommends the use of assays with a coefficient of variation of less than 10% at the 99$^{th}$ centile. Highly sensitive troponin assays, the current 'gold standard' in the diagnosis of MI, should have at least this level of precision.

### 1.3.6. Coronary angiography

Coronary angiography is an invasive radiological procedure for examination of the coronary arteries, first credited to Proudfit et al in 1966. The Seldinger technique (Seldinger, 1953) is used to introduce a catheter into the arterial system, common entry points being the femoral or radial arteries. Selective intubation of the right and left coronaries allows injection of radio-opaque contrast agent during which multiple frames are acquired in rapid succession (15 to 30 frames per second). After computer generated post-processing a black and white movie is produced of the opacification of coronary artery lumen; it is therefore more accurate to call this procedure 'lumenography'. Although this term is seldom used routinely it emphasises the fact that the image produced is only an outline of the lumen of the vessel. A lumenogram is two dimensional, but multiple angiograms in different planes are obtained by changing the projection of the image intensifier so that stenoses which are eccentric or hidden behind branches are not missed. The cardiologist records the approximate severity (usually expressing it as a percentage luminal compromise) and location of the stenoses. Broadly, the number and severity of stenoses and how proximally they are located are the main determinates in the decision to recommend medical management or revascularisation by either CABG or PCI, as discussed in the following section. A 'normal' coronary angiogram does not exclude the presence of extraluminal coronary disease.

## 1.4. Treatment of ACS

National cardiology societies have collaborated to produce detailed and comprehensive international guidelines which are frequently updated in accordance with emerging research results. Management of ACS is detailed separately in European

and American guidelines. The latest European guidelines on myocardial revascularisation were produced jointly by representatives from European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (Windecker et al, 2014).

In brief, contemporary management of ACS begins with the rapid initiation of strategies intended to relieve symptoms, to abort imminent death, to treat arrhythmias and to avoid or at least limit the extent of myocardial infarction. All patients are administered a range of pharmaceutical agents. Ventricular fibrillation and tachycardia are treated by D.C. cardioversion; bradycardia may require may require pacing. Selected patients will undergo myocardial revascularisation by either PCI or CABG.

Longer term management consists of managing residual ischaemic symptoms, psychological support and 'secondary prevention', involving strategies to prevent future cardiovascular events. In addition to pharmacological therapy, patients undergo 'cardiac rehabilitation' intended to restore/improve exercise capacity and encourage appropriate lifestyle modifications.

### 1.4.1. Pharmacology

Patients receive an evidence-based combination of medication including analgesics, antiplatelet agents, anticoagulants, vasodilators, ACE-inhibitors, beta-blockers and HMG-CoA reductase inhibitors ('statins'). Pharmacology at the time of PCI is discussed further in Section 1.5.5. Adequate antiplatelet therapy in particular is crucial to a successful outcome following PCI and antiplatelet agents are discussed further in Sections 1.6 to 1.8.

### 1.4.2. Invasive investigation and revascularization

It is recommended that the majority of patients with ACS undergo invasive investigation (a coronary angiogram) soon after presentation, leading to coronary revascularisation where indicated. There are two complementary methods of coronary revascularisation: CABG and PCI.

The first method of revascularization to be developed was coronary artery bypass surgery (CABG), and the first operation on a human being was carried out in the USA in 1960 (Goetz et al, 1961). CABG is a major undertaking requiring sternotomy and cardiopulmonary bypass, with considerable risk of morbidity and a recovery period of weeks to months. The risk is highest in the presence of co-morbidities; each patient's risk profile is often formalised by applying a risk score such as the EuroSCORE (Section 1-5-4-1). In ACS patients who are haemodynamically stable and pain–free,

with complex anatomy thought to be more suitable for CABG than PCI, revascularisation may be delayed to allow adequate planning, multidisciplinary team discussion and further investigations if required. Certain patterns of disease including multiple long and/or complex lesions, left main disease and chronic total occlusions (CTO) are preferentially revascularised surgically.

PCI (Section 1-5) requires less planning than CABG, can usually be undertaken rapidly and has overtaken CABG to become by far the more frequent method of revascularisation for ACS patients. PCI is a minimally invasive method of revascularization which has been developed using coronary angiography catheters to introduce balloons into the coronary arteries to widen the lumen (angioplasty). Any technique to treat coronary artery disease with catheter technology via peripheral arteries is currently known as percutaneous coronary intervention (PCI) (formally percutaneous transluminal coronary angioplasty, PTCA). When the coronary anatomy at angiography reveals one or more culprit lesions thought suitable for PCI, the operator may proceed directly to intervention at the same sitting. This is known as ‘ad hoc’ PCI and is recommended for patients with ACS.

STEMI is an indication for immediate revascularisation. Previously this was achieved by administration of an intravenous thrombolytic agent, but a high failure rate of this therapy led to development of 24 hour cardiac catheterisation services capable of providing primary PCI (PPCI).

## 1.5. Percutaneous Coronary Intervention (PCI)

### 1.5.1. Brief history of PCI

PCI was first performed on a human being in 1977 (reported in a letter to The Lancet in 1978) in Switzerland by a team led by Andreas Gruntzig. Initially the technique involved balloon angioplasty alone (plain old balloon angioplasty, or POBA). Subsequently, balloon-expanded stents were invented to provide a scaffold to prop the artery open following angioplasty, reducing the incidence of the two problems of abrupt vessel closure and restenosis. Bare metal stents (BMS) were first used in humans in 1986 in France. Puel reported the first cases in 1987, published in French; Sigwart then published a series of (presumably the same) cases later in 1987 in the English language. The first drug eluting stent (DES), the Cypher sirolimus-eluting stent, was approved in Europe in 2002. A history of the development of percutaneous coronary stent insertion is provided by Iqbal et al, 2013.

Stent technology is continually evolving. The most commonly used DES at the time of writing is the Xience everolimus-eluting stent (Abbott Vascular, U.S.A.), but even this single brand has now been produced in several different versions. The Xience V stent was comprised of a metallic scaffold, a polymer compound which coats the struts and a drug-containing compound which 'elutes' a cell division inhibiting agent. The stent struts are only 0.0032 inches (0.081 mm) thick and made of a complex alloy mainly composed of cobalt, chromium, nickel and tungsten. The stent is coated with poly n-butyl methacrylate (PBMA), a polymer that adheres to the stent and drug coating; and the drug coating is known as PVDF-HFP, which is comprised of vinylidene fluoride and hexafluoropropylene monomers. This latter compound is mixed with everolimus, a semi-synthetic cell division inhibiting drug derived from sirolimus. This stent was investigated in the SPIRIT series of trials, in which clinical superiority over an earlier drug-eluting stent was seen (SPIRIT IV, Stone et al, 2010).

### 1.5.2. Current indications and guidelines for PCI

Detailed and comprehensive international guidelines, already discussed in Section 1-4, are available which advise on all aspects of coronary revascularisation. Advances in technology and pharmacology and the rapid dissemination of new data require increasingly frequent guideline updates to remain in line with emerging evidence. Stable angina is managed differently to ACS. In the former, the emphasis tends to be on symptom control and there is less urgency to embark on invasive investigation and revascularisation. In the latter, early invasive investigation and revascularisation is encouraged. This has been reinforced by data (notably from the COURAGE trial, Boden, 2007) which indicated that PCI may not be a superior treatment to medical therapy in patients with stable coronary disease, contrary to the case for CABG which, in the original trials of the 1970s and 80s, was shown to have a mortality benefit in certain patient groups such as those with multivessel disease in combination with LV dysfunction (Yusuf, 1994).

That PCI in contemporary studies often fails to produce a clear mortality benefit is of concern and has led to much questioning of current interventional practice; but the interpretation of trial data is not straightforward. Overall mortality in IHD has decreased dramatically in recent years (Smolina et al, 2012) probably due to a combination of many improvements to treatment: revascularisation, novel pharmaceutical agents, improved infrastructure to provide emergency healthcare and the invention of implantable defibrillators. This low death rate makes achieving statistical significance in randomised studies difficult. Furthermore, high risk patients such as those with critical angiographic lesions, unstable symptoms or a large volume of ischaemic myocardium on a functional test are unlikely to be placed into trials in which they may be

randomised to medical therapy without revascularisation – such a trial would be unlikely to gain ethical approval – leading to selection bias. Therefore, the number of PCI cases continues to rise in spite of these apparent gaps in the published evidence; but two thirds of cases are now performed to treat ACS rather than stable angina (National Audit of PCI, British Cardiac Intervention Society, 2014).

### 1.5.3. Determining the culprit lesion in acute coronary syndromes

It does not always follow that an angiographically severe lesion is responsible for an ACS. Sometimes an isolated lesion in a coronary artery will be identified which corresponds with the distribution of the ECG changes and has signs of acute rupture, for example visible thrombus. The diagnosis and management become straightforward in these circumstances. However, angiography often cannot distinguish between atherosclerosis which is stable (histologically, predominantly fibromuscular or fibrocalcific) and which is acute (ruptured thin fibrous plaque overlying a lipid pool) or 'vulnerable' – with a high risk of future rupture. There are many confounding factors: the presence of multiple lesions, any or all of which may be unstable; a lack of localising ECG changes; or the unstable lesion may not be angiographically severely stenotic. This is discussed in detail in a review by Waxman et al, 2006. The merits of PCI to culprit lesions only vs. treating all lesions are currently being investigated (PRAMI and CULPRIT trials; Wald et al, 2013 and Gershlick et al, 2015).

Work is currently being undertaken to develop methods of highlighting areas of vulnerable plaque, for example by intravascular ultrasound (IVUS) and 'virtual histology', in the hope of more targeted therapy. Virtual histology has been used to demonstrate both the progression and regression of coronary lesions (Kubo et al, 2010). Angioscopy, a research technique in which a small camera is percutaneously inserted into the coronary artery lumen, can distinguish vulnerable high-lipid content coronary plaques, which appear yellow, from stable plaques which appear white. Angioscopically yellow plaque, shown to have a thin fibrous cap and large underlying lipid pool, is known to be associated with a higher rate of clinical events. Asakura et al in 2001 used this technique to examine 32 patients 1 month post PCI for STEMI. All patients had widespread disease and yellow plaque was seen in all three major coronary arteries. Most culprit plaques had associated thrombus.

IVUS virtual histology has yet to be validated, and angioscopy is technically challenging and increases the procedural risk. In the absence of technology to routinely identify acutely inflamed plaque, the current guidelines recommend revascularisation of obstructive proximal lesions with at least 70% stenosis, or at least 50% if the lesion is located in the left main stem (LMS). It is known that stable patients are less likely to

benefit from revascularisation in the absence of objective evidence of inducible myocardial ischaemia. If required, the functional significance of a stenosis can be determined by pressure wire assessment before proceeding to PCI; the FAME I study showing the benefits of fractional flow reserve included a proportion of ACS patients (Tonino et al, 2009).

### 1.5.4. Risk scoring systems to guide revascularisation

Many risk scoring systems are available in cardiology. For example, the TIMI and GRACE risk scores (both available as online calculators) were devised to allow risk stratification of ACS patients to help decide the management strategy. These scoring systems are used widely for research purposes and have been validated for prognosis following ACS (Antman, 2010; Elbarouni, 2009). They have not been generally adopted in contemporary day-to-day practice, where the main objective factors used to determine treatment are the ECG, Troponin level and clinical status of the patient. Other scoring systems have been created to estimate the risk posed by a revascularisation procedure, and in contrast to the above, are frequently used in routine clinical practice. Two of these, the EuroSCORE and the SYNTAX score, are discussed below.

#### 1.5.4.1. Clinical risk scoring: the EuroSCORE.

The EuroSCORE (Nashef et al, 1999) was developed from registry outcome data to predict the risk of in-hospital mortality following CABG. The score is calculated from the results of 17 readily available variables including age, significant past medical history and clinical presentation. Additive and logistic versions are available. Bhatti et al (2006) found that the logistic score was a reasonable predictor of death after CABG, although overestimated the risk; area under ROC 0.79; predicted mortality 5.7% and observed mortality 3.3% in 9995 patients. EuroSCORE has also been validated as a predictor of long term outcomes following PCI.

The EuroSCORE in the UK and Ireland is recorded for all patients undergoing surgical revascularisation and the statistics are available to the public (Society of Thoracic Surgery of Great Britain and Ireland). Between 2004 and 2008, information on 112251 operations for isolated coronary disease was analysed. 94764 (84.4%) of these were performed on patients in EuroSCORE group 0 to 5.9%; 9339 (8.3%) had a EuroSCORE over 10%. This indicates that estimated surgical risk (formalised as a EuroSCORE) of patients with severe coronary disease is crucial to the decision as to whether they are accepted for surgery. Many surgical turn-downs probably go on to have PCI, but we do not have data to support this, as there is no formal method for tracking these patients: surgical turndown patients are not recorded in surgical databases; EuroSCORE is not routinely recorded in PCI databases; ‘surgical turndown’

is never formally listed as a PCI indication in databases; and no PCI verses CABG outcome studies focusing on EuroSCORE have been undertaken.

Logistic EuroSCORE is comprehensive, but there are many other factors which can lead to the surgeon turning a patient down. Efforts to incorporate some of these in the score have led to the release of EuroSCORE II in 2012 (Nashef et al), which features several additional fields: DM, poor mobility (a general marker of frailty and poor prognosis), and renal function as a continuous rather than a dichotomous variable. The scale has also been adjusted to be more representative of current surgical mortality rates.

#### 1.5.4.2. Angiographic risk scoring: the SYNTAX score

The EuroSCORE does not include information on angiographic complexity, which is important in predicting outcomes after PCI. Lesion characteristics influence both procedural success and future prognosis (Yadav, 2013). Outcomes are worse with increasing number and length of stenoses requiring treatment, smaller vessel diameter, and the presence of diffuse disease, tortuosity, bifurcations, calcium and thrombus. The SYNTAX (Synergy between PCI with Taxus and Cardiac Surgery) trial was a randomised comparison of outcomes after revascularisation of patients with severe coronary disease treated by either CABG or PCI (Taxus stents). The SYNTAX score was developed from this trial.

The SYNTAX score is derived purely from assessment of the coronary angiogram. Angiographic reporting is subjective and there is substantial inter-interpreter variability. Nevertheless, terciles of SYNTAX score have been shown to predict outcome after PCI (Serruys a and b, 2009). In contrast, lesion complexity proved relatively unimportant with regard to CABG outcomes, providing there is a reasonable calibre distal target vessel to which a graft can be anastamosed. These patients were representative of the normal CABG population, with mean (SD) EuroSCORE of 3.9 (± 2.9). One third of patients had severe LMS disease, and the other two thirds had severe 3 vessel coronary disease (3VD).

There is now data to 4 years for this trial, which was separated into LMS and 3VD cohorts for presentation at the Transcatheter Cardiovascular Therapeutics conference in 2011. In the LMS disease group overall, the PCI patients fared slightly worse than the CABG patients in every outcome other than stroke; however, there was no difference in MACCE between the two revascularisation methods for LMS disease patients in the bottom two SYNTAX terciles (SYNTAX 0 to 22 and 23 to 32), with a big difference in MACCE in the top tercile (over 33) of 42.6% versus 26.3% for PCI and CABG

respectively. In the 3VD group, all SYNTAX terciles did worse with PCI than with CABG, although this difference was not statistically significant in the lower tercile. Interpretation of these results should be done with caution, as analysis of subgroups of subgroups is hypothesis generating only; however, this is the most comprehensive and contemporary data available in this area.

### 1.5.5. Adjunctive pharmacotherapy

The safety of PCI has been greatly increased by a number of pharmacological agents, both during and after the procedure.

*Anticoagulation*

Anticoagulation is used in every PCI case for the prevention of thrombus formation on angioplasty equipment. This is usually achieved by the administration of a weight-adjusted bolus of heparin at the start of the case, which may be topped up according to the ACT result. Low molecular weight heparins have been shown to be non-inferior to unfractionated heparin. The direct thrombin inhibitor, bivalirudin, has been approved for use as an anticoagulant during PCI in patients with ACS. This drug was developed to overcome some of the limitations of heparin such as unpredictability of response, heparin-mediated platelet activation and heparin-related thrombocytopenia. Trials in ACS patients have shown reduced rates of cardiovascular events and bleeding compared to heparin, although a more recent trial in STEMI patients showed high acute stent thrombosis rates (Shahzad, 2013). Anticoagulation in PCI is reviewed by Rao and Ohman, 2010.

*Antiplatelet agents*

All patients are pre-loaded with drugs which inhibit platelet activation and consequently the formation of platelet thrombus. These drugs are vital to prevent stent thrombosis during and after PCI, particularly in ACS patients in whom thrombus is already present. The agents with the most powerful antiplatelet effect are the intravenous IIb IIIa inhibitors. This subject is explored further in Chapter 3.

*Other medication*

Local anaesthetic, sedatives and opiate analgesic agents are used to limit discomfort during PCI, which is undertaken without general anaesthetic. Vasodilators such as nitrates, adenosine and verapamil may be used at various stages of the procedure. Antimuscarinic agents can be employed to correct bradycardia induced by autonomic disturbance, particularly a problem during right coronary artery PCI. Inotropic agents are used in cardiogenic shock.

### 1.5.6. Complications of PCI: early

PCI, in common with all invasive procedures, has a range of potential complications. These may be loosely categorised into peri-procedural (complications occurring during or shortly after PCI but before discharge) and post-procedural, usually occurring after discharge home. Most PCI clinical trials now use standardised definitions for reporting adverse events during the clinical follow up period. The Academic Research Consortium (ARC) (Cutlip et al, 2007) definitions for clinical endpoints are now used in clinical research when possible.

#### 1.5.6.1. Peri-procedural complications

The common or serious peri-procedural complications are listed on the patient consent form. They occur during the procedure or early after it.

#### 1.5.6.2. Procedural myocardial infarction, 'no-reflow' and acute stent thrombosis

Myocardial necrosis following PCI is frequent and may be of sufficient magnitude to induce permanent loss of myocardium detectable by cardiac magnetic resonance imaging (MRI); these studies are cited by Lansky and Stone in their 2010 review article on periprocedural MI. In 2005 Herrmann analysed 60 studies detailing procedural myocardial necrosis, excluding ACS patients. Post-procedural elevation above the upper limit of normal of CK-MB mass, troponin T, and Troponin I was found in 0 to 47%, 7% to 69%, and 5% to 53% (mean 23% ± 12, 23% ± 11, and 27% ± 12) of patients, respectively. Nearly all the studies showed an association between procedural biomarker elevation and future risk of adverse cardiovascular events. However, many baseline characteristics associated with procedural MI, such as lesion complexity, were also associated with long-term outcome. This clearly leads to confounding in such observational studies.

The Universal Definition of MI document defines a separate category for procedural MI: type 4a. By "arbitrary convention", the biomarker cut off is an increase in cardiac biomarker of more than three times the 99$^{th}$ percentile of the upper reference limit in a healthy population. The definition does discriminate between biomarkers of differing sensitivity – for example, more MIs would be diagnosed using troponin than CK-MB. The U.S.A. 2011 revascularization guidelines (Levine et al) recommend testing for procedural MI in patients with signs or symptoms of MI, or after a significant persistent angiographic complication (class I level C recommendation); and routine monitoring of all patients "may be reasonable" (class IIb, level C). Troponin I, Troponin T or CK-MB may be used. The latest European revascularisation guidelines (2014) do not appear to discuss procedural biomarker elevation.

Although many studies have shown an association between procedural myocardial necrosis and adverse outcome, there has been little to suggest a causal relationship. The value of screening for procedural MI in ACS patients (who often have elevated biomarkers pre-procedure, and who now make up the majority of PCI cases) is particularly uncertain, as it is difficult to distinguish the proportion of biomarker from iatrogenic myocardial injury from that produced by the spontaneous MI. Miller et al in 2006 found that pre-procedural, not post-procedural, troponin elevations influenced prognosis. Another contentious issue is how to manage the patient when procedural MI is diagnosed. For these reasons, procedural MI is not routinely monitored in many institutions, except in clinical trials where it often forms an important component of combined clinical end points.

There is some evidence to show that procedural MI may have independent prognostic importance. The frequency of peri-procedural MI in the TRITON-TIMI 38 trial (Wiviott et al, 2007), which tested prasugrel against clopidogrel in ACS patients, was 600/13608 = 4.4%. After adjustment for clinical characteristics, peri-procedural MI was associated with a 3.2% increase in the risk of cardiovascular death at 180 days, P=0.001. Rigorous criteria to define peri-procedural MI were used in this study, whose design preceded the publication of the universal MI document. Events were mostly identified using CK-MB, requiring a value 3 times the upper reference limit on 2 samples after PCI, or 5 times on a single sample within 48 hours of PCI. In addition, because this was a study of ACS (82% of TRITON patients had elevated biomarkers pre-PCI), for patients who were biomarker positive the biomarker had to be shown to be falling pre-procedure AND had to rise to >50% of the pre-procedural nadir to make the diagnosis. Therefore, procedural MI rate may have been underestimated according to universal definition of MI criteria.

The cause of most cases of low level myocardial necrosis in PCI is thought to be coronary micro-embolisation of particles of thrombus and atheromatous debris (Heusch et al, 2009), although other potential causes include procedural complications such as trapped side branches and no-reflow. Herrmann et al summarised known predictors of procedural MI into patient-, lesion- and procedure-related factors. Patient factors include advancing age, DM, LV dysfunction, renal failure and multi vessel disease. Lesion related factors associated with procedural MI include the presence of thrombus, saphenous vein graft (SVG) PCI and American Heart Association (AHA) Type C lesions. Procedural factors include the level complexity and the occurrence of procedural complications such as side branch occlusion, embolisation of atheromatous or thrombotic material to distal branches, and loss of a coronary vessel due to dissection.

There is evidence to show that procedural MI is more likely in patients with high platelet reactivity. Patti et al in 2008 found higher procedural MI with increasing PRU measured with the Verify Now device in 160 patients receiving clopidogrel prior to PCI: mean PRU levels were 258 ± 53 in patients with peri-procedural MI verses 219 ± 69 in patients without; P = 0.03. These procedural MIs were based on CK-MB > 3x the ULN; there was in fact no statistically significant variation in post-procedural troponin based on PRU level in this study.

It has been postulated that there are more subtle causes for periprocedural myocardial necrosis than mechanical obstruction of the vessel during PCI. A potential cause of procedure-related procedural MI is 'no-reflow', a poorly understood phenomenon in which there is poor circulation of blood through the microvasculature but in the absence of macroscopic obstruction. Chan et al (2012) found this complication in 4.8% of 5286 of patients, mostly transient. No-reflow was a predictor of 30 day major adverse clinical events, odds ratio 2.79 (95% confidence interval 1.84 to 4.25), P<0.001. No-reflow is unlikely to be due only to micro-vascular obstruction as described by Niccoli et al, 2009. It may also be related to 'reperfusion injury', explained in a review article by Yellon and Hausenloy, 2007. This is another poorly understood process in which restoration of arterial flow in an MI causes further myocardial infarction, ischaemic endothelial cell damage and microcirculatory dysfunction.

A specific type of peri-procedural MI due to definite macroscopic mechanic obstruction of the artery is acute thrombosis of the newly implanted stent, classified as MI type 4b in the universal MI document. Thrombosis can occur at any point during or after the procedure; however, ST before the end of the procedure is not a complication according to ARC definitions. Complete thrombotic occlusion of the stent causes chest pain accompanied by ST segment elevation on the ECG. It may result in further myocardial necrosis, but prompt reperfusion can usually be achieved by a repeat PCI procedure. Due to the combination of peri-procedural anticoagulant and antiplatelet medication, acute ST is rare; for example, Kuchulakanti et al (2006) reported only 5 cases of acute ST in 2574 (0.19%) patients within 24 hours of 1$^{st}$ generation DES implantation. In patients who have not been preloaded with antiplatelet medication with a high coronary thrombus burden, such as primary PCI for STEMI, acute ST may be more frequent. In a recent study by Shahzad et al (2014) acute ST was seen in 2.9% of 905 PPCI patients treated with bivalirudin (the rate was only 0.9% in the heparin arm). Subacute ST (> 24 hours, < 29 days) is discussed further in Section 1.5.7.3.

#### 1.5.6.3. Stroke

In the setting of PCI, stroke can occur due to embolisation to the brain of atheromatous material or thrombus which becomes dislodged by the procedure, and is a particular hazard in cases with a large volume of proximal coronary artery thrombus. There is also the potential for formation of thrombus on intravascular angioplasty equipment, although minimised by the use of anticoagulants. Less commonly, stroke can occur due to cerebral haemorrhage precipitated by anticoagulant and antiplatelet medication, and particularly by thrombolytic therapy.

In stable patients undergoing PCI, the rate of peri-procedural stroke is low but associated with high mortality. From a 2009 analysis of 706,782 patients from the multi centre National Cardiovascular Data Registry (U.S.A.) by Aggarwal et al, the incidence of in-hospital stroke after PCI was 0.22%. After multivariate analysis, factors strongly associated with stroke included a previous history of cerebrovascular disease, older age, admission with an ACS, and use of an intra-aortic balloon pump. Peri-procedural stroke patient in-hospital mortality was 30 times higher than in non-stroke patients. This study did not detail whether strokes were due to haemorrhage or infarction. However, Hoffman et al (2012) analysed stroke following PCI in another North American registry of over 20 000 patients from a single centre over 19 years was. The in-hospital cerebrovascular event rate was 0.37%; major predictors were age and previous stroke, but patients had also undergone more complex procedures and rotational atherectomy. Of these cases of stroke 7% were haemorrhagic.

#### 1.5.6.4. Coronary perforation and rupture

Loss of integrity of the coronary arterial wall may occur during PCI, detected by observing the extravasation of contrast during angiography. A perforation may be localised to the peri-arterial tissue, or may result in a leak of blood from the coronary artery into the pericardial space, which can cause rapid loss of cardiac output (cardiac tamponade). Proximal perforations may leak into the thoracic cavity and can cause hypovolaemic shock.

While small, self-limiting perforations with or without localised contrast staining (perforation types I and II) may be reasonably common (up to 3%), frank coronary rupture (type III) is extremely rare. Analysis of prospectively collected data from two institutions on 24465 interventions over a 16 year period (Al-Lamee et al, 2011) detected only 56 cases. 28.6% had tamponade requiring pericardiocentesis with a 14.8% in-hospital death rate. Ruptures in this series were sealed by a variety of methods including prolonged balloon inflation, deployment of a stent (standard or covered), emergency CABG and in one case coil embolisation.

#### 1.5.6.5. Damage to the peripheral vascular system and haemorrhage

Complications relating to the vascular access site are not infrequent in PCI, particularly when access is via the femoral artery. Ohlow et al (2009) found an incidence of arteriovenous fistula formation and pseudoaneurysm formation of 0.6% and 1.2% respectively in 18165 consecutive femoral access cases. Most of these cases could be treated conservatively and neither complication appeared to be associated with increased mortality.

Arterial bleeding following PCI is a more important complication - major bleeding after PCI has been associated with increased mortality, although whether this is cause-and effect or whether bleeding is just a general marker of high risk in a patient is unclear (Doyle et al, 2009). Note that bleeding complications are reported in multiple different ways by different trials; Bleeding Academic Research Consortium (BARC) definitions (Mehran et al, 2011) have been devised in an effort to standardise reporting. Bleeding may occur via the puncture site, from the GI or GU tracts, intracerebrally and intraocularly.

External puncture site bleeding is obvious and can be quickly identified and usually stopped by compression. However, femoral procedures can be complicated by the more insidious retroperitoneal haemorrhage, sometimes with huge loss of blood into the retroperitoneal space requiring blood transfusion and surgical repair. Efforts to devise strategies to minimise bleeding have resulted in a recent change from femoral to radial access: radial artery haemorrhage tends to be small volume and easy to stop. In a 2008 report from Rao et al on data from 593 094 PCI cases from the U.S.A. National Cardiovascular Data Registry, total bleeding (including access site bleeding, retroperitoneal bleeding, gastrointestinal bleeding, genitourinary bleeding, or other bleeding) complicated 1.83% of femoral procedures, but only 0.79% of the radial access procedures (only 1.32% procedures were radial).

#### 1.5.6.6. Allergy

Although unusual with modern contrast agents, allergic reactions including anaphylaxis may occur. Patients who are prone to allergy or who have had a previous contrast allergy may still undergo angiography if they are pre-treated with antihistamine and corticosteroid. Patients may also of course be allergic to any of the antiplatelet or other medications they are administered for PCI.

#### 1.5.6.7. Acute renal failure

Most cases of post-procedural renal failure occur at 3 to 5 days and are the result of contrast nephropathy (Persson et al, 2005). The 2 main risk factors for this are pre-

existing renal impairment and the volume of contrast administered. The risk can be limited by pre-and/or post-hydration with intravenous fluid, and by limiting the volume of contrast administered. Modern intra-arterial contrast agents are solutions of iodine which may be bound as an ionic or non-ionic (organic) compound; the latter have lower osmolality than the former. The incidence of nephropathy has been reduced by the use of non-ionic contrast agents (Rudnick et al, 1995); available molecules include iopromide (Ultravist 370 TM); and iodixanol (Visipaque 320 TM). Iodixanol has an osmolality of 290 mOsm/kg H2O, which is the same as blood (iso-osmolar).

More rarely, the kidneys can be damaged by embolisation of lipid particles, often accompanied by a necrotic rash on the lower limbs.

### 1.5.7. Complications of PCI: Late

These are complications due to problems with the stent or concomitant medical therapy occurring after the immediate recovery period.

#### 1.5.7.1. Bleeding

The increased risk of bleeding continues after PCI for the duration of antiplatelet therapy, as does the apparent association between bleeding and increased risk of other adverse clinical events. In a report from the E-SELECT post marketing surveillance registry of outcomes after SES stent implantation, in 15400 subjects the rate of major bleeding (STEEPLE criteria) was 1% at one year and had a 10% mortality rate, compared to 1.7% mortality for patients without bleeding (Urban et al, 2011). Note that patients at high risk of bleeding may not have had a DES implanted and so may be under-represented in this registry. Advancing age, use of warfarin and use of IIbIIIa inhibitors correlated with the occurrence of major bleeding.

#### 1.5.7.2. Radiation damage

PCI requires the use of ionizing radiation which can cause predictable, dose-related skin damage, from mild erythema to full thickness necrosis. There are also long term stochastic (unpredictable) effects, such as a probable increased risk of cancer (Pierce and Preston, 2000). A true picture of the negative effects of medical radiation is difficult to realise, as most of our information comes from nuclear bomb blast survivors. In the U.K. Ionizing Radiation (Medical Exposure) Regulations (IRMER) require that radiation dose (‘effective radiation dose’ calculated using body surface area; McCollough Schueler, 2000) is monitored and procedures are usually stopped before a prespecified threshold which varies between departments.

### 1.5.7.3. Sub acute, late and very late stent thrombosis

Thrombotic occlusion of a stent more than 24 hours after insertion is a serious post-procedural complication associated with a high rate of death. Sudden thrombotic occlusion of a coronary stent is an uncommon but frequently devastating complication of PCI. In contrast to the other major post-discharge complication of PCI, restenosis, ST has an important negative impact on survival (Urban et al, 2011). ST can occur at any time after the procedure, from hours to years. I will describe the risk factors for, incidence and sequelae of ST which have been reported in the literature.

The main problem encountered with the first stents was early re-occlusion. This was observed from the outset of PCI (Serruys et al, 1991) with early series reporting a rate of early abrupt vessel closure of 25%. It was rapidly recognised that changes in pharmacology and procedural technique could reduce this considerably, and subsequent series in the 1980s reported rates of 3 to 4% as discussed in the 2009 editorial by Cook and Windecker. It was also recognised that most cases were sub acute (occurring between 2 and 30 days), with few cases of acute ST (<24hrs) or late ST (>30 days). Clinical presentation was often insidious and, because ST usually occurred after the patient was discharged from hospital, rapid revascularisation often could not be achieved, resulting in high rates of MI and death. Subsequent PCI trials have observed ST at lower rates, but attempts to completely eradicate this complication have so far failed.

Late (30 days to 1 year) and very late (> 1 year) ST was initially an unrecognised problem and so not reported in earlier PCI trials. Large scale prospective registry data on stent thrombosis is now available. In a report by Armstrong et al, 2012, the definite angiographic late and very late stent thrombosis rates in 401 662 patients undergoing PCI in 2009 and 2010 (CathPCI Registry) were 0.3% and 1.1%. Wenaweser et al (2008) studied ARC defined ST in 8146 European patients receiving DES (Cypher and Taxus) between 2002 and 2005. There was an estimated cumulative incidence of 5.7% of definite/probable ST at 4 years (95% CI: 5.15% to 6.39%). Two thirds of cases occurred before 30 days, after which there was a steady rate of late/very late ST which did not appear to be levelling off at the end of the 4 year study period. Another study looked at 5 year rate of ARC defined ST in 12824 patients from the Japanese J-cypher registry, who had Cypher stents implanted from 2004 to 2006. Kimura et al (2012) reported a cumulative 5 year incidence of definite ST as 1.6%, with 27% of events occurring before 30 days followed by a low steady rate of ST during the subsequent 5 years, a pattern similar to that seen by Wenaweser et al (2008).

There are concerns that assessment of the true rate of ST, and comparison of rate of ST between studies, is likely to be confounded by difficulties with diagnosis. The only ways in which ST can be diagnosed unequivocally are by coronary angiography or post mortem examination. However, patients may present insidiously and the case may go unrecognised, especially in the elderly; or they may present in cardiogenic shock or pulmonary oedema, and may be too unwell for angiography. In addition, patients with stents who subsequently have an ACS may not be investigated invasively due to physician preference in the presence of frailty or co-morbidities, or patient preference. For those presenting with out of hospital cardiac arrest, many do not have cause of death confirmed by post mortem.

The ARC guidelines for reporting stent thrombosis were devised in an effort to overcome these diagnostic problems. Contemporary interventional trials now report ST based on these definitions. However, there is concern that the ARC definitions may result in under-reporting of ST. Cutlip et al (2011) applied the criteria to a post mortem (PM) registry of 139 patients with a history of coronary stenting with DES. 51 were found to have ST by PM. Although ARC definition specificity was high for definite (99%) and definite plus probable (83%) criteria, sensitivity was poor: 18% and 51% respectively. There is therefore the potential to under-report definite/probable ST by as much as half even when ARC criteria are used. The ARC definitions were particularly insensitive for late stent ST: sensitivity for definite/probable late ST was only 20% (CI: 7 to 39). This is of concern because many cases of ST result in death, as discussed below; but overall mortality rates after PCI are low, potentially obscuring the clinical importance of the problem.

In light of reservations about in the sensitivity of ARC criteria for later ST, and problems with completeness of data (some large centres reported no ST at all; the Japanese study did not include probable ST), the true ST rate in both these studies may be higher than reported. In the Wenaweser study, mortality after definite ST was 15.6% after 2 years. Definite/probable ST mortality was not given. In the Kimura study, all except one of the 611 patients with definite stent thrombosis were diagnosed by angiography after the patient presented with an ACS; the remaining one was diagnosed by PM. Two thirds presented with STEMI, one third with NSTEMI. 11% of early ST patients had had a cardiac arrest. Although >96% were treated by PCI, the cumulative risk of death after ST at 2 years was high at around 25%. Note that this mortality figure only applies to patients who actually reach hospital to have angiography – many more cases from this population may have gone unrecognised, as no effort was made to identify probable ST in the deceased patients. In the Wenaweser study, DM

independently predicted early ST; ACS, young age and Taxus stent independently predicted late ST.

#### 1.5.7.4. Restenosis and target lesion revascularisation

The term ‘restenosis’ refers to the re-narrowing of a coronary artery at the site of previous angioplasty; in-stent restenosis (ISR) when this occurs within a stent. Haemodynamically significant ISR is usually treated by target lesion revascularisation (TLR); ISR is the cause of most cases of TLR which occur after the first few weeks; before this procedural complications or ST are the cause of TLR. ISR is discussed in the following section. PCI injures the arterial wall. The healing process following angioplasty, with or without stent implantation, leads to the growth of a layer of tissue (neointima) on the luminal surface of the treated artery known as neointimal hyperplasia. Techniques such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) can accurately measure the thickness of this layer in vivo. The volume of neointima varies within stents, between stent type, between lesions within the same patient and between different patients. Restenosis may also be due to the formation of lipid-rich atherosclerotic tissue with foam cells, with the potential to rupture and cause ACS as in de novo lesions. The possible causes of restenosis, referred to as ‘late lumen loss’, are discussed in detail in a review article by Farooq et al, 2011.

Neointimal hyperplasia develops within all coronary arteries as they heal following angioplasty in response to direct mechanical injury to the vessel wall. Komatsu et al (1998) outlined this process after studying post mortem samples of stented human coronary arteries and observed the formation of thrombus at the site of injury, which rapidly become populated with macrophages. This was followed by the appearance of spindle-shaped cells which matured into smooth muscle cells, within a large volume of extracellular matrix. There was in addition a varying degree of inflammatory cell infiltration, the extent of which correlated with the volume of neointima formation. A layer of neoendothelium formed on the luminal surface of the neointimal layer, although complete re-endothelialisation did not occur for months. Some lesions underwent massive neointimal growth accompanied by extensive neovascularisation and extensive macrocyte infiltration – seen as severe ISR on angiography – the cause of which remains largely unexplained.

Both BMS and DES undergo neointimal hyperplasia. Chieffo et al (2009) performed histological analysis of coronary restenotic tissue obtained from directional atherectomy in both BMS and DES in 44 patients. DES was smaller volume and more frequently focal, but all types of stent produced basically the same histological type of neointima, with proteoglycan-rich smooth muscle cells and fibrolipidic regions.

However, although cell density was the same between samples, different stents had different smooth muscle types, possibly suggesting more than one mechanism of restenosis. 3 DES patients had an abundant inflammatory cell infiltrate.

Kornovski et al noted that, in animal models, the volume of neointima created was proportional to both the extent of injury (which potentially could be limited by correctly sizing stents) and the subsequent inflammatory reaction to the stent struts (which possibly could be controlled using ant proliferative agents). A range of factors have been postulated to explain why excessive neointimal hyperplasia causing ISR occurs in some stents and not others, summarised in reviews by Farooq et al (2011). Factors which are potentially modifiable are: resistance to the effect of the cytotoxic drug, thought to have a genetic basis; hypersensitivity reactions to the polymer (latest generation stents have biocompatible polymers which do not produce such excessive inflammatory responses); strut shape and thickness; and procedural factors such as incomplete stent expansion, geographical miss and barotrauma to un-stented segments.

Kastrati et al (2006) studied 1845 patients undergoing Cypher or Taxus stent implantation, all consenting to have follow-up angiography, for predictors of restenosis. 1495 underwent a follow-up coronary angiogram 193 ± 63 days after the index procedure; 350 patients either died before the angiogram was scheduled or declined the investigation. In this study the independent predictors of DES restenosis following multivariate analysis were female sex, history of CABG, chronic occlusions, vessel size, maximal balloon pressure, drug-eluting stent type, and final diameter stenosis. The most important factors were vessel size and stent type, with Taxus stents associated with higher ISR rates. Other studies have found length of stented segment and diabetes to be additional risk factors for ISR.

Comparing restenosis rate between different studies would be impossible without standardized definitions. By coronary angiography (quantitative coronary angiography (QCA) is often used in research studies), restenosis is traditionally said to have occurred if more than 50% of the lumen of the vessel is compromised. This is known as the ‘binary restenosis rate’ – binary refers to the two states of no restenosis and restenosis. There are several problems with this method. A 50% stenosis may not cause functional ischaemia: meta-analysis from Cutlip et al (2002) found a disparity between binary restenosis rate and clinical outcome as only about half required repeat revascularisation. Furthermore, neointimal tissue volume is a continuous rather than a binary variable.

Late lumen loss may be a more useful way of assessing hyperplasia following stent implantation: this is the minimal luminal diameter immediately post-procedure, minus the minimal luminal diameter at follow up, measured using QCA in the same

projection. Yet a third way of characterising neointimal hyperplasia is using IVUS. IVUS can clearly delineate both the stent and the position of the endothelium and from this calculate the volume of neointimal hyperplasia. IVUS can also determine the cross sectional area of the lumen: less than 4 $mm^2$ in a proximal epicardial vessel is correlated with ischaemia and worse outcomes.

In the very earliest studies of PCI, following balloon angioplasty alone (POBA), the incidence of restenosis was reported to be 30-40%. Restenosis has become less common since the introduction of stents, and has decreased further with improved stent design. The exact rate of ISR is difficult to compare between trials because of differing rates of angiographic follow up: contemporary large scale revascularisation trials do not perform routine follow-up coronary angiography in the absence of symptoms. Instead, studies report a range of different surrogate endpoints, often composite, such as ischaemia-driven revascularization or target vessel failure. In Kastrati's study, 211 (14.1%) patients developed ISR defined as diameter stenosis >50% by QCA. The method chosen to identify ISR in this study was binary restenosis, which may be a poor judge of haemodynamic and therefore clinical significance.

Target lesion revascularisation (TLR) is another way of effectively comparing ISR rates between trials. In an influential meta analysis of double blinded, randomised trials of DES vs. BMS (2009), Kirtane et al reported TLR rates of 23.6% and 7.8% respectively for pooled studies of BMS vs. SES, median follow-up 4.0 years, 1748 patients; and 20.0% and 10.1% for pooled studies of BMS vs. PES, median follow-up 3.2 years, 3513 patients. In all 9 trials in the analysis a high proportion of subjects (42.5% to 97% depending on the study) underwent routine follow up angiography at between 6 and 9 months post PCI, reducing the possibility of missing clinically silent ISR. Note that the TLR rate is not interchangeable with ISR rate: TLR rate also includes patients with stent thrombosis in the absence of ISR; and some patients with ISR may not have undergone further revascularisation.

Registry data provides another way of quantifying the frequency of ISR. Fröbert et al (2009) reported an angiographic restenosis rate of 3.5% after 1 year and 4.9% after 2 years in 19 004 patients from the Swedish coronary angiography and angioplasty registry (SCAAR). These patients had any of 4 types of DES inserted (Cypher, Taxus Express, Taxus Liberté and Endeavor) and were not consented for routine follow-up angiography. This study has the advantage of including every patient who had PCI in an entire country (Sweden) so is definitely 'real-world'; however, the true restenosis rate will be underestimated, as a proportion of patients will have had asymptomatic restenosis not leading to angiography and therefore remaining undiagnosed.

In contemporary practice, SES and PES are being superseded by newer generation stents. Claessen et al (2009) reported the 2 year angiographic and IVUS results of 152 patients from the SPIRIT II trial, which randomised patients to receive a Xience V everolimus eluting stent (EES) or a Taxus paclitaxel eluting stent (PES) in a 3 to 1 ratio. 95 patients had 2 year angiography and IVUS. For EES and PES respectively, in-segment binary angiographic restenosis at 2 years was 5.2% and 8.6%, P=0.44; in–segment mean late loss was 0.21±0.37 and 0.34±0.34, P=0.63; mean percentage volume obstruction by IVUS was 5.18±6.22 and 5.80±6.31, P=0.65; and TLR was 3.7% and 6.5%. It is likely that the true rate of haemodynamically significant ISR lies somewhere around the 5% mark with Xience V stents at 2 years. However, this may be reduced even further with the latest stent designs such as Xience Prime (Abbott Vascular) and Resolute Integrity (Medtronic), both 'third generation' devices with very thin struts, good radial strength and biocompatible polymers.

ISR can manifest clinically with exertional angina or with ACS. Adlam et al (2012) reported the indication for repeat revascularisation in 10509 patients who had undergone a first stenting procedure for any indication, over a median follow up of 3.8 years (60% BMS only, 35% DES only, 4% both BMS and DES). In this registry, 11.2% required repeat PCI and 2.0% required CABG. Of patients having a second PCI, in 250 (20.9%) the procedural indication was 'stent related disease', mainly ISR; there were also some cases of stent thrombosis, but exact figures are not given (of the remaining patients, nearly half had new disease unrelated to the stent and a quarter had planned (staged) PCI). Excluding staged PCI, the presenting syndrome leading to repeat stenting was stable angina in just over half the patients; troponin negative UA in about a quarter; and MI (STEMI and NSTEMI) in the remaining patients (again, exact numbers are not reported).

Severe ISR or even complete vessel occlusion may also be asymptomatic. Ruygrock et al (2001) found that of 2690 patients following PCI with POBA or BMS, 607 had binary angiographic restenosis which was clinically silent in 55% of cases. However, only in 20% of those with restenosis was the stenosis >70%, so it is likely that many of these cases were not haemodynamically significant.

Work to reduce ISR led to the invention of the DES, in which the metal scaffold of the stent has a polymer coating impregnated with an antimitotic drug, often one of the 'limus' family, to inhibit neointimal growth. The drug is released (eluted) slowly and has a high concentration at the stent site but very low concentration elsewhere in the body, minimising systemic effects of the drug. DES use reduces the requirement for TLR, and possibly also mortality, compared to BMS. However, not all patients are

considered suitable for DES, such as those thought to be at high risk of bleeding who cannot take long term dual antiplatelet therapy (DAPT). Furthermore, restenosis due to intimal hyperplasia still occurs with DES, albeit at a lower rate than with BMS.

Angiographic ISR may be treated conservatively if thought not to be haemodynamically significant (non-ischaemic ISR). Repeat revascularisation of obstructive lesions may take place by either PCI or CABG. Before the DES era, efforts to treat BMS ISR by POBA, cutting balloon or further BMS implantation resulted in a high re-restenosis rate. A technique of irradiating the restenotic segment with locally delivered ionizing radiation called brachytherapy was developed. Brachytherapy was an effective short-term ant proliferative strategy but was limited by logistical difficulties and high rates of long-term target lesion revascularisation (Ruef, 2007).

### 1.5.8. Adverse Event Reporting: ARC and BARC Definitions

In recognition of the importance of standardised reporting of clinical events, a collaboration between European and North American research institutions with representation from the U.S. Food and Drug Administration (FDA) and commercial device companies known as the Academic Research Consortium (ARC) has produced specific criteria for the most important safety and efficacy endpoints after PCI:

- **Death**; ARC recommend reporting all-cause mortality and also death classified by cause into (i) cardiac, (ii) vascular and (iii) non-cardiovascular. 'Cardiac death' is any death of unknown cause, including "*unexpected death even in patients with coexisting potentially fatal noncardiac disease*"; and any procedure-related death
- **MI** (see below for categories)
- **Repeat revascularisation**; the ARC recommend reporting this as 'target lesion revascularisation' (TLR) (clinically and non-clinically indicated) and 'target vessel revascularisation' (TVR)
- **Stent thrombosis** (ST). **Acute** (less than 24 hours), **subacute** (24 hours to 30 days), **early** (<30 days; acute plus subacute), **late** (30 days to 1 year) and **very late** (more than one year). **Definite ST** must be diagnosed angiographically (must also be accompanied by clinical evidence of myocardial ischaemia) or by post mortem examination. **Probable ST** is any unexplained death within 30 days of PCI, or any MI (at any time point) documented to be in the territory of the stented segment in the absence of angiographic evidence. **Possible ST** is any unexplained death occurring more than 30 days after PCI.

Bleeding is another major complication following PCI; the risk of bleeding is increased due to antiplatelet and anticoagulant medication. Each major trial group – for example the TIMI and GUSTO groups - has used different definitions consisting of a combination of laboratory parameters (decreases in haemoglobin and haematocrit scores) and clinical bleeding events combined to create some form of ranking of severity. This impedes direct comparison of true bleeding rates between trials. However, another group of researchers has recently devised a new system, weighted towards clinical severity and prognosis, which is simple to use and may help standardise reporting of bleeding. The proposed Bleeding Academic Research Consortium (BARC) definitions are as follows:

- **Type 0**: No bleeding
- **Type 1**: Minor bleeding that is not actionable; for example, bruising or a small self-limiting nosebleed
- **Type 2**: minor bleeding which requires action (a test, a treatment or withdrawal of a drug) from a health care professional, but not sufficient to cause significant blood loss or compromise to any organ system; for example, a nosebleed requiring temporary cessation of antiplatelet medication.
- **Type 3**: severe bleeding.
- **Type 3a** is any overt bleed requiring a transfusion with a Hb drop of 3 to 5g/dL
- **Type 3b** is life-threatening bleeding: it includes overt bleeding requiring a transfusion with a Hb drop of more than 5g/dL; cardiac tamponade; bleeding requiring surgical intervention to stop it; or bleeding with shock requiring inotropes
- **Type 3c** is intracranial or intraocular haemorrhage
- **Type 4**: CABG related bleeding
- **Type 5**: fatal bleeding

## 1.6. Platelets, antiplatelet agents and platelet function testing

Antiplatelet drugs are the cornerstone in the prevention of coronary thrombosis during and after PCI. This section contains a brief overview of platelet function and mode of action; and describes the available antiplatelet medications and their limitations.

### 1.6.1. Platelets and thrombosis

Platelets are anucleate cells which are produced in the bone marrow and circulate for approximately 10 days. They have a vital role in rapid haemostasis following injury,

their primary function being the initiation of thrombosis at the site of arterial damage by the processes of adhesion, activation and aggregation.

#### 1.6.1.1. Platelet adhesion

Platelets do not adhere to healthy endothelial cells, which have several antiplatelet properties: they carry a negative charge, and they secrete the antiplatelet agents nitric oxide, prostacyclin and ADP-ase. However, where the endothelial layer is interrupted in spontaneous plaque rupture or angioplasty, platelets rapidly bind to exposed subendothelial collagen. As described by Ruggeri and Ware in 1993, in the presence of the high sheer forces found in fast flowing blood in small vessels and around arterial stenoses, this attachment is largely mediated by von Willebrand factor (vWF). This glycoprotein, the largest known soluble globular protein, is generated in endothelial cells and released into both the blood and the subendothelium. It is also produced by megakaryocytes, the cells which give rise to platelets, and is stored in platelet α-granules. vWF changes shape from a globular structure to an extended chain conformation both on binding to collagen and under conditions of shear stress above a critical value (Siedlecki et al, 1996). This conformational change allows the binding of platelets via platelet membrane glycoprotein receptors such as GP 1bα. Platelet adhesion triggers activation of the platelet.

#### 1.6.1.2. Platelet activation

Activation is a complex collection of different molecular processes and conformational changes which must occur before the aggregation of platelets into a stable platelet plug. Several platelet activating agents are known including collagen and vWF/thrombin complex in subendothelial tissue, and the soluble agonists ADP, thrombin and thromboxane $A_2$ ($TXA_2$), each with its specific platelet membrane receptor (see below). Binding of an agonist rapidly causes a marked rise in platelet intracellular calcium, triggering a number of events. The platelet loses its cytoskeletal structure to change shape from a disc to a globule with multiple arm-like extensions and blebs. The blebs are shed as microvesicles and the arms increase the surface area available for platelet-platelet binding. Highly pro-coagulant factors are released from α- and dense granules into the immediate vicinity; and the surface of the platelet become highly pro-coagulant due to the redistribution of phospholipid molecules. Platelet activation also leads to a conformational change in the IIbIIIa (Integrin $\alpha_{IIb}\beta_3$) complex in the platelet membrane (Seiss, 1989), the final event required for agonist-mediated aggregation.

Activation is propagated by several mechanisms, including the binding of the adenine nucleotide ADP (released from platelet granules) to the purinergic $P2Y_1$ and $P2Y_{12}$ G protein-coupled platelet receptors.

#### 1.6.1.3. Platelet aggregation

Activated platelets form stable clumps by the cross linking of cell membrane IIbIIIa molecules. IIbIIIa is part of the integrin family, large proteins which span the cell membrane. Integrins have 2 main functions: to mediate cell attachment to surrounding tissue, and as cell signalling intermediaries (they have also been studied for their potential roles in invasion of tissue by metastatic cancer cells, the functioning of viruses and allergic disorders such as asthma). IIbIIIa is the most abundant platelet glycoprotein, with approximately 80 000 molecules per platelet (Scarborough et al, 1999).

Recent work has shown that platelets are also capable of forming clumps without activation, independent of soluble agonists and IIbIIIa, in response to high shear stress. Nesbitt, Jackson et al (2009) showed that discoid platelets could form stable clumps, via membrane tethers, in injured non-stenosed mouse vessels. These initially formed downstream from the site of vascular injury and had sufficient strength to occlude vessels. Secondary, agonist-dependent consolidation of the platelet clump then occurred at the site of tissue injury in the areas of lower shear created around the developing thrombus.

### 1.6.2. Platelet agonist receptors and inhibitors used in PCI

Identification of the various cell receptors involved in platelet function has led to the development of drugs designed to block these receptors in order to reduce arterial thrombosis in patients with cardiovascular disease. A detailed review of contemporary antiplatelet therapy has been provided by White, 2011.

#### 1.6.2.1. IIbIIIa inhibition

Platelet activation leads to a conformational change in the IIbIIIa molecule in the platelet cell membrane and allows permanent cross-linking of platelets and the formation of a platelet plug. Activated platelet IIbIIIa can bind to multiple soluble ligands, such as fibrinogen, fibronectin and vWF. Intravenously administered IIbIIIa inhibiting drugs have been in routine clinical use for many years to treat coronary thrombosis (Nurden et al, 2009). Intravenous IIbIIIa inhibitors are still used during PCI, particularly when coronary thrombus is present or when flow appears poor, although less than before. An unexpected event in the evolution of antiplatelet therapy was that in all 5 trials of oral IIbIIIa agents, increased mortality and increased bleeding rates and,

paradoxically, increased thrombotic events. Chew et al presented these results in a 2001 meta-analysis. These findings were never fully explained but use of oral IIbIIIa inhibitors ceased and attention was turned to different antiplatelet agents affecting alternative platelet receptors.

### 1.6.2.2. Thromboxane A2 and aspirin

$TXA_2$ is produced by platelets from arachidonic acid by the cyclooxygenase (COX-1) pathway, involved in prostaglandin synthesis (Vane, 1971). Platelets have 2 variants of $TXA_2$ receptor, TPα and TPβ. Both are G-protein linked receptors which trigger an increase in intracellular calcium levels and further platelet activation. $TXA_2$ is also widely produced by other mechanisms, such as the COX-2 pathway, by several other cell types. Roth and Majerus in 1975 suggested that aspirin exerted its antiplatelet effect by the rapid and permanent acetylation and inactivation of platelet cyclooxygenase. Aspirin has been shown to improve cardiovascular outcomes in secondary prevention in several studies e.g. in ISIS-2, 1988, the death rate 34 days post MI was 9.4% on aspirin and 12.0% on placebo, risk ratio 0.78. Aspirin's role in primary prevention is less clear, when bleeding may outweigh the benefit.

### 1.6.2.3. Adenosine diphosphate (ADP) and $P2Y_{12}$ receptor inhibitors

ADP is released by damaged endothelial cells and erythrocytes, but is also excreted (in much larger amounts) by activated platelets, causing a self-propagating cascade of platelet activation leading to the formation of a platelet plug at the site of an endothelial breach. It is thought that the binding of ADP to $P2Y_1$ is responsible for the change in shape of activating platelets via changes in intracellular calcium level. The binding of ADP to $P2Y_{12}$ inhibits adenylate cyclase, a transmembranous G protein-coupled enzyme catalysing the conversion of ATP to cAMP. The inhibition of cAMP production reduces the activity of protein kinases, which can no longer phosphorylate vasodilator stimulated phosphoprotein (VASP). VASP phosphorylation is required for GP IIb/IIIa receptor inhibition; reduced cAMP therefore results in activation of IIb/IIIa receptors and promotion of platelet aggregation (Jin and Kunapuli, 1998). $P2Y_1$ is widely expressed throughout the body and so has not been a focus of antiplatelet research in humans. $P2Y_{12}$ is expressed only in platelets and the brain; several $P2Y_{12}$ inhibitors have been developed which are in clinical use, including the thienopyridines clopidogrel and prasugrel; and ticagrelor, a nucleoside analogue (White, 2011).

### 1.6.2.4. Thrombin and thrombin inhibitors

Thrombin is an essential component in thrombus formation. It is the final protease in the coagulation cascade, cleaving fibrinogen to form a fibrin clot. Antithrombin is the

primary natural inhibitor of blood coagulation proteases, which only exerts its effect when in contact with heparin-like glyocosaminoglycans with which it forms a ternary complex (Li, 2004) – the basis of heparin as an anticoagulant. However, thrombin also has a direct effect on platelets unrelated to its interaction with fibrinogen (Davey and Luscher, 1967), and is a potent platelet agonist.

Two human platelet thrombin receptors have now been demonstrated: the protease activated receptors (PAR) 1 and 4 (Kahn 1998). PARs are G protein-coupled receptors, very widespread in all organs, which have complex interactions with multiple agonists and antagonists. Vorapaxar and atopaxar are novel oral drugs in development, both targeting PAR1, for potential use in the treatment of ACS. A major clinical trial in which vorapaxar was added to standard therapy after NSTE-ACS was halted early due to excess bleeding without a reduction in the composite primary endpoint. Intracranial haemorrhage rates were 1.1% in the vorapaxar group vs. 0.2% in the placebo group (Tricoci et al, 2012). Atopaxar has been tested in Phase II clinical trials with promising results (Wiviott, 2011).

## 1.7. Platelet function testing

The ability to test platelet reactivity has been used in clinical research to assess the effect of antiplatelet therapy and has been particularly useful in the study of ‘clopidogrel resistance’. Several of the available point-of-care devices have been studied for their clinical utility in routine care but at the time of writing none are recommended for routine clinical use, although studies are ongoing. There is no ‘gold standard’ platelet function test, but several different techniques have been used to measure the different aspects of platelet function (for example platelet reactivity, quantification of activated platelets, platelet-platelet and monocyte-platelet aggregates and platelet micro particles). Generally the test involves the response of platelets to the addition of an agonist such as ADP or thrombin. Platelet function testing for clinical purposes has recently been extensively reviewed by Gorog and Fuster, 2013. Briefly, the available methods of platelet function assessment are:

- Bleeding time
- Platelet aggregometry (turbidometry or electrical impedance)
- Flow cytometry VASP MoAbs
- Testing for urinary or blood markers of platelet activation

Bleeding time is the time taken for a small standardized iatrogenic wound to stop bleeding. It is prolonged in any disorder of platelet function, but not in haemophilia or

warfarin therapy. Rodgers and Levin (1990) reviewed 1083 studies in humans and found the test generally unsatisfactory, being neither specific nor reproducible – and it leaves a scar. Plasma assays of platelet factor 4, soluble P-selectin, b-thromboglobulin and plasma and urinary assays of thromboxane A2 metabolites have all been used as indirect markers of platelet activation. However, aggregometry and flow cytometry appear to be more reliable methods of platelet function.

### 1.7.1. Aggregometry

Platelet aggregometry is the measurement of platelet aggregation (a marker of the extent of activation), used as an indicator of platelet reactivity. Aggregation may be measured in the laboratory by detecting changes in light transmission in a sample of platelet-rich plasma, which may have been exposed to an agonist of activation, a method sometimes called 'turbidometry', as explained by Michelson (2004). Activated platelets form clumps and fall out of suspension, so increasing light transmission. This is usually compared to the light transmission through a sample of platelet-poor plasma; hence, each patient acts as his own control. Platelet aggregation can also be demonstrated in whole blood by measuring changes in electrical impedance caused by platelet aggregation on the electrodes.

Light transmission aggregometry (LTA) is impractical for routine large scale clinical use due to the complexity of the technical aspects of the procedure. However, a number of point of care assays have now been developed, including the VerifyNow system (formerly known as the Ultegra rapid platelet function analyzer), Accumetrics, California, discussed below. Several electrical impedance devices have also been developed. Of available bedside devices, VerifyNow has the largest body of supporting evidence and has been used most frequently in clinical trials of platelet function (Breet et al. 2010).

### 1.7.2. Flow cytometry

Platelet aggregation may also been studied using flow cytometric techniques. A flow cytometer is a device for studying the various properties of large numbers of small particles. Fluid containing the particles is streamed through the device; the particles are fluorescently labelled; a laser is directed at the stream; and the characteristic fluoresced light patterns can be analysed. Flow cytometry allows the accurate study of even very small numbers of individual particles. Platelets can be studied both for their degree of activation, degree of reactivity and response to individual agonists in their physiological environment of whole (anticoagulated) blood. They are labelled using monoclonal antibodies to the various platelet receptors. (Michelson 2004). Drawbacks of the

technique are that it requires a flow cytometer, is labour and expertise intensive and is not conducive to routine clinical use.

### 1.7.3. VerifyNow $P2Y_{12}$ receptor point-of-care assay

The VerifyNow device is a rapid whole blood assay of platelet function based on optical aggregometry, used for point of care platelet function testing. It was first marketed as the Integra Rapid Platelet Function Analyzer by Accumetrics incorporated, San Diego, California. Separate assays are available to test the response to $P2Y_{12}$ inhibitors, aspirin and IIbIIIa inhibitors. Van Werkum et al (2006) described the principles behind its operation and its accuracy compared to LTA, and described the evidence for its clinical application (2008). Briefly, citrated blood is mixed with fibrinogen coated beads to which activated platelets will bind. In the presence of a platelet agonist, platelet-coated beads fall out of suspension so that light transmission through the sample will gradually increase over time. The rate of change in transmission is altered in the presence of antiplatelet drugs which will tend to keep the beads in suspension for longer and so increase sample turbidity.

The $P2Y_{12}$ assay has individual chambers in which different agonists can be mixed with the blood sample. One chamber mixes blood with PAR-1 and PAR-4 (platelet thrombin receptors) agonists, which act independently of the $P2Y_{12}$ and thromboxane receptors to cause very complete platelet aggregation (thrombin is the most potent platelet agonist known). A proprietary algorithm is applied to produce a value known as the 'baseline PRU' value (PRU stands for $P2Y_{12}$ Reaction Units). There is a wide normal range given by the manufacturer for the baseline value of 212 to 398. If the value is less than this range, the device will not report the result and reads 'error' – a low baseline value implies abnormal platelet function due to factors other than $P2Y_{12}$ inhibition, such as IIbIIIa treatment or a platelet disorder. The higher the value, the more reactive the platelets are.

A second chamber mixes blood with 20 µmol of ADP and 22 nmol of prostaglandin E1 (the addition of the latter compound reduces the contribution of ADP binding to $P2Y_1$ receptors to aggregation). This generates a 'PRU' value which corresponds with the extent of ADP/ $P2Y_{12}$-mediated aggregation.

A percent inhibition value is then generated using the following equation:

$$Percent\ platelet\ inhibition = \frac{Base\ value - \ PRU\ value}{Base\ value} \times 100$$

All 3 values are displayed by the device. This method allows the effect of the $P2Y_{12}$ inhibiting drug to be quantified using a single blood sample, rather than before- and after-dose samples.

The VerifyNow test appears to be precise: Paniccia et al in 2011 reported the coefficient of variation (CV) for the $P2Y_{12}$ test to be 3.2% when tested in patients with coronary artery disease. Furthermore, the results appear to correlate well with platelet reactivity measured by conventional LTA. Using 10 μmol/L ADP for LTA, concordance with VerifyNow was 79.1%, ($\kappa = 0.43$, $P < 0.0001$; $\rho = 0.64$, $P < 0.0001$) (same study). Several studies have correlated antiplatelet resistance as defined by the VerifyNow device with subsequent adverse cardiovascular outcomes. Moreover, the VerifyNow $P2Y_{12}$ test appears to compare favourably with other available point-of-care tests with regard to subsequent clinical events (Breet et al, 2010).

## 1.8. Platelet reactivity, drug resistance and clinical outcome

Trip et al (1990) were among the first investigators to show that platelet reactivity following MI correlates with recurrent ischaemic events. Spontaneous platelet aggregation was tested in 149 survivors of MI at intervals of 6 months using light transmission aggregometry. This study recruited patients prior to 1990, when aspirin was the only antiplatelet in routine use. Patients were asked to stop aspirin for 1 week before each blood test. The relative risk of cardiac death in the group with a high level of aggregation compared to the group with low aggregation was 5.4 (95% confidence interval 2.2 to 13.4).

Despite the widespread contemporary use of DAPT (and other beneficial medical therapies) in ACS and PCI, there remains a significant rate of recurrent cardiovascular events. As an example, in the large HORIZONS trial (Mehran et al, 2009), the major adverse cardiovascular event rate between 30 days and 1 year in 3602 patients presenting with STEMI who underwent primary PCI was around 7% (7.3% in the control group, 6.8% in the bivalirudin group). This was a randomised controlled trial; ‘real world’ patients may have even higher rates. It is currently thought that an important number of these cases of recurrent ischaemia were due to antiplatelet resistance, particularly to the thienopyridines.

### 1.8.1. Aspirin resistance

Aspirin was discovered in the 1970s to be an inhibitor of platelet arachidonic acid (AA) metabolism, leading to inhibition of thromboxane A2 production and a decrease in platelet reactivity (see Section 1-6-2-2). Aspirin is recommended in all patients undergoing PCI, unless contraindicated, as part of the standard dual antiplatelet therapy

(DAPT) regime. Variability in response to aspirin resulting in adverse clinical outcomes has been noted by many authors. Chen et al (2007) found procedural myonecrosis (CK-MB) was increased in 151 patients undergoing elective PCI on DAPT as measured by the Ultegra Rapid Platelet Function Assay.

However, as pointed out in a 2011 review by Fitzgerald and Pirmohamed, although aspirin resistance may be a clinically important entity, there is currently no accepted definition of aspirin resistance, no recognised gold standard test, and no clear indication of how to treat it when detected. At present, the International Society on Thrombosis and Haemostasis working group on aspirin resistance and the international PCI guidelines do not recommend testing for aspirin resistance. Furthermore, it is thought to be much less common than resistance to $P2Y_{12}$ inhibitors.

### 1.8.2. $P2Y_{12}$ inhibitor resistance

$P2Y_{12}$ inhibitors suppress ADP-induced platelet activation and aggregation, thereby inhibiting arterial thrombosis. Clopidogrel is currently the most widely used agent with a strong evidence base to demonstrate reduced MACE after ACS and PCI when compared to aspirin alone. It is known on the basis of laboratory platelet function testing that there is a wide range of response to clopidogrel: a large proportion of the population, thought to be about 30%, are completely or partially resistant to its action as a $P2Y_{12}$ inhibitor. The other thienopyridine currently in routine use, prasugrel, also has evidence of resistance although much less commonly than clopidogrel.

In contrast to the case with aspirin resistance, PCI guidelines approve the concept of testing for clopidogrel resistance, because studies have shown a correlation with bedside platelet function testing and clinical events. Brar et al in 2011 produced a collaborative, patient level meta-analysis of 6 of these studies looking at clinical outcome following PCI in a total of 3059 patients treated with clopidogrel who had a VerifyNow test. There was a 4% increase in frequency of the primary composite outcome of death, MI and stent thrombosis for every 10-unit increase in PRU (HR: 1.04; 95% CI: 1.03 to 1.06; $P < 0.0001$. The event rate at 2 years was 5.5% in the lowest PRU quartile and 15.8% in the highest ($P < 0.001$). Using a receiver-operator curve statistical method, the authors suggest that a PRU cut off of >230 offers the greatest discriminatory value for predicting adverse events.

Other authors have observed worse outcomes in patients with both high and low on-treatment platelet reactivity. Mangiacapra et al, 2012, observed optimal clinical outcomes in patients with VerifyNow PRU within the range 179 to 238. Patients with

higher PRU had more ischaemic events and those with lower PRU more bleeding events.

## 1.9. Hypothesis, Aims and Objectives

### 1.9.1. Main hypothesis

The main hypothesis of this work is that adverse clinical events following PCI, such as stent thrombosis, bleeding and in-stent restenosis, may be predicted from the identification of markers of risk at the time of PCI, particularly by the use of risk scores, platelet function testing and measuring biomarker levels.

### 1.9.2. The secondary hypotheses

- P2Y12 inhibition with clopidogrel is variable and 'clopidogrel resistance' may be measured.
- Thrombotic adverse cardiovascular events in patients undergoing PCI can be related to clopidogrel resistance.
- Bleeding can be related to high levels of $P2Y_{12}$ inhibition.
- Further revascularisation/ISR can be related to angiographic and procedural complexity (SYNTAX score, procedural complications).
- Procedural MI can be diagnosed with novel biomarker HFABP.
- Procedural MI is important for long-term prognosis.

### 1.9.3. The aims of this work

- To record the clinical outcome (MACCE) of patients with ACS undergoing PCI.
- To correlate adverse events with already known or postulated risk factors including baseline clinical features, procedural factors, clopidogrel resistance and genetic profile.
- To define procedural MI using the novel biomarker HFABP.
- To identify novel risk factors for adverse outcomes following PCI.

# Chapter 2 Methods: the OPERA study

The Outcomes from PCI by Evaluation of Risk Attributes (OPERA) study was established in 2005 as a registry of patients with ACS undergoing PCI. The purpose was to record a comprehensive array of clinical and procedural characteristics, including procedural complications, biomarkers, clopidogrel resistance and genotyping, and prospectively follow patients for adverse clinical events.

## 2.1. Study structure, protocol and patients

### 2.1.1. Ethical approval

Version 4 of the study protocol (see appendix) was approved by Leeds (West) Research Ethics Committee on 25 Oct 2005 (ref 05/Q1205/54).

### 2.1.2. Consent, inclusion and exclusion criteria

Patients were recruited to the study if they had presented to hospital with an ACS – UA, NSTEMI or STEMI – and went on to have PCI. Patients who were unwilling or unable to provide written consent were not included, hence this study does not contain patients with an impaired conscious level such as in cardiogenic shock; and patients with a terminal illness were not recruited. There were no other exclusion criteria.

For patients where PCI was planned, informed consent was obtained before the procedure where possible after reading the information sheet. For ad hoc PCI, verbal assent to take part and remove a blood sample was sought after the angiogram but before PCI. The patient then underwent the informed consent process when they were feeling well enough, usually on the ward within a few hours of the procedure.

### 2.1.3. ACS diagnosis

The final diagnosis in suspected ACS patients was made by the local medical team responsible for the patient's clinical care. Unstable angina (UA) was diagnosed if the symptoms were attributable to myocardial ischaemia in the absence of elevated cardiac biomarkers. Myocardial infarction was diagnosed when ischaemic symptoms were accompanied by elevated cardiac biomarker levels and categorised as NSTEMI or STEMI on the basis of the ECG changes. The STEMI patients recruited into this study may have had primary, rescue (failed thrombolysis) or convalescent PCI.

The standard cardiac biomarker was Troponin I. Because patients were admitted to several different hospitals over a period of several years, TnI assays by a range of different manufacturers were used. Note that most of the assays used over this period of

time had not been tested for precision at the level of rigor required to meet the definition of a ‘high-sensitivity’ troponin assay according to the most recent recommendations.

### 2.1.4. Participating hospitals

All PCI was undertaken at a single tertiary cardiology centre (Leeds General Infirmary). However, the patient may have initially presented to any one of ten or more surrounding district general hospitals within the West Yorkshire cardiac network; see Table 3.1.

### 2.1.5. Standard medical ACS care

Unless contra-indicated, all ACS patients received contemporary standard evidence-based medical therapy including dual antiplatelet therapy (DAPT), statins, beta blockers, ACE inhibitors and anticoagulant agents. The antiplatelet regime was 300mg aspirin administered soon after first presentation, often by paramedic staff pre-hospital, followed by 75 mg per day. The standard second antiplatelet agent was clopidogrel, given as a loading dose of 300 mg on arrival to hospital followed by 75 mg per day. If the total clopidogrel dose ingested was less than 600 mg at the time of PCI, additional clopidogrel was administered pre-procedure to bring the total to 600 mg. In 2009 prasugrel was introduced for use in PPCI or for patients with clopidogrel sensitivity, given as a 60 mg loading dose followed by 10 mg per day, or 5 mg per day if aged over 75 years.

Anticoagulants were prescribed shortly after arrival to hospital to patients in whom ACS was strongly suspected, administered subcutaneously, and continued for a minimum of 48 hours in patients found to be troponin positive. The standard anticoagulants were weight-adjusted low molecular weight heparin or fondaparinux.

IIbIIIa inhibitors, usually tirofiban, were administered by continuous intravenous infusion to patients with ongoing pain with ECG ischaemia after the initial medical therapy, or for recurrent symptoms. Abciximab tended to be reserved for administration in the cath lab.

Patients with STEMI underwent urgent reperfusion therapy by thrombolysis and/or PPCI.

### 2.1.6. PCI operators, techniques, methods and decision to treat

PCI was undertaken by several different operators. Equipment, pharmacology, devices and implantation techniques were selected by each operator according to his individual preferences but within local procedural guidelines and availabilities.

Diagnostic coronary angiography was usually performed by the district general hospital (DGH) for patients presenting to hospitals other than Leeds General Infirmary, so in many cases the PCI strategy could be planned in advance and discussed with colleagues at an MDT meeting if required. In unstable cases and in patients presenting directly to the Leeds General Infirmary, 'ad hoc' emergent PCI was performed (decision to treat made on-table after diagnostic images obtained; PCI carried out directly during the same procedure).

## 2.2. Study Funding

The materials used in the study were partially funded by unrestricted educational awards from Eli Lilly and the Medicines Company. Assistance with nursing support was provided by the NIHR. Randox funded biomarker analysis.

## 2.3. Data collection

Data was collected by the study nurses and cardiology registrar using a paper case report form (CRF). The data was then transferred to a computer database by the study nurse, medical staff and data entry staff.

## 2.4. Data storage and security

Paper CRFs were stored in numerical order in a locked filing cabinet. The electronic database was located in the University of Leeds and was password protected.

## 2.5. Data fields

See for the CRF in the Supplementary Material for data points collected.

### 2.5.1. Identification and demographic data

- Name, date of birth, gender, general practitioner details, address and telephone numbers
- Mode (UA, NSTEMI, STEMI, PPCI), date and hospital of presentation, and occurrence of cardiac arrest or malignant arrhythmia.

### 2.5.2. Previous medical background

- History of coronary disease and prior revascularisation details
- Co-morbid medical conditions (respiratory problems, cerebrovascular disease)
- Cardiovascular risk factors (diabetes mellitus, hypertension, smoking history, family history, hypercholesterolaemia)

### 2.5.3. Pre-PCI clinical details

- Medication etc

### 2.5.4. Angiographic data, PCI procedural details and complications

Coronary angiograms were reported by UMS and IRP. The location of each lesion was recorded by allocating the lesion to a numbered coronary segment: 1 segment for the LMS, 5 segments for the LAD territory, 4 for the Cx territory and 4 for the RCA territory. The following angiographic and procedural details were recorded for each lesion:

- Visual estimate of vessel diameter and percent stenosis
- Presence of thrombus, calcification
- ACC/AHA classification of lesion complexity – see table 2-1
- TIMI grade flow before and after procedure. Note: *TIMI 0 flow* is absence of antegrade flow; *TIMI 1 flow* is faint antegrade coronary flow with incomplete filling of the distal coronary bed; *TIMI 2 flow* is reduced flow but with complete filling of the distal territory; *TIMI 3 flow* is normal brisk flow (ref)
- Use of pre- or post-dilatation and balloon sizes
- Types and sizes of each stent
- Procedural success/complications

**Table 2-1 ACC/AHA lesion complexity characteristics; Ellis modification, Circulation 1990; 82:1193-1202**

| Type A lesion | Type B lesion | Type C lesion |
|---|---|---|
| High success rate, low risk | Moderate success, moderate risk<br>(Type B1 lesions: one B characteristic only; Type B2 lesions: more than one B characteristic) | High risk, low success rate |
| Discrete (<10mm in length) | Tubular (10 to 20mm in length) | Diffuse lesion (>20mm in length) |
| Concentric | Eccentric | Excessive tortuosity |
| Readily accessible | Moderate tortuosity | Extremely angulated (>90°) |
| Non-angulated | Moderately angulated (45 to 90°) | Inability to protect major side branch |
| Smooth contour | Moderate calcification | Degenerated vein graft |
| Little or no calcium | Irregular contour | Total occlusion > 3 months old |
| No thrombus | Some thrombus | |
| Not located at ostium or bifurcation | Requires double guidewires | |
| | Ostial lesion | |
| | Bifurcation lesion | |
| | Total occlusion < 3 months old | |

### 2.5.5. Procedural success and complications

Angiograms and clinical records were reviewed independently by UMS and IRP for complications and procedural success.

#### 2.5.5.1. Technical success

Technical success was said to have occurred if at the end of the procedure:

- There was no angiographically significant obstructive disease (<50% residual angiographic stenosis) within 10 mm either side of the treated lesion
- There was brisk flow (TIMI III) in all major branches (>1.5 mm in diameter) involved in the treated lesion.

NB if a side branch was angiographically ‘pinched’ or ‘trapped’ but with brisk flow, as per convention this was reported as technical success for the lesion.

For lesions without technical success an explanation was recorded, classified into the following categories:

- Unable to penetrate the lesion – chronic total occlusion
- Lesion crossed but unable to dilate – heavy calcium
- Lesion crossed and dilated but residual stenosis >50%, but with good flow
- Lesion crossed and dilated but residual stenosis >50%, but with poor flow (<TIMI III)
- No mechanical obstruction/stenosis but poor flow at the end of the case due to microvascular obstruction: 'no-reflow' phenomenon
- Loss of any significant calibre vessel (>1.5 mm)

#### 2.5.5.2. Periprocedural complications

Periprocedural complications were given a detailed description and further categorized into an intra-procedural thrombotic event*, MACCE event† or BARC bleeding event

- Distal embolization of thrombus
- Slow flow/no reflow main vessel
- Thrombus formation
- Transient vessel closure
- Localised edge dissection requiring no treatment or one further stent without loss of flow to vessel (does not include intended 2 stent strategy)
- Extensive dissection requiring multiple extra stents
- Loss of a side branch – less than TIMI III flow in the side branch at the end of the procedure; vessels greater than 1.5 mm in diameter
- Angiographic pinching/trapping or dissection of side branch but normal flow
- Coronary perforation
- Ventricular fibrillation
- Complete heart block or other bradycardia requiring temporary pacing
- Acute neurological symptoms/signs compatible with ischaemia i.e. stroke
- Acute neurological symptoms/signs compatible with contrast reaction
- Allergic reaction
- Cardiac tamponade requiring drainage
- False aneurysm
- GI bleed
- Retroperitoneal bleed
- Intracranial bleed

- Access site haemorrhage
- Peripheral arterial occlusion requiring any intervention
- Acute stent thrombosis (within 24 hours)
- Infection
- Acute renal failure
- Pulmonary oedema
- Death on table
- Non VF cardiac arrest
- Catheter tip dissection (not related to treatment of a lesion)

* 'Intra-procedural thrombotic event' is a composite of distal embolisation, slow/no flow, new thrombus formation and transient vessel closure

† 'MACCE event' is major adverse cardiac or cerebrovascular event

### 2.5.6. Reporting the SYNTAX score

A single operator (U. M. Sivananthan) calculated SYNTAX scores using the online calculator.

### 2.5.7. Pre- and post-procedural ECG

The ECG with the most marked ischaemic changes was used as the 'pre ECG'; the 'post ECG' was taken within an hour after PCI in the recovery ward. If the patient's admission ECG changes had resolved by the time of PCI, the post ECG recorded new changes relative to the immediately pre-procedure ECG rather than the admission ECG, in order to capture changes which would indicate a procedural MI. ECGs were reported by a cardiologist or experienced study nurse. The following parameters were recorded:

- ST deviation or T wave changes
- Distribution of changes (anterior, inferior, posterior, lateral)
- Bundle branch block
- Paced rhythm

## 2.6. Follow up

Cardiac symptoms, further hospital admissions, MACCE events (ACS, stroke, unplanned revascularisation or death), and bleeding events were recorded. Attempts were made to contact every patient by telephone; the GP was contacted for information about medication or clinical details. DGHs were contacted to supply copies of medical notes when there had been an admission or outpatient clinic review when this pertained to the study.

The NHS Information Centre provided the certified date and cause of death. When the death had been cardiovascular, the hospital notes were inspected where possible. When a post mortem examination had taken place the pathologist's report was obtained by application to the coroner's office.

## 2.7. Arbitration of clinical events

All MACE events were defined using ARC criteria (see introduction for discussion) after case review by an events committee.

- **Death**: total, cardiac, vascular and non-cardiovascular as per ARC criteria
- **Spontaneous MI**: the ARC has agreed to define MI according to Universal Criteria of MI definitions. A troponin positive event which was provoked by a non-cardiac acute illness such as chest infection, without symptoms or ECG changes, was not counted as an MI.
- **Stent thrombosis**: as per ARC criteria for definite, probable and possible
- **Target lesion revascularisation** as per ARC
- **Target vessel revascularisation** as per ARC
- **Stroke**. No ARC criteria. Stroke was defined as a sudden neurological event attributable to acute cerebral ischaemia or intracranial haemorrhage. CT or MRI scan reports were obtained where possible.
- **Bleeding** was defined according to BARC criteria

**Procedural MI** was diagnosed according to the Universal Definition of MI 2012 as follows:

If troponin below 99th percentile of upper reference limit (URL) of the normal range in a healthy population at baseline, then must rise to greater than 5 times this level.

If elevated above $99^{th}$ percentile URL at baseline and the level is stable or falling, the level must rise to greater than 20% of the initial value.

If biomarkers are rising (as in primary PCI patients) procedural injury cannot be reliably calculated

The 2012 Universal Definition requires additional clinical features in addition to biomarker elevation for the diagnosis of procedural MI. These are: (i) symptoms suggestive of myocardial ischaemia or (ii) new ischaemic ECG changes or (iii) angiographic findings consistent with a procedural complication or (iv) the loss of viable myocardium or a new regional wall motion abnormality demonstrated by cardiac

imaging. Biomarker elevation following PCI without these clinical features is referred to as ‘procedural myocardial injury’ rather than ‘procedural MI’.

## 2.8. Blood tests

Study blood samples were collected at 3 time points: baseline (from the arterial sheath just prior to PCI), 4 hours post PCI and 12 hours post PCI (by standard venesection).

### 2.8.1. VerifyNow test methods

Blood was drawn from the arterial sheath pre-PCI into a 2ml citrated (3.2%) Greiner tube and processed according to the manufacturer’s instructions. In brief, samples were allowed to stand for at least 20 minutes and were analysed within 4 hours. The collection tube was inserted into an individual assay platform and then into the analyser, after which point the test was fully automated. The analyser was frequently calibrated using its own calibration system as per manufacturer’s instructions, usually before each new sample but at least once per day. The test result, available in less than 3 minutes, was immediately recorded on the paper CRF front sheet and later transcribed into the e-CRF.

### 2.8.2. Biomarker measurement methods

Blood samples for biomarker measurement were drawn into 4ml clot activating gel separator serum Greiner tubes and spun at 1500 RPM for 10 minutes. The serum was transferred to a freezer tube and placed in a -21°C freezer situated at the research work station for temporary storage. Samples were transferred in batches every few days to permanent storage at -80°C. In addition, 2 × 4ml citrate Greiner tubes were filled from the arterial sheath pre PCI and were spun and stored as above.

All samples were labelled in the following ways:

- Each freezer tube had an individual patient code written in permanent marker pen, derived from the first 3 letters of the surname, first 3 letters of the first name and the date of birth. For example, the code for John Smith with date of birth 10$^{th}$ May 1950 would be SMIjoh100550.
- The time the sample was taken was indicated by the colour of the tube’s cap: red for time 0, orange for 4 hour and green for 12 hour. A blue cap was used to indicate the (time 0) citrated sample.
- An adhesive label with a unique barcode was applied to each tube, with a copy of the barcode placed on the front sheet of the paper CRF with the type

of sample and time taken clearly indicated. At a later date these barcodes were scanned into the electronic CRF.

The stored serum was couriered in liquid nitrogen to the Randox laboratory, N. Ireland, for biomarker measurement. Results were delivered by e-mail to the Leeds cardiology data manager (R.G.), matched using the unique barcode number allocated when the sample was first taken and imported into the Leeds study database for analysis by A.S.H. and I.R.P.

Biomarker measurement at Randox was undertaken using the patented RANDOX cardiac biomarker array. The array measures 6 cardiac biomarkers: heart-type fatty acid binding protein (HFABP), myoglobin, glycogen phosphorylase BB, TnI, CK-MB mass and carbonic anhydrase III. See Supplementary Appendix (CD) for further product information. Note that carbonic anhydrase III is not a cardiac biomarker, being found exclusively in skeletal muscle, but is used with myoglobin (not a cardiac-specific biomarker) to indicate the proportion of the latter which may be from a cardiac origin.

In addition, a high sensitivity cardiac troponin I level (Advia Centaur TnI Ultra, Siemens) was measured at Randox Laboratories. For this assay the published 99$^{th}$ centile of a healthy pop of 648 individuals (aged 17 to 91 years) testing 1845 fresh samples was 0.04 µg/L; CoV is <10% at this level. The minimum detectable concentration was 0.006 µg/L.

These biomarkers have differing release kinetics following myocardial injury: CK-MB peaks at approximately 18 to 36 hours and is cleared over several days; myoglobin peaks after a few hours and is rapidly cleared; and cTnI peaks at 12 to 24 hours but may not be cleared for 2 to 3 weeks. HFABP rises and falls very rapidly compared to the other biomarkers, within minutes to hours. Hence we have studied myocardial injury using the baseline and 12 hour samples for CK-MB and cTnI; and baseline and 4 hour samples for myoglobin and HFABP.

### 2.8.3. Sample storage for genetic analysis

Blood for genetic analysis was taken from the arterial sheath pre PCI (3 9ml EDTA Greiner tubes). The tubes were labelled with the patient code as above and frozen at -21°C. Twice weekly, batches were processed to remove the red cells and lyse the white cells, producing a stable solution containing DNA. This solution can be stored for several years at room temperature. Each sample was labelled with a unique lab number which was recorded in a lab book with the patient code. The lab number was also entered into the electronic CRF.

### 2.8.4. Other blood parameters

Pre procedure full blood count and renal function were recorded from the patient notes. Lipid levels were documented when these had been checked.

## 2.9. Statistical analysis

The population was described using crude numerical data (with percentages) for categorical variables; and by medians with interquartile range (IQR), or mean with standard deviation (SD) when normally distributed, for continuous variables. Groups were compared using two-sided Student's t-tests and one-way ANOVA for continuous parametric data and the Mann-Whitney U (if two categories) and Kruskal-Wallis (if more than two categories) tests for non-parametric variables. Categorical data were compared using a two-sided Pearson Chi-squared test. A linear by linear association Chi-squared test was used to identify differences between the expected and observed frequencies if more than 2 categories.

Survival time was calculated from the date of PCI to the date of censorship (the first on-treatment adverse event or last clinical contact). Unadjusted survival estimates, stratified by the presence of risk factors, were depicted using Kaplan–Meier curves, and the Mantle log rank test used to compare distributions. Survival was studied using Cox proportional hazards models, proportional assumptions were tested and not violated. Initially, models were fitted using clinically and statistically ($p<0.01$) significant patient and treatment characteristics as univariables, from which a parsimonious models were built for time to the combined endpoint. Regression model parameter estimates were represented by adjusted hazard ratios (aHR) and 95% confidence intervals (CI). All analyses were conducted using IBM SPSS Statistics Version 20.

# Chapter 3 Description of overall patient population

## 3.1. Selection of patients for this analysis

- 1018 patients provided informed consent to be included in the study
- 9 patients subsequently withdrew consent and their data was removed from the electronic database (PAT IDs: 1038, 1039, 925, 928, 930, 513, 994, 995, 1042)
- 6 patients provided prior written consent but did not undergo PCI or attempted PCI (5 had normal coronaries, 1 underwent IVUS examination only); their data was retained but the patients were excluded from all analyses (PAT IDs: 174, 427, 481, 706, 998, 1032)
- 35 patients either had no record of ACS prior to PCI and were re-categorised as elective stable patients, or the ACS was very remote (the initial management strategy was medical and the patient discharged home without a plan for PCI: patients were effectively elective/stable at the eventual time of PCI); data for these 35 were retained in the database but not included in this analysis (PAT IDs: 67, 83, 246, 397, 446, 550, 583, 683, 684, 685, 686, 693, 699, 702, 703, 710, 717, 733, 734, 735, 736, 737, 741, 746, 749, 750, 760, 765, 767, 769, 771, 772, 773, 778). Patients for whom the initial management strategy was revascularisation but for whom this was unusually delayed for any reason (for example co-morbid conditions requiring medical work-up, patient indecision, outstanding investigations or surgical assessments) were not excluded, hence prolonged time from ACS to PCI for some patients.
- Therefore, data for **968 patients** with valid written informed consent, presenting with ACS and undergoing PCI, are included in these analyses.

## 3.2. Recruitment timescale

The first patient had PCI on 1st June 2006 and the final patient 6th July 2011. Figure 3-1 illustrates recruitment rates over the 5 year period. There were 2 distinct recruitment time phases; no patient recruitment took place between 29th Oct 2008 and 1st June 2010.

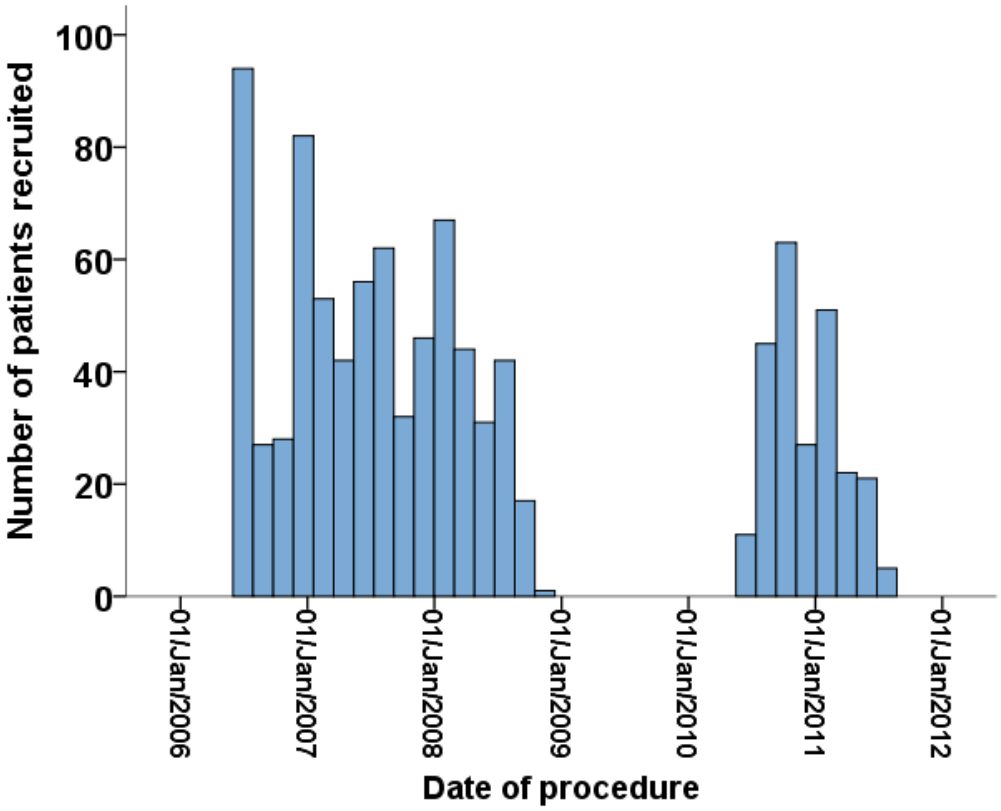



**Figure 3-1 Histogram showing the changes in patient recruitment rate over time**

## 3.1. Time from ACS to PCI

The median (IQR) time from ACS to PCI was 6 (6) days for 873 patients presenting with ACS, excluding PPCI patients. 941 (97%) patients had PCI within 30 days of first hospital admission. 5 patients had severely delayed PCI (PAT IDs 755, 181, 889, 697, 715 and 761 had PCI 119, 129, 138, 140, 150 and 176 days following ACS). Patients presenting directly to the PCI centre (Leeds) received intervention sooner than those presenting to a district general hospital (DGH), shown in Table 3-1.

**Table 3-1 Time from admission to PCI for patients presenting to different hospitals**

| Admitting Hospital | Number of days from ACS to PCI (Excluding 96 patients presenting with PPCI and 4 patients with missing data) | |
|---|---|---|
| | **Median days** | **Number of patients** |
| Leeds (LGI or St James') | 4 | 248 |
| Airedale | 7 | 51 |
| Bradford | 8 | 12* |
| Dewsbury | 5 | 57 |
| Harrogate | 6 | 23 |
| Huddersfield | 7 | 90 |
| Pinderfields (Wakefield) | 10 | 107 |
| Pontefract | 11 | 64 |
| Calderdale (Halifax) | 6 | 80 |
| York | 7 | 136 |
| All | 6 | 868 |

*Bradford Royal Infirmary had PCI capability during the study recruitment period, hence the small numbers of referrals relative to the other hospitals.

## 3.2. Data completeness and patient follow-up

### 3.2.1. Missing clinical and procedural characteristics

For the 968 patients in these analyses, data was >98% complete for all fields except:

- A **VerifyNow $P2Y_{12}$ receptor Platelet Function Test** result was missing in 103 (10.6%) cases. Of these, 63 patients had received pre-treatment with a IIbIIIa inhibitor making testing impossible; in the most of the remainder the test was deliberately not performed because of inadequate $P2Y_{12}$ inhibitor loading time in PPCI patients. According to the study protocol, testing was to be carried out using blood drawn at the time of PCI; we therefore did not attempt to bring patients with missing results back for a test at a later date
- In 491 (50.7%) patients we could find no record of an assessment of **Left Ventricular Systolic Function** in the Leeds or the DGH medical case files or electronic records, either pre-procedurally or during follow-up. Although this is an important prognostic variable, the requesting of tests to assess LV function was not part of the study protocol and an LV function measurement was often omitted from routine medical care
- Pre-procedural **Renal Function** was not documented for 21 (2.2%) patients; and post-procedural renal function was unrecorded for the majority of patients (777, 80%)
- A pre-procedural **Full Blood Count** was unrecorded for 78 (8.1%) patients
- Data on pre-procedural **ACE-inhibitor** use and **B-blocker** use were unrecorded for 55 (5.7%) and 52 (5.4%) patients respectively
- A **SYNTAX score** was missing for 20 (2.1%) patients (angiograms irretrievable)
- A post-procedural **ECG** was not stored for 70 (7.2%) patients

### 3.2.2. Length and completeness of patient follow-up

The median (IQR) time from PCI to final clinical follow-up or death was 3.56 (1.31) years, giving a total of 3,226 patient-years of clinical follow-up.

The median (IQR) time from PCI to death (censored 13th June 2013) was 5.35 (3.42) years, giving a total of 4590 patient-years of mortality tracking.

All surviving patients had at least 1 episode of clinical follow-up by telephone or outpatient clinic. Of the 940 patients alive at 1 year, 14 (1.5%) patients had less than 12 months clinical follow-up. These patients are listed in Table 3-2.

**Table 3-2 Patients with less than 12 months clinical follow-up**

| Patient ID | Time from PCI to last clinical follow-up | Reason |
|---|---|---|
| 75 | 31 days | Patient emigrated, lost to F/U |
| 907 | 36 days | No record of 1 year F/U on database |
| 840 | 99 days | Unable to trace patient |
| 432 | 184 days | No record of 1 year F/U on database |
| 987 | 223 days | No record of 1 year F/U on database |
| 1045 | 248 days | No record of 1 year F/U on database |
| 1020 | 249 days | No record of 1 year F/U on database |
| 831 | 253 days | Had CABG at 6 months, censored |
| 494 | 277 days | Unable to trace patient |
| 1000 | 300 days | No record of 1 year F/U on database |
| 990 | 315 days | No record of 1 year F/U on database |
| 909 | 341 days | No record of 1 year F/U on database |
| 984 | 343 days | No record of 1 year F/U on database |
| 988 | 343 days | No record of 1 year F/U on database |

## 3.3. Baseline characteristics

In this section are presented the baseline clinical characteristics on admission of the OPERA study patients. I have included some of the important procedural characteristics, including pharmacotherapy and stent type. This study recruited patients over a long time period (2006 to 2011) and in 2 distinct phases. While the incidence of many characteristics remains stable over time, several important characteristics became more or less frequent; these are discussed in the following sections. In this section I have also highlighted the inter-relationship between many of the important baseline characteristics.

### 3.3.1. Age

The mean (SD) age on the date of PCI of the total cohort was 62.2 (12.0) years. The age distribution is shown in Figure 3-2. Only 25 (2.6%) patients were under 40 years. The youngest patient was 27, a previously well South Asian male smoker who presented with a NSTEMI. He was treated with 3 *'Cypher'* sirolimus-eluting stent to his LAD.

There were 70 (7.2%) patients aged 80 years or more; the oldest patient was 90. He presented with a NSTEMI on a background of previous CABG, previous PCI and diabetes. He had PCI to his native circumflex artery with a *'Xience'* everolimus-eluting stent.

The age of the overall cohort did not change significantly by year of presentation, see Figure 3-3. However, when patients with UA or NSTEMI were considered (STEMI excluded), the mean age increased slightly between the 2 recruitment phases: 62.7 (12.0) years vs. 65.0 (12.9) years in the first and second phases, p = 0.04 (Figure 3-4). STEMI patients tended to be younger and formed a greater proportion of the patients recruited in the second phase, discussed further in Section 3.5.6.

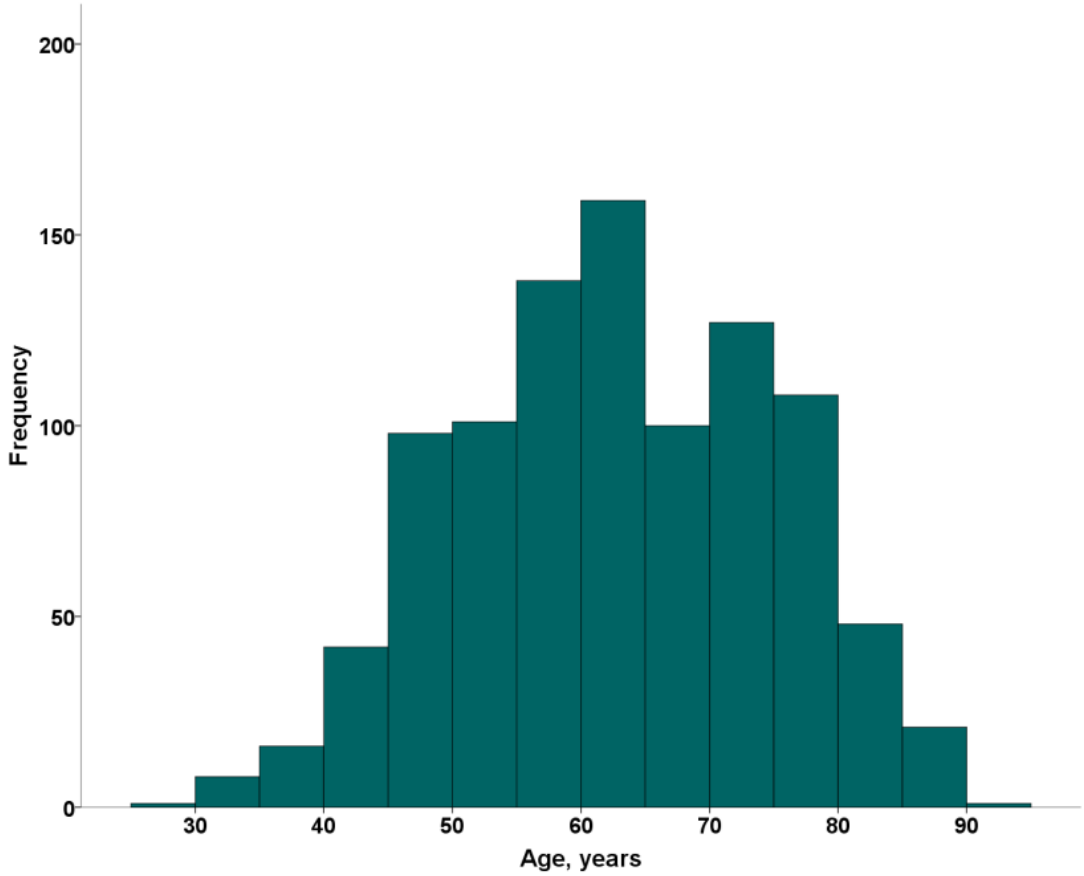



**Figure 3-2 Histogram showing age distribution in the OPERA cohort**

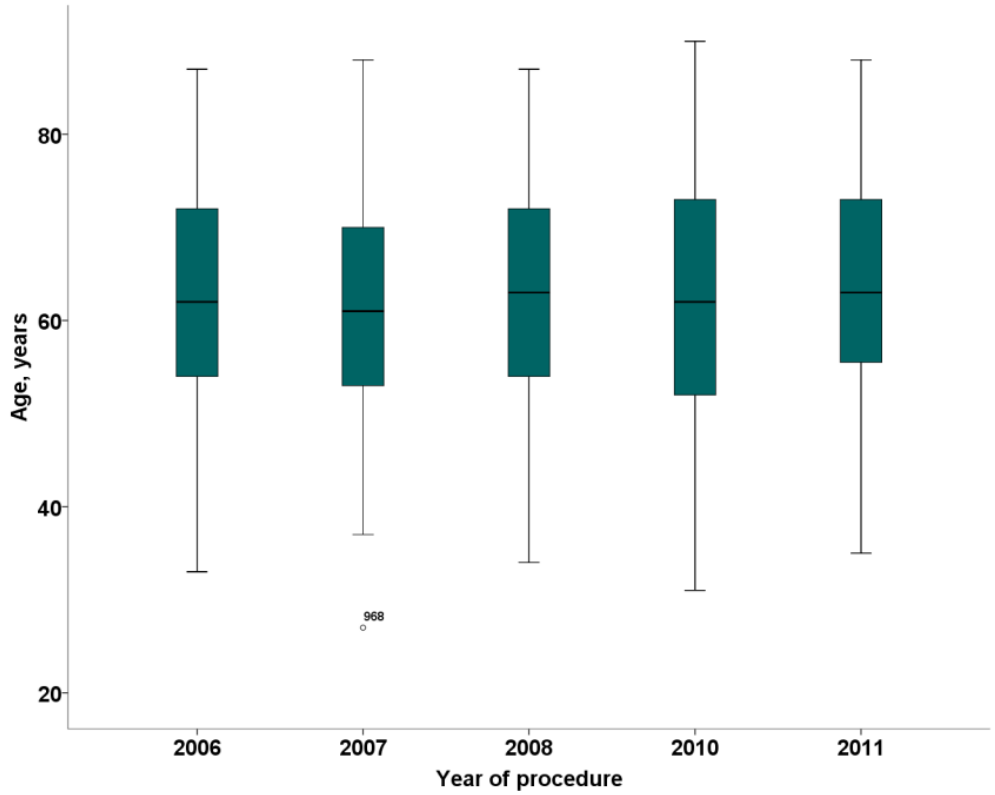



**Figure 3-3 Comparison of patient age by year of procedure**

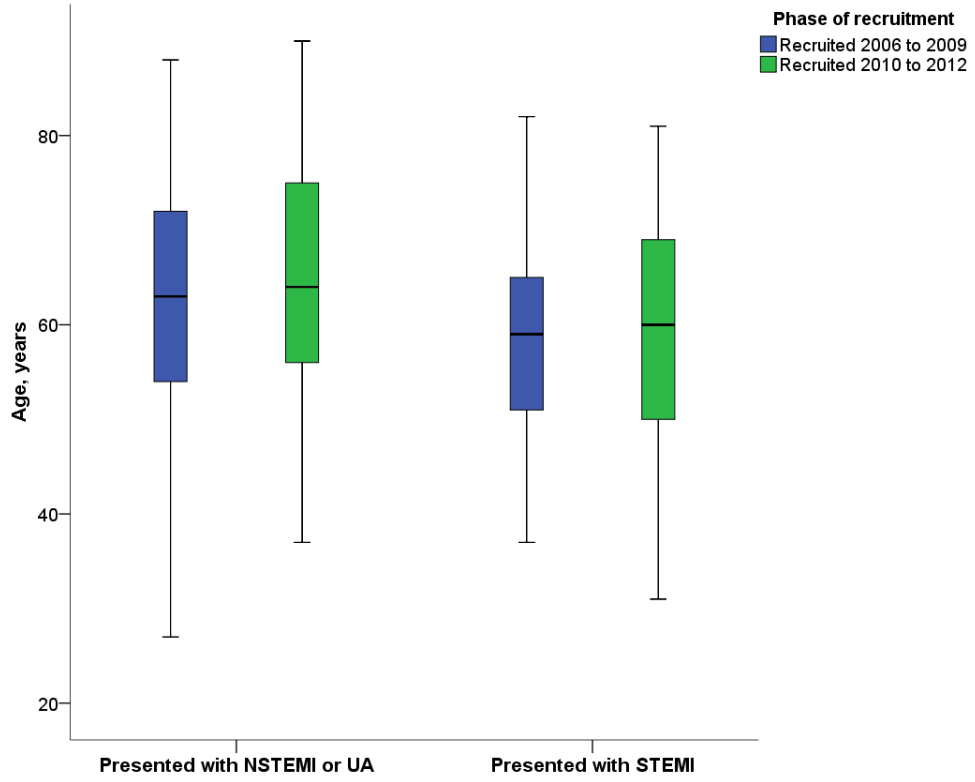



**Figure 3-4 Comparison of age of patients in first and second recruitment phases, stratified according to clinical syndrome**

### 3.3.2. Gender and other baseline characteristics

Women comprised 25% of all patients recruited into this study, a proportion which remained stable over the time course of the recruitment period. There were 2 main differences in baseline characteristics between men and women. Firstly, there was a difference in clinical syndrome at presentation between men and women, with women being more likely to present with UA and men with STEMI, see Table 3-3. Secondly, women were older than men by nearly 6 years on average in this study: 66.3 (12.1) years vs. 60.8 (11.7) years, $p < 0.01$.

There were no significant differences in the rates of other baseline characteristics or co-morbidities between the 2 sexes.

### 3.3.3. Body Mass Index; incidence of overweight and underweight patients

The median (IQR) BMI was 28 (6) for the entire cohort. The lowest BMI was 15 and the highest was 76 (this patient was only 105 cm tall but weighed 86 kg). According to the classification proposed on the NHS Patient Choices website, 80.4% of patients in this study were either 'overweight, obese or very obese' with a BMI $\geq 25$. Of the total cohort, 37.1% of patients had a BMI $\geq 30$, and in 3.8% the BMI was $\geq 40$. Only 7 (0.7%) patients had a BMI < 20 (underweight).

Patients with diabetes had higher BMIs than those without, 30 (7) vs. 28 (6), $p < 0.01$. Also, BMI increased slightly between the first and second recruitment phases: 27.9 (6) vs. 28.9 (5), $p = 0.01$. There was no significant difference in BMI between men and women, or between patients presenting with different clinical syndromes.

### 3.3.4. Diabetes and other baseline characteristics

The proportion of patients known to have had diabetes mellitus (DM) of any type was 16.5% for the overall cohort. The incidence of Type I DM was 2.6% and of Type II DM was 13.8%, with missing data in 2 patients. Patients with diabetes tended to be older: the mean age in those with and without DM was 65.5 (12.1) vs. 61.6 (11.9) years, $p < 0.01$.

The proportion with DM (any type) was slightly higher (17.9%) when PPCI patients were excluded from the analysis. Some of the PPCI patients may have had undiagnosed diabetes, whereas undiagnosed DM would be very unlikely in UA and NSTEMI patients who have spent several days in hospital. We did not record the incidence of new diagnoses of DM following emergent PCI for ACS in this study.

Patients with DM had an increased incidence of co-morbidities compared to those without. These included a previous history of angina (67.9% in those with DM vs. 40.0% in those without DM, $p < 0.01$); previous MI (37.7% vs. 20.7%, $p < 0.01$); previous PCI (18.9% vs. 10.2%, $p < 0.01$); a history of CABG (14.5% vs. 6.1%, $p < 0.01$); a previous diagnosis of heart failure (5.0% vs. 0.9%; $p < 0.01$); a history of hypertension (67.9% vs. 47.2%, $p < 0.01$); a history of hyperlipidaemia (65.4% vs. 54.7%, $p = 0.01$); a history of obstructive airways disease (18.9% vs. 9.0%, $p < 0.01$); renal failure (Cr >200 µmol/L) (6.3% vs. 1.6%, $p < 0.01$); and a past history of stroke (8.2% vs. 4.5%, $p = 0.05$). Patients with DM were also more likely to be anaemic (Haemoglobin < 10g/dL) (6.2% vs. 1.1%, $p < 0.01$).

The proportion of patients with DM increased over the time course of the study from 14.7% during the first recruitment phase to 21.6% in the second, $p = 0.01$. See Figure 3-5 for numbers of patients with and without DM per year of recruitment.

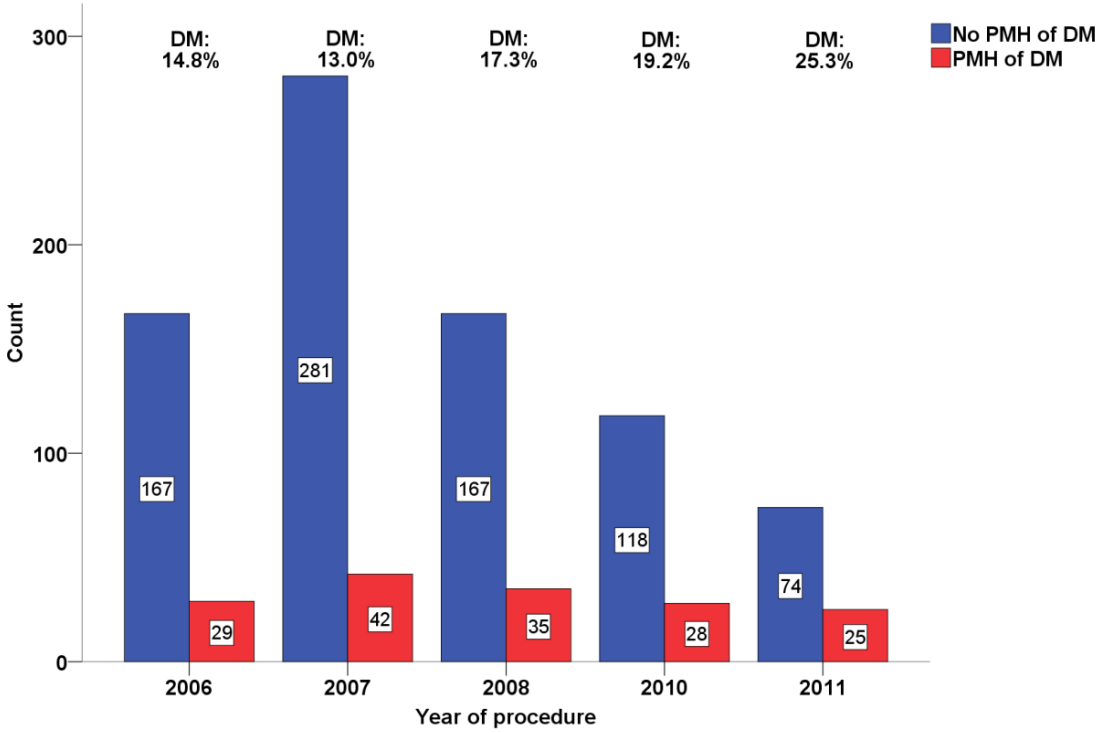



**Figure 3-5 Bar chart showing the numbers of patients with and without diabetes by year of procedure**

### 3.3.5. Previous ischaemic heart disease and other baseline characteristics

Of the total cohort 44.6% of patients reported a previous history of angina and 23.5% a previous history of MI. 17.1% had undergone revascularisation previously, 11.6% by PCI and 7.4% by CABG. Overall, nearly half of the total cohort (462 patients, 47.7%) had a previous history of some type of clinical/symptomatic IHD.

The clinical syndrome at presentation was different in patients with and without previous symptomatic IHD. Most of the patients presenting with UA had a prior history of IHD (81.5%), compared to 47.8% of the NSTEMI patients but only 28.1% of the STEMI patients, $p < 0.01$.

Previous IHD (any type) was associated with a higher incidence of DM (24.3% vs. 9.3%, $p < 0.01$); obstructive airways disease (13.0% vs. 8.5%, $p = 0.02$); a history of stroke (7.1% vs. 3.2%, $p < 0.01$); and a history of renal failure (serum creatinine > 200 µmol/L) (4.3% vs. 0.6%, $p < 0.01$). Patients with prior IHD were older than those without: 65.0 (12.3) years vs. 59.7 (11.3) years, $p < 0.01$. There was no difference in the incidence of previous IHD between men and women.

The proportion of patients presenting with a past history of clinical IHD decreased over time: 50.2% in the first phase of recruitment compared to 40.4% in the second phase, $p < 0.01$. Likewise the proportion of patients with a previous history of angina decreased over the course of the study from 48.7% during the first phase to 32.7% in the second phase, $p < 0.01$. Conversely, there was a small increase from the first to the second recruitment phase in the number of patients having had a previous MI (21.9% vs. 28.2%, $p = 0.04$). The proportion of patients with previous revascularisation was not significantly different over time.

Most patients in this study (93.4%) underwent PCI to ‘de novo’ lesions – i.e. lesions in native coronary arteries which have undergone no previous revascularisation attempts. However, several patients had PCI following an ACS which resulted from failure of a previous revascularisation. There were 31 cases of saphenous vein graft (SVG) PCI in patients with degenerated bypass grafts, 33 cases of PCI to treat in-stent restenosis after an earlier PCI, and 3 cases of SVG PCI which were re-interventions due to restenosis of a bypass graft following a previous PCI.

### 3.3.6. Clinical syndrome and other baseline characteristics

Of the total cohort, 124 (12.8%) patients presented with unstable angina (UA), 627 (64.8%) with NSTEMI, and 217 (22.4%) with STEMI. Of the STEMI patients, 121

(12.5% of the total cohort) received pharmacological thrombolysis followed by convalescent PCI; and 96 (9.9% of the total cohort) received PPCI.

Patients unable to provide informed consent such as those in cardiogenic shock were not recruited into the OPERA study as retrospective consent was not part of the protocol. However, a few patients did receive some form of cardiopulmonary resuscitation prior to PCI: 8 (1.4%) patients with NSTEMI; and 11 (5.2%) patients with STEMI.

Patients presenting with UA, NSTEMI and STEMI had different baseline characteristics, see Table 3-4. Patients with STEMI were younger than patients with NSTEMI or UA, were more likely to be current smokers and were much less likely to have a previous history of IHD. STEMI patients were also less likely to have a prior diagnosis of several co-morbidities including hypertension and diabetes; and they were less likely to have poor renal function. Patients presenting with MI were more likely to have a history of smoking and were more likely to be male compared to UA patients.

Despite these differences in baseline characteristics there was no significant difference in either EuroSCORE or in SYNTAX score between patients presenting with different clinical syndromes. Median (IQR) EuroSCORE and median SYNTAX score were both relatively low in the OPERA study: for the overall cohort these were 3.1 (4.5) and 9.0 (10.0) respectively.

Note: ethnicity is omitted from Table 3-4. Of the whole cohort, 920 (95.0%) of the patients were White, 43 (4.4%) South Asian (India, Pakistan or Bangladesh), 3 Afro-Caribbean, 2 South East Asian (China), and 1 patient described himself as Mixed Race.

### 3.3.7. Changes in clinical syndrome over time

Over the course of the recruitment period of this study a regional primary PCI service was established resulting in changes in the proportions of patients admitted with the different clinical syndromes. PCI for STEMI became more frequent and PCI for UA less so. In the first and second recruitment phases the proportions with UA, NSTEMI and STEMI were 15.5%, 67.8% and 16.7% and 4.9%, 55.9% and 39.2%, $p < 0.01$. PPCI gradually replaced thrombolysis as the treatment for STEMI across the region during the study recruitment period: only 1 of the 44 STEMI patients recruited in 2006 received PPCI, compared to 32 of the 35 STEMI patients recruited in 2011; see Figure 3-6

**Table 3-3 Comparison of baseline characteristics in patients presenting with unstable angina, NSTEMI and STEMI**

| **Characteristic**<br>*Dichotomous characteristics expressed as Number (% within clinical syndrome)* | **UA**<br>N = 124 | **NSTEMI**<br>N = 627 | **STEMI**<br>N = 217 | **P value** |
|---|---|---|---|---|
| *Demographics* | | | | |
| Mean (SD) age, years | 61.8 (10.9) | 63.4 (12.4) | 59.0 (10.9) | <0.01 |
| Female | 38 (30.6) | 160 (25.5) | 44 (20.3) | 0.09 |
| Median (IQR) Body Mass Index | 28.1 (7) | 27.9 (6) | 28.1 (5) | 0.81 |
| Recruited in first phase (2006 to 2009) | 112 (90.3) | 490 (78.1) | 121 (55.8) | < 0.01 |
| Recruited in second phase (2010 to 2012) | 12 (9.7) | 137 (21.9) | 96 (44.2) | |
| Any history of chronic smoking | 74 (59.7) | 431 (69.2) | 161 (74.5) | 0.02 |
| Ex-smoker | 52 (42.4) | 243 (39.0) | 63 (29.2) | <0.01 |
| Current smoker (within 4 weeks of ACS) | 22 (17.6) | 188 (30.2) | 98 (45.4) | <0.01 |
| Family history of IHD, 1st degree relatives | 69 (57.5) | 313 (52.3) | 107 (51.4) | 0.53 |
| *Medical history* | | | | |
| Angina | 98 (78.4) | 288 (45.9) | 46 (21.2) | <0.01 |
| Previous MI | 35 (28.0) | 157 (25.0) | 35 (16.1) | 0.01 |
| Previous PCI | 29 (23.3) | 67 (10.7) | 16 (7.4) | <0.01 |
| Previous CABG | 17 (13.6) | 49 (7.8) | 6 (2.3) | <0.01 |
| Diabetes, any type | 22 (17.6) | 112 (17.9) | 25 (11.5) | 0.09 |
| Hypertension | 73 (58.4) | 334 (53.3) | 82 (37.8) | <0.01 |
| Lung disease, any type | 14 (11.2) | 72 (11.5) | 17 (7.8) | 0.32 |
| Previous stroke, any type | 4 (3.2) | 37 (5.9) | 8 (3.7) | 0.26 |
| Peripheral vascular disease | 2 (1.6) | 35 (5.6) | 6 (2.8) | 0.06 |
| Clinical heart failure, any class | 0 | 14 (2.2) | 1 (0.5) | 0.06 |
| Untreated moderate or severe aortic stenosis | 2 (1.6) | 10 (1.6) | 0 | 0.17 |
| *Pre PCI blood results* | | | | |
| Median (IQR) serum creatinine, µmol/L | 92 (30) | 95 (24) | 94 (19) | 0.51 |

| | | | | |
|---|---|---|---|---|
| Serum Cr ≥ 200 µmol/L | 1 (0.8) | 19 (3.0) | 3 (1.4) | 0.18 |
| eGFR <60ml/min/1.73m$^2$ | 38 (30.6) | 211 (34.6) | 44 (20.8) | <0.01 |
| Median Haemoglobin, g/dL | 14.1 (2.0) | 14.0 (2.1) | 14.1 (2.2) | 0.58 |
| Haemoglobin <10 g/dL | 1 (0.9) | 13 (2.3) | 3 (1.5) | 0.55 |
| Median platelet count x10$^9$/L | 244 (71) | 248 (87) | 253 (106) | 0.07 |
| Median (IQR) *VerifyNow Base* value* | 318 (94) | 309 (80) | 296 (103) | 0.06 |
| Median (IQR) *VerifyNow PRU* value* | 259 (156) | 241 (116) | 239 (148) | 0.69 |
| Median (IQR) VerifyNow %inhibition value* | 21 (41) | 20 (39) | 18 (42) | 0.93 |
| *PPCI patients excluded from VerifyNow analysis | | | | |
| *Medication at PCI* | | | | |
| Aspirin | 120 (96.0) | 609 (97.3) | 209 (97.2) | 0.73 |
| Clopidogrel | 124 (100.0) | 626 (99.8) | 165 (76.0) | <0.01 |
| Prasugrel | 0 | 3 (0.5) | 52 (24.0) | <0.01 |
| Dual antiplatelet therapy | 119 (96%) | 609 (97.3) | 209 (97.2) | 0.73 |
| Loaded with $P2Y_{12}$ inhibitor <24hrs before PCI | 9 (7.2) | 16 (2.6) | 99 (45.6) | <0.01 |
| Loaded with $P2Y_{12}$ inhibitor >3 days before PCI | 105 (84.8) | 512 (81.7) | 98 (45.2) | <0.01 |
| Heparin during PCI | 121 (98.4) | 576 (91.9) | 137 (63.4) | <0.01 |
| Bivalirudin during PCI | 3 (2.4) | 54 (8.6) | 89 (41.6) | <0.01 |
| IIbIIIa inhibitor use before/during PCI | 27 (21.6) | 207 (33.0) | 78 (35.9) | 0.02 |
| ACE inhibitor | 56 (47.9) | 447 (75.1) | 114 (56.4) | <0.01 |
| B blocker | 93 (78.2) | 483 (81.2) | 116 (57.1) | <0.01 |
| HMG-CoA reductase inhibitor | 110 (88.8) | 559 (89.2) | 134 (61.8) | <0.01 |
| Proton pump inhibitor | 44 (35.2) | 210 (33.5) | 40 (18.4) | <0.01 |
| Warfarin pre-ACS (stopped before PCI) | 2 (1.6) | 13 (2.1) | 4 (1.8) | 0.93 |
| *Risk scores* | | | | |
| Median (IQR) EuroSCORE 1 | 3.1 (3.5) | 3.1 (4.9) | 3.0 (3.2) | 0.77 |
| Median (IQR) SYNTAX score | 9.0 (11.0) | 9.0 (11.0) | 8.0 (11.0) | 0.40 |

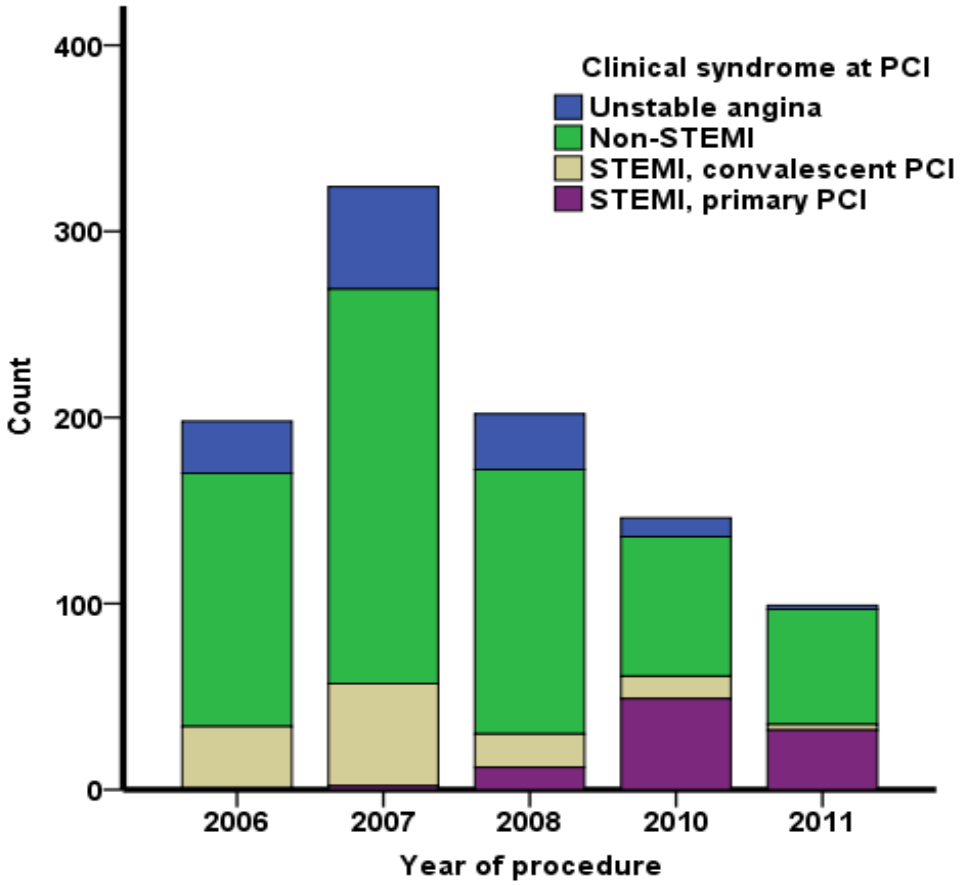



**Figure 3-6 Bar chart showing the change in proportions of patients with different clinical syndromes over time**

### 3.3.8. Pharmacology and other baseline characteristics

Dual oral antiplatelet therapy (DAPT) was administered prior to PCI to 97.3% of patients. 28 (2.3%) patients received single antiplatelet therapy (29 patients clopidogrel only, 1 prasugrel only) due to aspirin intolerance. More than 80% of the UA, NSTEMI and convalescent STEMI patients received loading with a $P2Y_{12}$ inhibitor 3 or more days prior to PCI, and only 28 (3.2%) received a $P2Y_{12}$ inhibitor less than 24 hours prior to PCI. By far the most commonly used $P2Y_{12}$ inhibitor was clopidogrel. Prasugrel was introduced in July 2010 and this drug was mainly used in PPCI (48 of the 55 patients receiving prasugrel underwent PPCI).

The most commonly used IIbIIIa inhibitor was abciximab in 261 (26.9% of the total cohort) followed by tirofiban (40, 4.1%), with eptifibitide used only in 2 patients. IIbIIIa inhibitors were administered more frequently in the presence of 'high-risk' angiographic characteristics such as SVG PCI (48.6% vs. 31.6%, $p = 0.03$), bifurcation lesions (36.3% vs. 29.7%, $p = 0.03$), Type C lesions (43.9% vs. 24.7%, $p < 0.01$) and thrombotic lesions (48.8% vs. 26.7%, $p < 0.01$). Patients receiving a IIbIIIa inhibitor

were more likely to have an angiographic procedural complication reported (48.4% vs. 29.9%, $p < 0.01$) and more commonly had presented with an MI than with UA (33.8% vs. 21.8%, $p < 0.01$). The use of IIbIIIa inhibitors declined steadily over the course of the recruitment period, from 47.0% in 2006 to 23.2% in 2011.

The majority of patients were anticoagulated during their procedure with unfractionated heparin (86.3%), but bivalirudin was also used throughout the course of the study and was received by 146 (15.1%) patients, half of whom had PPCI (78 of the 94 PPCI cases featured Bivalirudin use).

At the time of PCI, Beta-blockers were received by 75.5%, ACE-inhibitors by 67.6% and 'Statins' by 83.1% of patients overall. The utilization of these medications at PCI appears to have decreased over time: the proportions receiving the same drugs in 2006 and 2011 were 80% vs. 62.8 %, 70.5% vs. 54.3% and 94.4% vs. 68.7%. However, when PPCI patients (who are taken immediately to the catheter laboratory on arrival at hospital) are excluded from the analysis this decrease is no longer seen (80.4% vs. 87.3%, 70.8% vs. 73.0% and 94.4% vs. 89.6% in 2006 and 2011 for Beta-blockers, ACE-inhibitors and Statins).

### 3.3.9. Arterial access route

The most frequent arterial access route in the study, femoral, was used in 762 (78.8%) patients, while 205 (21.2%) had radial access procedures (data missing for 1 person). Over the course of the study recruitment period radial access gradually became more frequent than femoral, performed in only 3.5% of procedures in 2006 but in 71.7% in 2011.

Radial access was used more frequently in patients undergoing PPCI (66.3%) compared to the other clinical syndromes.

### 3.3.10. Stent selection and other baseline characteristics

Both 'first generation' and 'second generation' stents were used in this study. The 'first generation' DES used in this study were the '*Taxus*' paclitaxel-eluting and '*Cypher*' sirolimus-eluting devices. The 'second generation' stents were the *'Endeavor'* zotarolimus-eluting and *'Xience'* everolimus-eluting devices. Small numbers of other DES types were used e.g. 8 patients received a *'Biomatrix'* absorbable-polymer DES, and 2 patients received a *'Resolute Integrity'* DES.

At least 1 DES was deployed in 571 (59.0%) patients and at least 1 BMS in 423 (43.7%), with 44 (4.5%) patients receiving both types. POBA ('Plain Old Balloon Angioplasty') only was used in 12 (1.2%).

There was a tendency for more females than males to be treated with DES (62.0% vs. 55.4%, p = 0.08); and patients receiving DES were younger than those receiving BMS (mean age 61.2 years vs. 63.4 years, p < 0.01). Patients receiving BMS had shorter total stent length than those receiving DES (median 18mm vs. 27mm, p < 0.01), although the diameter of the smallest stent used was the same for DES and BMS (median 3.0mm).

The types of stent implanted changed over the course of the study, shown in Figure 3-7. BMS were used in half of the patients during the first phase of recruitment, but only in 11.4% recruited in the second phase. Even during the first recruitment phase, second generation DES use had superseded first generation DES use (24.7% vs. 18.1%). In the second recruitment phase, only 7% received a first generation DES. BMS was the most frequently implanted stent type in the first recruitment phase (49.5%), followed by *'Cypher'* DES (16.2%). *'Xience'* DES was the most frequently implanted stent type in the second phase (76.7%) followed by BMS (11.4%).

### 3.3.11. Angiography screening (fluoroscopy) time

The total fluoroscopy (length of X-ray screening) time was recorded for every procedure in this study. After arranging all fluoroscopy times in ascending order as a graph (Figure 3-8), there appears to be a relatively normal range for this parameter, up to about 10 minutes. After this time there is an inflection point in the graph, which may prove useful as a cut-off to define more complex cases.

### 3.3.12. Intravascular imaging

Intravascular ultrasound (IVUS) was not a recorded data field; its use during this study was very limited. Optical coherence tomography (OCT) was not yet available.

### 3.3.13. Rotational atherectomy

Rotational atherectomy was used in only 4 patients, 2.2% of the patients with heavily calcified lesions.

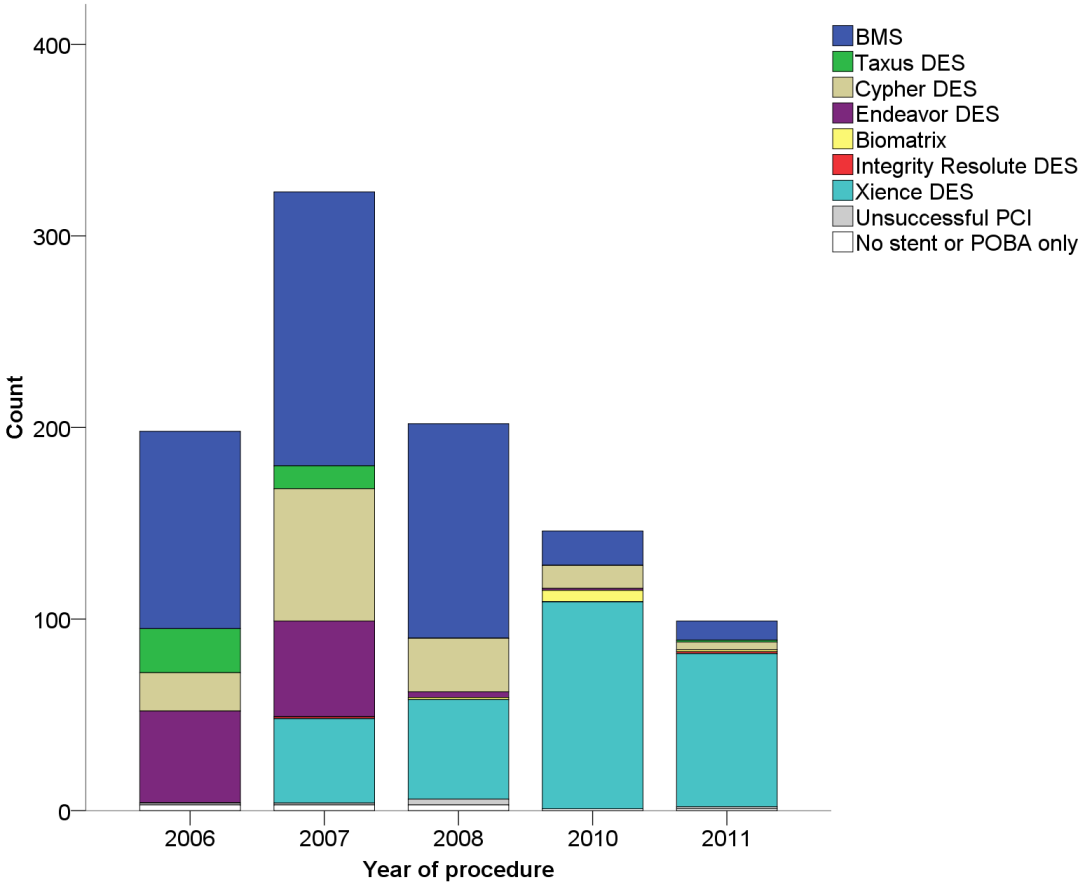



**Figure 3-7 Bar chart showing changes in stent selection over time**

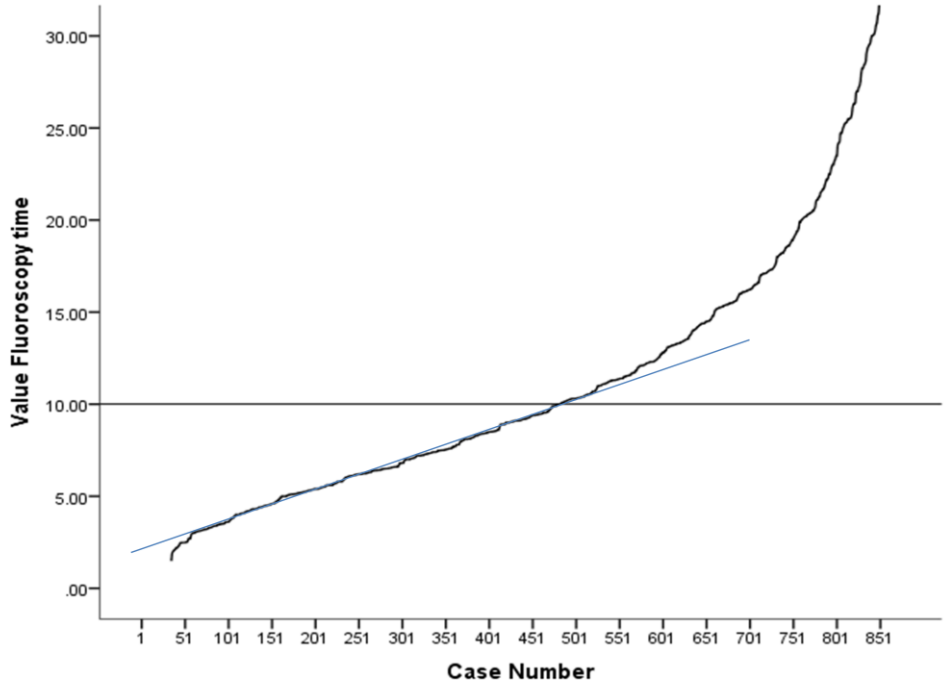



**Figure 3-8 Fluoroscopy times for all cases arranged in ascending order; straight line indicates approximate normal range**

### 3.3.14.SYNTAX score and other baseline characteristics

The median (IQR) SYNTAX score for the whole cohort was 9.0 (10.0); Figure 3-8 shows the distribution of scores. The SYNTAX score was different in patients with different baseline characteristics, shown in Table 3-4. Elderly patients had higher scores, as did those with a previous history of IHD (but not PCI), DM, hypertension, stroke and heart failure. Patients with renal dysfunction and anaemia had higher SYNTAX scores. Patients in the lowest EuroSCORE quartile had lower SYNTAX scores, and those in the highest quartile had higher SYNTAX scores, but the overall correlation between SYNTAX and EuroSCORE was only modest; correlation coefficient 0.359 (Spearman's rho); $p < 0.01$.

SYNTAX score also increased between the first and second recruitment phases.

A history of current or past smoking was, conversely, associated with lower SYNTAX scores. There was no apparent difference in SYNTAX score between men and women, or between patients presenting with different clinical syndromes.

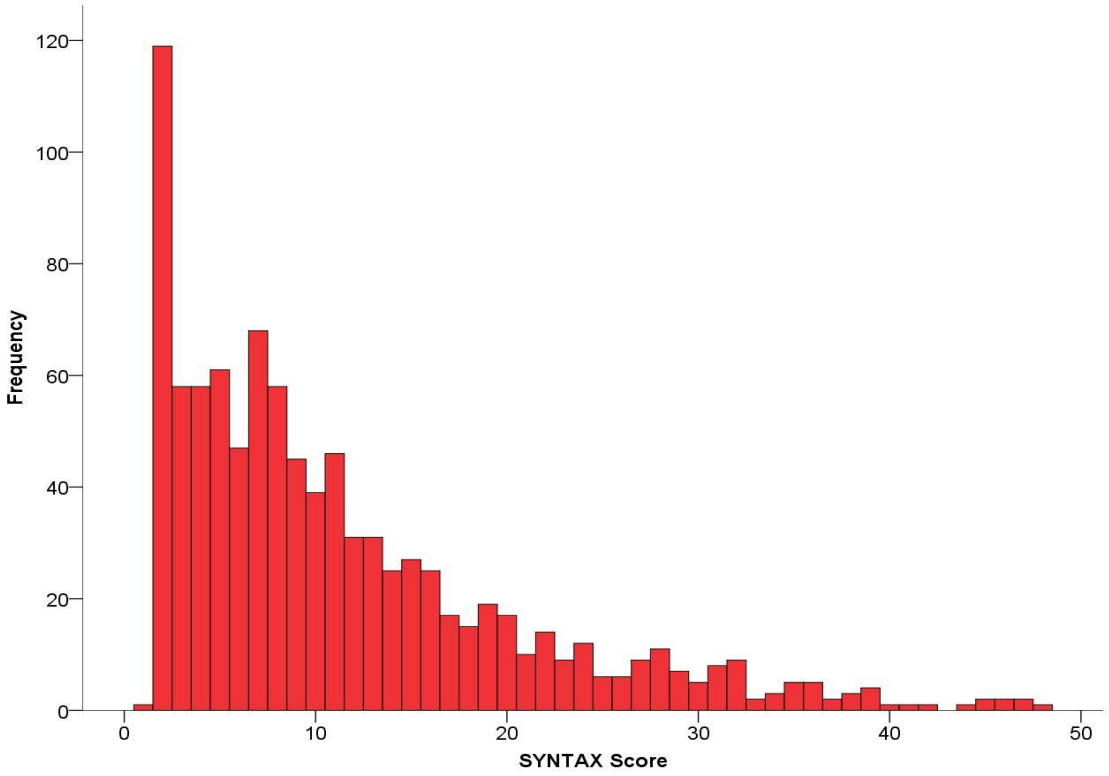



**Figure 3-9 Histogram showing distribution of SYNTAX score for whole cohort**

**Table 3-4 Comparison of median SYNTAX scores in patients with and without selected baseline characteristics**

| Baseline characteristic | Number of patients with characteristic (proportion of total cohort) | Median (IQR) SYNTAX score when characteristic present | Median (IQR) SYNTAX score when characteristic absent | P value |
|---|---|---|---|---|
| Female | 236 (24.9) | 8.5 (10.0) | 9.0 (11.0) | 0.73 |
| Age ≥ 80 years | 69 (7.3%) | 19.0 (18.0) | 8.0 (10.5) | < 0.01 |
| First recruitment phase | 718 (75.7) | 8.0 (10.0) | 11.0 (13.6) | < 0.01 |
| History of smoking | 651 (69.0%) | 8.0 (10.0) | 10.0 (11.0) | < 0.01 |
| Presented with UA | 124 (13.1%) | 9.0 (11.0) | 8.0 (11.0) | 0.17 |
| Presented with NSTEMI | 624 (65.8%) | 9.0 (11.0) | 9.0 (11.0) | 0.66 |
| Presented with STEMI | 200 (21.1%) | 8.0 (11.0) | 9.0 (10.0) | 0.54 |
| Previous history of MI | 222 (23.4%) | 12.0 (16.6) | 8.0 (9.1) | < 0.01 |
| Previous history of PCI | 110 (11.6%) | 10.0 (12.0) | 8.0 (11.0) | 0.26 |
| Previous history any clinical IHD | 456 (48.1%) | 11.0 (12.0) | 7.0 (8.0) | < 0.01 |
| DM | 157 (16.6%) | 12.0 (14.3) | 8.0 (10.0) | < 0.01 |
| Hypertension | 477 (50.3%) | 10.0 (11.0) | 8.0 (10.0) | < 0.01 |
| Obstructive airways disease | 101 (10.7%) | 9.0 (12.0) | 8.0 (11.0) | 0.40 |
| Previous history of stroke | 45 (4.7%) | 12.0 (21.6) | 8.0 (11.0) | 0.08 |
| Previous history of heart failure | 15 (1.6%) | 23.5 (12.0) | 8.0 (11.0) | < 0.01 |
| Renal dysfunction (eGFR < 60 ml/min/1.73m$^2$) | 287 (31.0%) | 12.0 (14.0) | 8.0 (9.0) | < 0.01 |
| Anaemia (Hb < 10 g/dL) | 16 (1.8%) | 20.5 (14.4) | 8.0 (11.0) | < 0.01 |
| Lowest EuroSCORE quartile (1.5 to 1.9) | 247 (26.1%) | 7.0 (7.5) | 10.0 (12.0) | < 0.01 |
| Highest EuroSCORE quartile (> 6.4) | 230 (24.3%) | 14.3 (17.1) | 7.0 (9.0) | < 0.01 |

### 3.3.15. Changes in patient risk profile over time

The risk profile of the patients, as measured by EuroSCORE, increased between the first and second phases of recruitment. For the whole cohort, median EuroSCORE (IQR) was 2.8 (4.1) in the first phase and 4.4 (7.7) in the second. This change appeared to be due to an increase in EuroSCORE in patients undergoing non-emergent procedures: excluding patients undergoing PPCI (i.e. emergent cases), median EuroSCORE was 2.7 (4.0) in the first phase and 3.4 (8.4) in the second, $p < 0.01$. For PPCI patients only, EuroSCORE was not significantly different between the 2 phases of recruitment: 4.8 (8.1) in the first and 4.9 (7.1) in the second, $p = 0.73$.

## 3.4. Summary points and discussion for Chapter 3

- The OPERA cohort contained nearly one thousand patients presenting with all types of acute coronary syndrome, all undergoing PCI or attempted PCI.
- There were no formal exclusion criteria, but no patients with cardiogenic shock could be recruited owing to lack of informed consent.
- Data completeness was generally very good, except for left ventricular function where only 50% of patients had a formal assessment.
- Follow-up of at least 1 year was achieved in 98.5% of patients surviving to 1 year; median length of detailed follow-up was 3.5 years and mortality was tracked for a median of 5.4 years.
- There were several important differences in baseline characteristics of patients presenting with STEMI compared to the other clinical syndromes, including younger age and much higher rate of current smoking (45%).
- A high proportion, nearly 50%, had a previous clinical history of IHD.
- Patients were recruited over a broad time frame of 5 years, in 2 distinct cohorts separated by a gap of about 18 months. There were several important changes in baseline characteristics over this time

### 3.4.1. Comparison of baseline characteristics of OPERA patients with patients from contemporary data sources

It is useful to examine the clinical outcomes of patients from the OPERA study in the context of published data. However, we first need to establish that the OPERA patient cohort is broadly comparable in baseline characteristics and treatment to patients in contemporary studies. There are 2 very large, international, multicentre studies of PCI oral antiplatelet therapy in ACS patients contemporary with the OPERA study which should have a comparable patient population: the TRITON-TIMI 38 trial (Wiviott et al, 2007) and the PLATO invasive study (Cannon et al, 2009). Regarding registry data, the British Cardiovascular Intervention Society (BCIS) publishes its audit returns annually. Table 3-5 compares some of the key baseline characteristics of patients from OPERA with those 3 data sources. Note that the BCIS figures refer to PCI patients presenting with all clinical syndromes including stable elective patients unless indicated; also that PLSATO Invasive contained 5.8% of patients having CABG rather than PCI, all other studies were PCI only.

The age and gender balance of the patient cohorts in these studies are similar, but other characteristics are considerably different. For example, STEMI patients are clearly over-represented in the PLATO Invasive trial, a fact which has not been commented

upon by the authors. In both randomised trials, patients with DM appear to be over-represented and patients with previous MI under-represented compared with UK data. Note that both these trials had exclusion criteria, mainly to do with perceived risk of bleeding. Therefore although the patients are broadly comparable, caution should be used when interpreting the OPERA results in the context of these published data.

**3-5 Crude comparison of important baseline characteristics of patients in the OPERA, TRYTON-TIMI 38 and PLATO Invasive studies and BCIS ACS patients in 2007 and in 2010**

| | OPERA | TRYTON 2007 | PLATO 2010 | BCIS 2007 | BCIS 2010 |
|---|---|---|---|---|---|
| Mean age, years | 62 | 61 | 61 | 64 | 65 |
| Female | 25% | 26% | 25% | 26% | 26% |
| Cardiogenic shock | 0% | 0% | 0% | 3% | Not given |
| Presented with STEMI | 22% | 26% | 49% | 25%* | 39%* |
| DM | 17% | 23% | 23% | 18% | 17%* |
| Previous MI | 24% | 18% | 17% | 30% | 29% |
| Mean BMI | 28 | 28 | Not given | Not given | Not given |
| IIbIIIa use | 31% | 55% | 35% | 40%‡ | 24%‡ |
| DES use | 59% | 47% | Not given | 57%† | 67%† |

* ACS patients only †UA/NSTEMI patients only ‡NSTEMI only

### 3.4.2. Possible impact of baseline characteristics and missing data on study outcomes

The OPERA study has a relatively large patient cohort for a single centre platelet function study which should help to limit any potential bias when comparing groups. We have a very long clinical follow up period so both early and late complications of PCI can be detected. Follow-up was thorough and it is unlikely that any major MACCE events have been missed. Furthermore the OPERA study cohort does appear broadly representative of contemporary ACS PCI patients (see above), with the exception of patients with cardiogenic shock. These patients were also excluded from TRYTON-TIMI 38 and PLATO. However, this is a small (3% of total UK PCI) and uniquely high-risk category of patient requiring special study for which OPERA was not designed; their omission from this study is unlikely to detract from the overall results.

LV systolic function is possibly a more important missing prognostic variable – with only 50% of available data we excluded this parameter from most analyses. This data is also missing from both PLATO Invasive and TRYTON-TIMI 38. In an ACS population an estimation of LV systolic function at around the time of PCI is of questionable value. Myocardium may be temporarily dysfunctional ('stunned') due to ischaemia and may well improve with time, particularly following successful revascularisation. The time length required for complete recovery is unknown but probably several months should be allowed. Therefore, LV function at the time of recruitment somewhat loses its relevance as a reliable prognostic variable in this type of population and its absence becomes less important to the overall results.

Some STEMI patients have been recruited into the OPERA study. However, STEMI patients had different baseline characteristics to the UA/NSTEMI group – they were younger, more likely to smoke and had less co-morbidities. Separate in-depth analysis of this sub-group is difficult owing to its small relative size, particularly as 2 different treatment modalities were used: half the STEMI patients had convalescent PCI following thrombolysis and half PPCI. During the platelet function analyses (one of the most important aspects of this study) emergent PCI patients have been excluded due to uncertain validity of the VerifyNow test in patients without adequate clopidogrel preloading. Therefore, the findings from this study are probably more applicable to the UA/NSTEMI group of ACS patients rather than STEMI patients.

### 3.4.3. Possible impact of prolonged recruitment time scale on study outcomes

There were many changes in treatment which occurred over the recruitment period of the OPERA study, including:

1. There was an increase in numbers of patients undergoing primary PCI and a decrease in those presenting with UA.
2. The baseline risk of the patients increased: there was more diabetes, higher BMI, older age, higher SYNTAX score and higher EuroSCORE in the 2$^{nd}$ recruitment phase than in the 1$^{st}$ phase.
3. Stent type changed from BMS and older first generation DES to modern low-profile DES.
4. Arterial access site changed from mostly femoral to mostly radial over the course of recruitment.
5. There was a decrease in the amount of IIbIIIa inhibitor used and an increase in bivalirudin used.

These factors are important but, providing they are born in mind when interpreting results, are not necessarily detrimental to the study. The 2 distinct recruitment phases allow separate analysis of the associations between baseline characteristics and clinical outcomes and could in fact improve the ability of this study to achieve its primary purpose in detecting possible causes of recurrent ACS. In particular, the impact of changing stent type can be studied by this method. Furthermore, arguably the most important factor influencing clinical outcome in these patients from the above list is the oral antiplatelet regime, strongly linked to both thrombotic and bleeding events over 12 months. This remained largely unchanged throughout the OPERA study with aspirin and clopidogrel given to most patients and so, despite the relatively long recruitment span, this cohort studied as a whole should be expected to provide important and valid associations.

# Chapter 4 Procedural outcomes, restenosis and further revascularisation

## 4.1. Procedural success

Procedural 'technical success', as defined by <50% residual stenosis and good flow in all major branches in all attempted lesions, was achieved in 942 (97.2%) cases. Table 4-1 shows the reasons for lack of procedural success, the most common reason being the inability to penetrate a total occlusion. Procedural success rates were not significantly different between the first and second recruitment phases (2.9% vs. 2.4%, p = 0.71).

**Table 4-1 Details of cases lacking procedural success**

| Reason for lack of procedural success | Number of cases |
|---|---|
| Unable to cross a total occlusion | 11 |
| Persistent poor flow in, or occlusion of, a major side branch | 10 |
| Lesion crossed but unable to dilate | 2 |
| Lesion dilated but unable to deliver stent | 2 |
| Guide catheter dissection of non-culprit artery, procedure abandoned | 1 |
| Side branch was mistaken for main vessel, hence main vessel left untreated | 1 |

## 4.2. Incidence of procedural complications and associations with baseline characteristics

Procedural angiographic and clinical complications are listed in table 4-2 (haemorrhagic complications are discussed in detail in Chapter 6). Angiographic complications as reported by UMS occurred in 128 cases (13.2%), the majority of these complications being either a localised dissection or a trapped side branch. Angiographic complications rates were not significantly different between the first and second recruitment phases (12.2% vs. 13.9%, p = 0.49). Other (non-angiographic, non-haemorrhagic) procedural complications were less frequent in the first recruitment phase compared to the second (0.6% vs. 3.3%, p < 0.01). This was due mainly to the increase in the proportion of patients undergoing PPCI in the second recruitment phase, as PPCI patients had the highest non-angiographic, non-haemorrhagic complication rate. In patients with UA, NSTEMI, convalescent STEMI and PPCI the rates were 0%, 1.3%, 0% and 4.2%, p = 0.02.

**Table 4-2 Frequency of sub-types of procedural complications as reported by UMS**

| **Type of procedural complication** (Patients could have more than 1 type of complication) | **Number of events (% of all patients)** |
|---|---|
| Angiographic complication | 128 (13.2%) |
| Localised dissection | 44 (4.5%) |
| Trapped side branch | 43 (4.4%) |
| No-reflow (transient or persistent) | 23 (2.4%) |
| Unable to dilate lesion fully or under-deployed stent | 12 (1.2%) |
| Extensive coronary dissection | 4 (0.4%) |
| Thrombus formation during procedure | 1 (0.1%) |
| Perforation | 1 (0.1%) |
| Procedural Bleeding | 25 (2.6%) |
| Groin haematoma (≥BARC 2) | 16 (1.7%) |
| Pseudo-aneurysm | 3 (0.3%) |
| Required blood transfusion post procedure | 4 (0.4%) |
| Upper GI bleed with melaena | 1 (0.1%) |
| Retroperitoneal bleed | 1 (0.1%) |
| Other procedural clinical complication | 12 (1.2%) |
| VF arrest during procedure | 6 (0.6%) |
| Complete heart block – temporary pacing wire | 1 (0.1%) |
| Pulmonary oedema | 1 (0.1%) |
| Allergic reaction | 1 (0.1%) |
| Ischaemic leg (fatal) | 1 (0.1%) |
| Contrast nephropathy | 1 (0.1%) |
| TIA (transient expressive dysphasia) | 1 (0.1%) |
| Total procedural clinical complications of any type | 165 (17.1%) |

Baseline clinical characteristics were not different between those with and without complications. Regarding procedural characteristics, angiographic complications were more frequent in patients with bifurcation lesions (18.5% vs. 11.0% in non-bifurcation lesions, P < 0.01) and in lesions with reduced TIMI flow at the start (26.2% vs. 12.0% in lesions with TIMI 3 flow at the start, P < 0.01). Heavy calcification did not predispose to angiographic complications (12.6% vs. 12.6% without heavy calcification). Median SYNTAX score (IQR) was higher in those with angiographic complications: 11.0 (10.0) vs. 8.0 (11.0), p < 0.01.

### 4.2.1. Procedural death

There was 1 death which resulted directly from a procedural complication (this was the only peri-procedural death). This patient had presented with a NSTEMI and had a history of peripheral vascular disease, severe airways disease and a number of other co-morbidities. He was unsuitable for CABG due to a calculated EuroSCORE of 41 and maximal medical therapy had failed to alleviate his symptoms of intractable angina, which had led to frequent hospital admissions over the previous 18 months. His only vascular access route was via a femoro-popliteal bypass graft conduit. PCI was complicated by no-reflow and he was administered a IIbIIIa inhibitor during the procedure. Several hours after the procedure he developed bleeding from the puncture site leading to leg ischaemia and hypovolaemic shock. He died despite emergent vascular surgery.

The 1 year mortality rates in patients with and without a non-haemorrhagic complication were numerically, but not significantly, different: all-cause mortality, 5.1% vs. 2.5%, p = 0.10; and cardiac mortality, 3.6% vs. 1.7%, p = 0.13. A lack of ‘procedural success’ had no obvious association with cardiac or all-cause mortality – however, this is a very small group.

## 4.3. Follow-up coronary angiography and staged PCI

In all, 197 patients (20.4% of the total cohort) underwent at least 1 further coronary angiogram during follow-up. Further angiography in most (170, 17.6%) cases was to investigate recurrent angina or following a further ACS, but an additional 27 patients (2.8%) underwent planned angiography for ‘staged’ PCI. There were no unstable symptoms or adverse clinical events between the 2 procedures in patients undergoing staged PCI. Staged PCI took place in all of the 27 (2.8%) patients in whom it was planned.

More follow-up angiography took place in those with a history of coronary revascularisation prior to the index procedure (34.3% vs. 17.5%, p < 0.01), those having

PCI to more than 1 vessel (32.8% vs. 17.0%, $p < 0.01$), patients having PCI to restenotic lesions (38.9% vs. 19.6%, $p = 0.02$) and in patients receiving small diameter ($\leq$ 2.5mm) stents (24.5% vs. 19.2%, $p = 0.04$).There was a tendency for higher rates of follow-up angiography in men compared to women (21.6% vs. 16.5%, $p = 0.06$).

## 4.4. Further revascularisation and binary restenosis rates

### 4.4.1. Incidence and types of unplanned revascularisation

Unplanned further revascularisation for any reason was undertaken in 118 patients (12.2% of the whole cohort) over long-term follow-up. Further unplanned revascularisation rates at 30 days and 1 year, and the Kaplan-Meier estimated rate at 3.3 years (median follow-up), were 0.7%, 6.9% and 12.3%. Further revascularisation was attempted by PCI in 101 cases and by CABG in 17 cases. In 49 patients, further revascularisation took place urgently following a recurrent episode of ACS; and in 69 patients, further revascularisation took place electively following recurrent angina.

Note: these figures do not include further revascularisation which was planned at the time of the index PCI (‘staged’ procedures).

### 4.4.2. Incidence of angiographic binary restenosis

Binary restenosis (>50% stenosis by angiography) was diagnosed in 60 patients, making the long-term ‘clinically-driven’ detected restenosis rate 6.2% over long-term follow-up (the subclinical restenosis rate is of course unknown as only 1 in 5 had follow-up angiography). The median (IQR) time from PCI to further revascularisation in patients with restenosis was 307 (489) days.

### 4.4.3. Incidence of target lesion versus non-target lesion revascularisation

Of 118 cases of further unplanned revascularisation, 63 (53.4%) were classified as target lesion revascularisation (TLR). The Kaplan-Meier estimated TLR rates at 1 year and 3.3 years were 3.7% and 6.4%. However, there was almost as much non-target lesion revascularisation (non-TLR), occurring in 55 patients over long-term follow-up. The 1 year and 3.3 year rates of non-TLR were 3.2% and 5.8%. Of the cases of non-TLR, there were 20 cases of target vessel/non target lesion revascularisation (16.9% of all further revascularisation) and 35 cases of non-target vessel revascularisation (29.7% of all further revascularisation). Note: I have not included ‘staged PCI’ (planned at the time of the index procedure but deferred) in the further revascularisation figures.

Figures 4-1 and 4-2 show KM curves of the cumulative incidence of TLR and non-TLR, showing a frequency and distribution of events which is strikingly similar for

each. There are higher rates early after the index PCI which gradually decline during median follow-up; and there are similar numbers of events in each category.

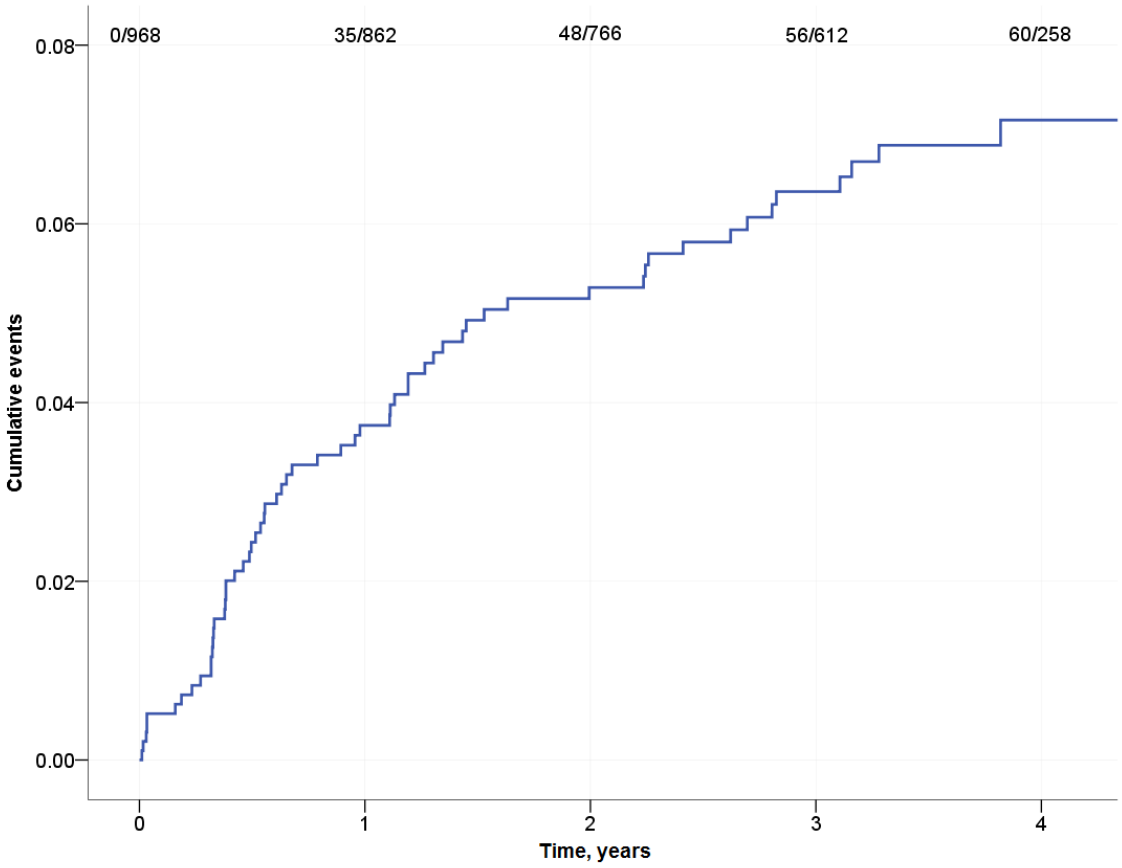



**Figure 4-1 KM estimated incidence of target lesion revascularisation**

### 4.4.1. Clinical characteristics of further revascularisation

Further revascularisation was mostly attempted by repeat PCI, occurring in 90% of TLR and 80% of non-TLR, with the remaining patients undergoing CABG. All of the 17 patients who had CABG were referred directly for surgery after angiography without further attempts at PCI. The clinical syndrome leading to further revascularisation was recurrent ACS in many cases, especially TLR: of the 63 patients undergoing TLR, 49.2% required emergent/urgent treatment compared with 32.7% in those undergoing non-TVR.

Restenosis was a major cause of further revascularisation, known to have occurred in half of the 49 patients undergoing urgent further revascularisation and 28 of the 65 patients undergoing elective further revascularisation over long-term follow-up for the entire cohort (restenosis is discussed further in Section 4.5). In patients undergoing TLR, severe in-stent restenosis was the causal factor in 51 (81%) cases and stent thrombosis in 9 (14%) cases. Of the other 3 cases of TLR, 2 cases took place for a

further attempt at a previously unsuccessful PCI; and there was 1 case of balloon angioplasty of a large trapped side branch without actual stent restenosis.

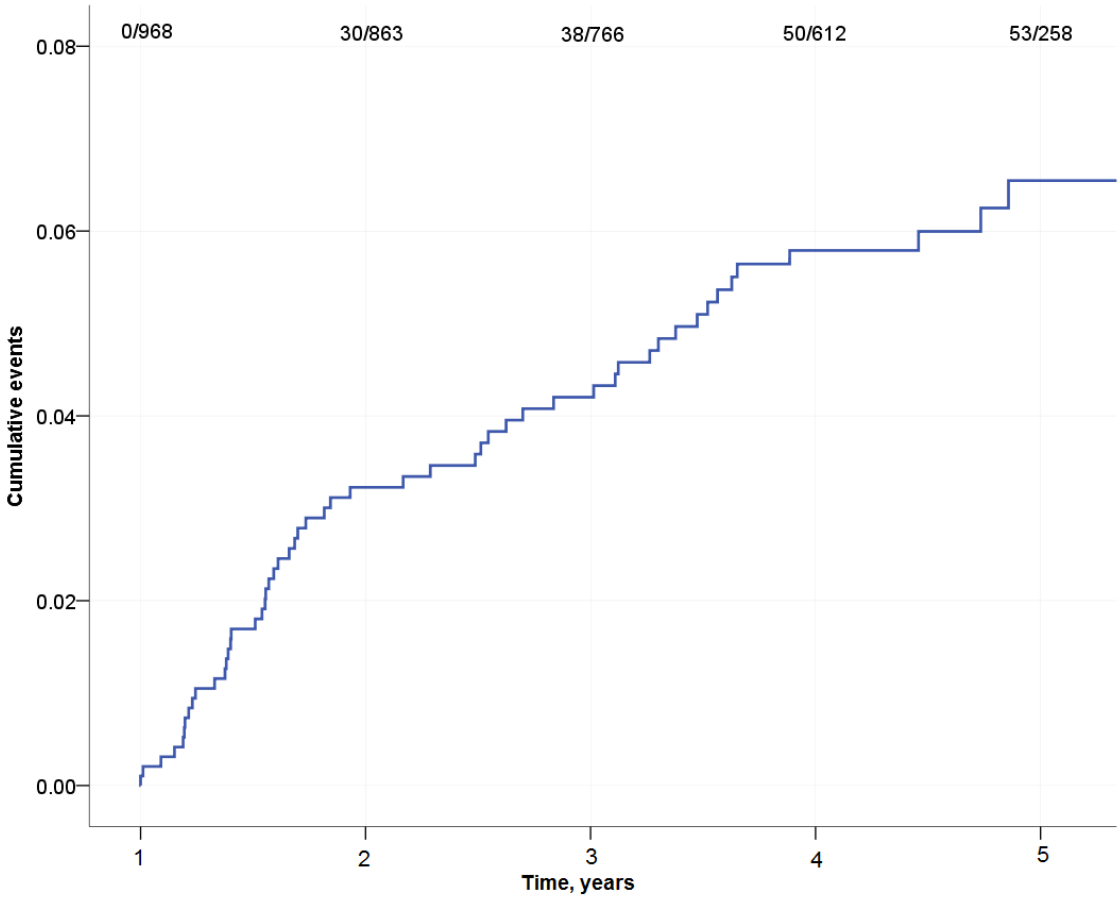



**Figure 4-2 KM estimated incidence of non-target lesion revascularisation**

### 4.4.2. Revascularisation rates at 1 year and recruitment phase

Neither the overall 1 year TLR nor the 1 year total unplanned revascularisation rates changed significantly per year of PCI, although it should be noted that comparisons are hampered by low event numbers. In the first and second recruitment phases there were 29 and 6 cases of TLR up to 1 year, giving 1 year TLR rates of 4.0% vs. 2.4% (p = 0.26). The 1 year total unplanned revascularisation rates in the first and second recruitment phases were 6.6% vs. 6.9% (p = 0.87).

In patients receiving only DES, the 1 year TLR in the first and second recruitment phases were 3.5% vs. 2.4% (p = 0.46); and 1 year total unplanned revascularisation rates in the first and second recruitment phases were 4.8% vs. 5.7% (p = 0.65). In patients receiving at least 1 BMS, the rates of these same events over the 2 recruitment periods were 4.3% vs. 3.3% (p = 0.80) and 7.9% vs. 16.7% (p = 0.10).

## 4.5. Baseline characteristics in patients undergoing further revascularisation and in patients with restenosis

Baseline and procedural characteristics for patients without restenosis or any further revascularisation, patients with binary restenosis and patients undergoing any further revascularisation are shown in Table 4-3.

### 4.5.1. Associations of baseline characteristics with further revascularisation

Unplanned further revascularisation was less frequent in females than males and in patients undergoing convalescent PCI following thrombolysis for STEMI than in PPCI, NSTEMI or UA; but was more frequent in those with a previous history of angina, any known IHD or any previous revascularisation (but not in previous MI). In patients requiring repeat revascularisation, total stent length was greater; and they were more likely to have received a IIbIIIa inhibitor than those not requiring further revascularisation. There was no difference in EuroSCORE between patients who did and did not undergo further revascularisation.

Patients receiving only DES were less likely to require repeat revascularisation, as were patients receiving only 'second generation' DES and only *'Xience'* stents. There was a trend towards more unplanned revascularization among patients receiving only BMS. A similar trend was also seen for patients with heavily calcified lesions. There was a strong trend for lower TLR rate in patients receiving only *'Xience'* DES compared to other stent types, 1.8% vs. 4.3% at 1 year ($p = 0.07$) and 3.8% vs.7.0% at 3 years (Kaplan-Meier estimates; Log Rank $p = 0.05$).

There was no association between angiographic or other procedural complications and unplanned revascularisation rates.

**Table 4-3 Comparison of baseline characteristics in patients with and without binary restenosis, and with and without any further unplanned revascularisation, at any time during long-term follow-up**

| **Characteristic**<br>Binary characteristics shown as Number (% within restenosis/revascularisation groups) | **No restenosis or revascularisation**<br>N = 844 | **Binary restenosis**<br>N = 60 | **P value** | **Any unplanned revascularisation**<br>N = 118 | **P value** |
|---|---|---|---|---|---|
| *Demographics and clinical syndrome* | | | | | |
| Mean (SD) Age, years | 62.3 (12.1) | 61.2 (12.1) | 0.50 | 62.1 (11.6) | 0.98 |
| Female | 222 (26.4) | 11 (18.0) | 0.19 | 20 (16.9) | 0.03 |
| Recruited during first phase | 626 (74.2) | 52 (86.7) | 0.03 | 92 (78.0) | 0.38 |
| Recruited during second phase | 218 (25.8) | 8 (13.3) | | 26 (22.0) | |
| Median (IQR) Body Mass Index | 28 (6) | 28 (6) | 0.68 | 28 (6) | 0.75 |
| Any history of smoking | 578 (68.7) | 46 (75.4) | 0.28 | 83 (70.9) | 0.66 |
| Family history of IHD | 419 (51.9) | 38 (63.3) | 0.09 | 67 (58.3) | 0.21 |
| Unstable angina | 99 (11.8) | 17 (28.3) | <0.01 | 24 (20.3) | 0.02 |
| Non-STEMI, | 546 (64.6) | 37 (61.7) | 0.61 | 77 (65.3) | 0.89 |
| ST elevation MI: Convalescent PCI | 112 (13.3) | 3 (5.0) | 0.07 | 8 (6.8) | 0.05 |
| ST elevation MI: Primary PCI | 87 (10.3) | 3 (5.0) | 0.19 | 9 (7.6) | 0.38 |
| ST elevation MI: any | 199 (23.6) | 6 (10.0) | 0.02 | 17 (14.4) | 0.03 |
| *Previous medical history* | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Angina | 358 (42.4) | 43 (71.7) | <0.01 | 69 (58.5) | <0.01 |
| MI | 197 (23.3) | 18 (30.0) | 0.21 | 28 (23.7) | 0.93 |
| PCI | 84 (9.9) | 19 (31.7) | <0.01 | 25 (21.2) | <0.01 |
| CABG | 55 (6.5) | 11 (18.3) | <0.01 | 15 (12.7) | 0.02 |
| Any previous revascularisation | 127 (15.0) | 25 (41.7) | <0.01 | 36 (30.5) | <0.01 |
| Any previous known IHD | 386 (45.7) | 44 (73.3) | <0.01 | 70 (59.3) | <0.01 |
| Diabetes mellitus | 138 (16.4) | 12 (20.0) | 0.45 | 21 (17.8) | 0.68 |
| Hypertension | 421 (49.8) | 35 (58.3) | 0.21 | 65 (55.1) | 0.28 |
| Hyperlipidaemia | 473 (56.2) | 37 (62.7) | 0.31 | 67 (57.3) | 0.85 |
| Peripheral vascular disease | 40 (4.7) | 1 (1.7) | 0.28 | 3 (2.5) | 0.29 |
| Respiratory disease | 87 (10.3) | 8 (13.3) | 0.48 | 15 (12.7) | 0.43 |
| Stroke | 43 (5.1) | 3 (5.0) | 0.98 | 6 (5.1) | 0.99 |
| Untreated aortic valve disease | 9 (1.1) | 2 (3.3) | 0.13 | 3 (2.5) | 0.17 |
| Heart failure | 12 (1.4) | 2 (3.3) | 0.25 | 3 (2.5) | 0.35 |
| | | | | | |
| *Blood results (pre-procedural)* | | | | | |
| Mean (SD) eGFR, ml/min/1.73m$^2$ | 76 (30) | 83 (41) | 0.30 | 74 (31) | 0.79 |

| | | | | | |
|---|---|---|---|---|---|
| eGFR < 60 ml/min/1.73m$^2$ | 264 (32) | 18 (30.5) | 0.94 | 28 (24.6) | 0.11 |
| Mean (SD) Hb concentration, g/dL | 14.0 (1.7) | 14.1 (2.4) | 0.88 | 13.9 (1.8) | 0.69 |
| Mean (SD) platelet count, x10$^9$/L | 256 (68) | 244 (93) | 0.66 | 244 (112) | 0.68 |
| Mean white cell count, x10$^9$/L | 8.9 (3.4) | 8.8 (3.8) | 0.99 | 8.8 (3.1) | 0.86 |
| Median (IQR) PRU *(VerifyNow)* | 247 (127) | 247 (147) | 0.73 | 247 (148) | 0.67 |
| | | | | | |
| *Risk scores* | | | | | |
| Median (IQR) EuroSCORE I | 3.1 (4.5) | 3.2 (6.0) | 0.57 | 3.3 (5.1) | 0.60 |
| Median (IQR) SYNTAX score | 8.0 (11.0) | 11.5 (12.6) | 0.06 | 11.5 (12.0) | <0.01 |
| Lowest SYNTAX score quartile (≤4.0) | 217 (26.2) | 11 (18.3) | 0.23 | 19 (16.4) | 0.02 |
| Highest SYNTAX score quartile (≥15.5) | 191 (23.1) | 19 (31.7) | 0.19 | 40 (34.5) | <0.01 |
| | | | | | |
| *Medication* | | | | | |
| Aspirin | 815 (96.7) | 59 (100) | 0.17 | 118 (100) | 0.05 |
| Clopidogrel | 793 (94) | 59 (98.3) | 0.18 | 116 (98.3) | 0.05 |
| Prasugrel | 53 (6.3) | 1 (1.7) | 0.17 | 2 (1.7) | 0.05 |
| Dual antiplatelet therapy | 814 (96.7) | 59 (100) | 0.17 | 118 (100) | 0.05 |

| | | | | | |
|---|---|---|---|---|---|
| $P2Y_{12}$ inhibitor loading <24 hours before PCI | 110 (13) | 5 (8.3) | 0.29 | 14 (11.9) | 0.75 |
| Heparin | 725 (86.1) | 54 (90.0) | 0.34 | 103 (87.3) | 0.75 |
| IIbIIIa | 259 (30.7) | 26 (43.3) | 0.06 | 50 (42.2) | 0.01 |
| Bivalirudin | 129 (15.4) | 7 (11.7) | 0.44 | 17 (14.4) | 0.81 |
| Thrombolysis on admission | 70 (8.3) | 2 (3.3) | 0.17 | 8 (6.8) | 0.59 |
| | | | | | |
| *Procedural characteristics* | | | | | |
| Arterial access: radial route | 180 (21.3) | 7 (11.7) | 0.06 | 24 (20.3) | 0.81 |
| Worst lesion treated: Type A | 124 (14.7) | 8 (13.6) | 0.84 | 15 (13.2) | 0.68 |
| Worst lesion treated: Type B1 | 208 (24.8) | 10 (16.9) | 0.17 | 24 (21.1) | 0.39 |
| Worst lesion treated: Type B2 | 199 (23.6) | 9 (15.3) | 0.14 | 21 (18.4) | 0.21 |
| Worst lesion treated: Type C | 310 (36.8) | 32 (54.2) | <0.01 | 54 (47.4) | 0.03 |
| Number vessels treated: 1 | 671 (79.4) | 46 (76.7) | 0.66 | 88 (74.6) | 0.21 |
| Number vessels treated: 2 or 3 | 174 (20.6) | 14 (23.3) | 0.66 | 30 (25.4) | 0.21 |
| Number vessels treated: 3 | 13 (1.5) | 0 | 0.35 | 0 | 0.18 |
| LMS treated | 19 (2.2) | 4 (6.7) | 0.04 | 6 (5.1) | 0.07 |
| LAD treated | 415 (49.1) | 27 (45.0) | 0.60 | 51 (43.2) | 0.24 |

| | | | | | |
|---|---|---|---|---|---|
| Circumflex treated | 258 (30.5) | 22 (36.7) | 0.31 | 37 (31.4) | 0.88 |
| Right coronary artery | 340 (40.2) | 20 (33.5) | 0.23 | 53 (44.9) | 0.32 |
| Restenotic lesion | 26 (3.1) | 8 (13.3) | <0.01 | 7 (5.9) | 0.17 |
| Bypass graft lesion | 28 (3.3) | 4 (6.7) | 0.19 | 6 (5.1) | 0.36 |
| Ostial lesion | 52 (6.2) | 5 (8.3) | 0.50 | 8 (6.8) | 0.78 |
| Chronic total occlusion | 27 (3.2) | 1 (1.7) | 0.53 | 1 (0.8) | 0.14 |
| Heavily calcified lesion | 152 (18.0) | 14 (23.3) | 0.35 | 29 (24.6) | 0.09 |
| Bifurcation lesion | 318 (37.7) | 22 (36.7) | 0.85 | 44 (37.3) | 0.90 |
| Lesion with angiographic thrombus | 212 (25.1) | 11 (18.3) | 0.21 | 32 (27.1) | 0.61 |
| Rotational atherectomy | 3 (0.4) | 1 (1.7) | 0.12 | 1 (0.8) | 0.43 |
| TIMI flow <III pre-PCI in any treated lesion | 37 (4.4) | 2 (3.3) | 0.69 | 5 (4.2) | 0.96 |
| Median (IQR) width of smallest stent, mm | 3.0 (0.8) | 3.0 (0.5) | 0.35 | 3.0 (0.8) | 0.46 |
| At least 1 small stent implanted (≤2.5mm) | 192 (23.1) | 16 (27.1) | 0.45 | 24 (20.9) | 0.54 |
| Median (IQR) total length all stents, mm | 24 (21) | 27 (27) | 0.03 | 28.0 (24.0) | 0.03 |
| 2 or more stents implanted | 229 (27.4) | 23 (39.0) | 0.07 | 44 (38.3) | 0.02 |
| DES only | 468 (55.5) | 33 (55.0) | 0.94 | 54 (45.8) | 0.04 |
| BMS only | 325 (38.5) | 23 (38.3) | 0.89 | 55 (46.6) | 0.08 |

| | | | | | |
|---|---|---|---|---|---|
| Combination of DES and BMS | 37 (4.4) | 3 (5.0) | 0.86 | 6 (5.1) | 0.76 |
| No stent, POBA only | 11 (1.3) | N/A | N/A | 1 (0.8) | 0.68 |
| 1st generation DES only | 125 (15.0) | 13 (22.0) | 0.13 | 17 (14.8) | 0.89 |
| 2nd generation DES only | 328 (39.6) | 17 (28.8) | 0.14 | 30(26.1) | <0.01 |
| *'Cypher'* DES only | 101 (12.0) | 10 (16.7) | 0.26 | 14 (11.9) | 0.94 |
| *'Taxus'* DES only | 24 (2.8) | 3 (5.0) | 0.31 | 3 (2.5) | 0.81 |
| *'Xience'* DES only | 247 (29.3) | 10 (16.7) | 0.04 | 21 (17.8) | <0.01 |
| 'Endeavor' DES only | 81 (9.6) | 7 (11.7) | 0.51 | 9 (7.6) | 0.51 |
| Staged PCI | 22 (2.6) | 2 (3.3) | 0.79 | 5 (4.2) | 0.31 |
| | | | | | |
| *Procedural complications* | | | | | |
| Unsuccessful procedure | 23 (2.7) | N/A | N/A | 4 (3.4) | 0.67 |
| Angiographic complication, any (see table) | 104 (12.3) | 10 (16.7) | 0.33 | 16 (13.6) | 0.74 |
| Angiographic localised dissection (UMS) | 34 (4.0) | 3 (5.0) | 0.86 | 9 (7.6) | 0.09 |
| Angiographic trapped side branch (UMS) | 42 (5.0) | 1 (1.7) | 0.28 | 1 (0.8) | 0.04 |
| Other procedural clinical complication (non-haemorrhagic) | 9 (1.1) | 1 (1.7) | 0.76 | 3 (2.5) | 0.17 |

### 4.5.2. SYNTAX score and further revascularisation

SYNTAX scores were higher in those requiring further unplanned revascularisation. When patients were stratified into SYNTAX score quartiles, further revascularization rates over long-term follow-up were: Q1 = 8.1% (SYNTAX score < 5.0), Q2 = 9.8% (5.0 to 8.0), Q3 = 13.9% (9.0 to 15.0) and Q4 = 17.1% (≥ 15.5), p = 0.01. The Kaplan-Meier cumulative incidence of further unplanned revascularisation of any sort, for patients stratified into SYNTAX score quartiles, are shown in Fig 4-3. Note that SYNTAX scores were different in patients with a variety of clinical characteristics, shown in Table 3-4, Section 5.5.13.

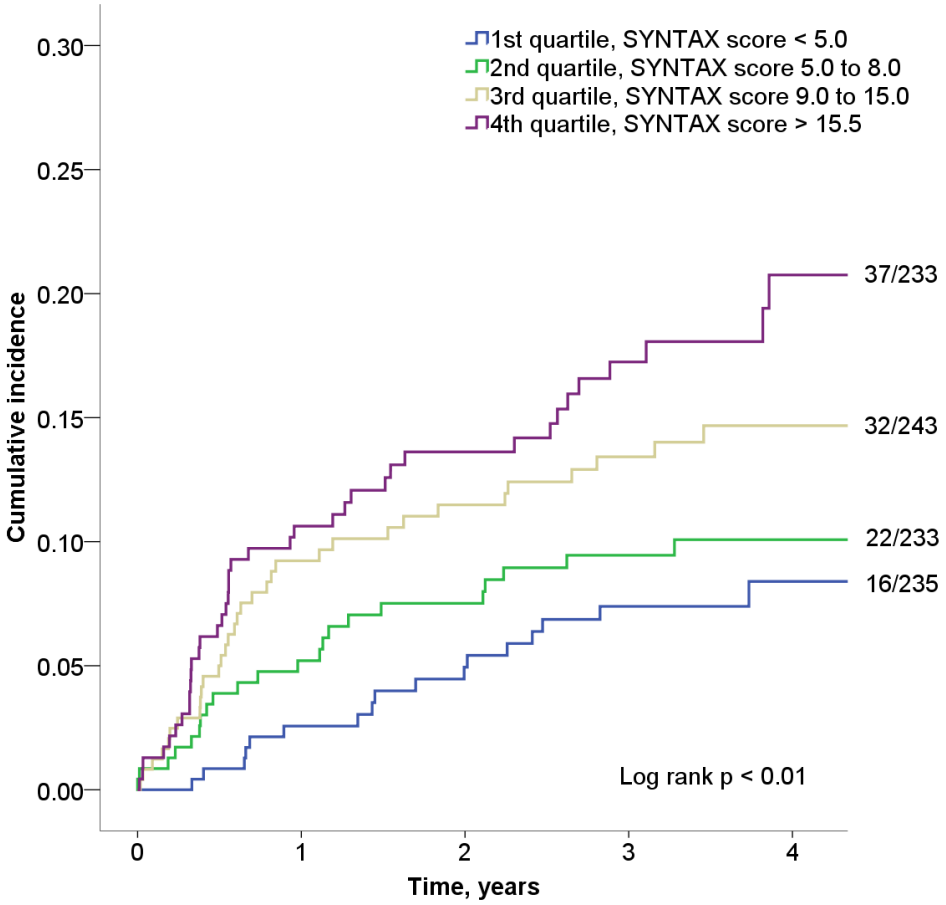



**Figure 4-3 KM estimated cumulative incidence of any type of unplanned revascularisation over median follow-up, stratified by SYNTAX score quartiles**

### 4.5.3. Associations of baseline characteristics with restenosis

There were significant differences in baseline characteristics in patients who developed restenosis compared to those who did not. Those with restenosis were more likely to have presented during the first recruitment phase than the second. They were more likely to have presented with unstable angina, but less likely to have presented with STEMI. They had a greater incidence of a previous history of IHD and to have undergone prior revascularisation (PCI, CABG or both). More often they had undergone

PCI to Type C lesions, the index lesion treated was more likely to have been itself restenotic, and the total length of implanted stents was slightly greater. The restenosis rate in bypass grafts was not significantly higher than that in native vessels, although only 10 of the 35 patients having graft PCI underwent an angiogram during follow-up and subclinical graft occlusion rate is, therefore, unknown.

Patients who experienced restenosis had a trend towards higher SYNTAX scores; however the difference in restenosis rates between patients stratified into SYNTAX score quartiles was not statistically significant. EuroSCOREs were not different in patients with and without restenosis. The occurrence of procedural complications was not associated with restenosis.

The incidence of restenosis in patients receiving DES and BMS did not appear to be different: the rate in those receiving only DES was 6.4% and in those receiving at least 1 BMS was 6.1%; p = 0.84 (but note that there were important differences in baseline and procedural characteristics between patients receiving DES and those receiving BMS, see Section 3.5.4). By individual stent type, '*Xience'* stents appeared to have the lowest incidence of restenosis over long term follow-up: only 3.7% of patients treated exclusively with *'Xience'* stents experienced restenosis compared to 7.2% in patients not receiving Xience stents; p = 0.05. The KM estimated rate of target lesion revascularisation at 1 year was 2.0% in patients with only Xience stents vs. 4.8% in other patients, log rank P = 0.053; see Figure 4-4.

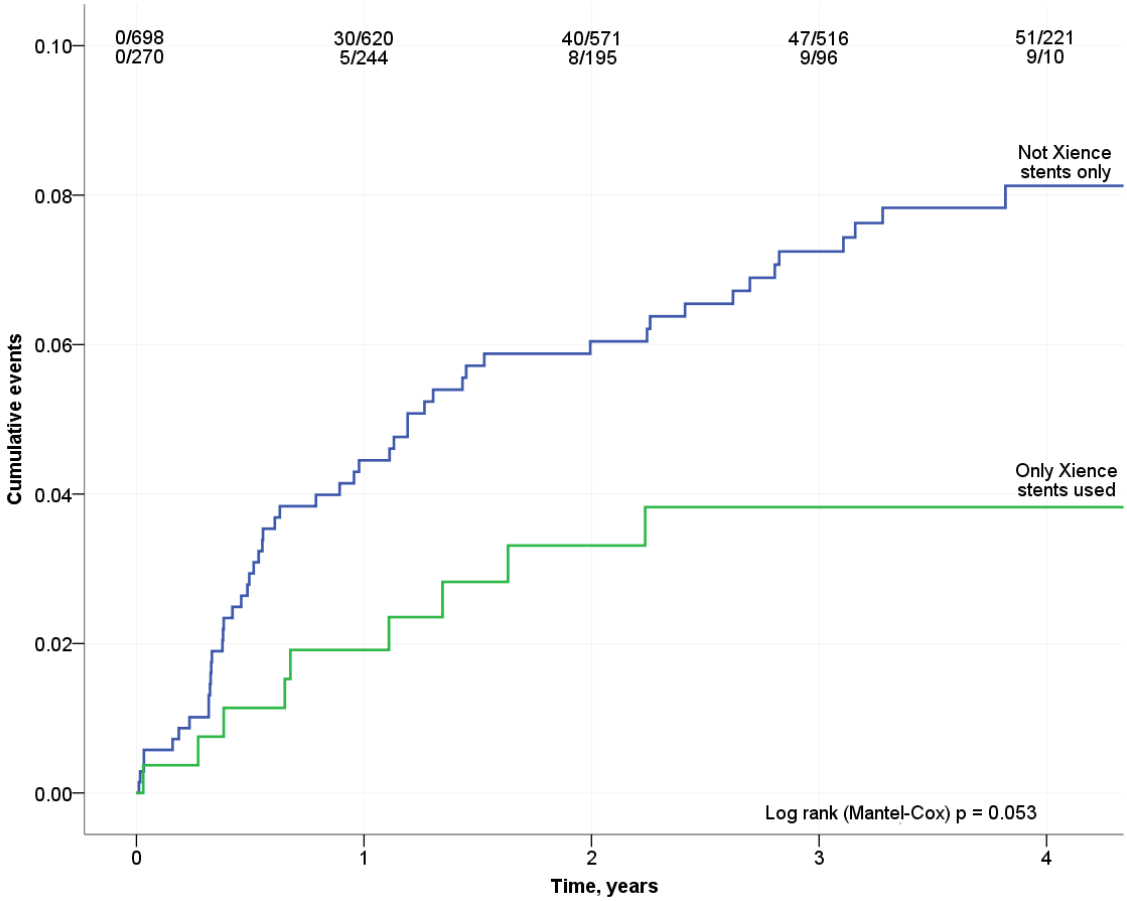



**Figure 4-4 KM estimates of rate of target lesion revascularisation, stratified by Xience stent use only vs. other stent types/combinations**

### 4.5.4. Clinical outcomes in patients with restenosis

Of the 60 patients with restenosis, 54 (90.0%) underwent further revascularisation (compared to 63 (6.9%) of those without restenosis over long term follow-up) and 51 (85.0%) underwent target lesion revascularisation (TLR). More specifically, restenosis was managed as follows:

- No further revascularisation, 6 patients
- Revascularisation of non-target vessel, 3 patients
- Target lesion PCI with DES, 36 patients
- Target lesion PCI with BMS, 5 patients
- Target lesion PCI with balloon angioplasty, 5 patients
- CABG, 5 patients

Further revascularisation for restenosis was undertaken electively after the patient presented with recurrent angina in 30 patients, 14 patients required urgent revascularisation after being admitted with troponin positive ACS, and 9 presented with troponin negative ACS (1 had missing data).

The median (IQR) time from index PCI to TLR for restenosis was 326 (588) days.

The patients who were found to have restenosis but did not undergo TLR are listed in Table 4-4 with details explaining the management decision.

**Table 4-4 Details of patients who did not undergo revascularisation of a restenosis**

| PAT ID | Reason for no further revascularisation of restenotic target lesion |
|---|---|
| 453,757 | No viability/reversible ischaemia in myocardium subtended by restenotic vessel, managed medically |
| 68, 72 | Completely occluded native vessel stent, unattractive for PCI, PCI to another vessel |
| 754 | Completely occluded native vessel stent, unattractive for PCI, managed medically |
| 993 | Completely occluded SVG, managed medically |
| 677 | Completely occluded SVG, PCI to another vessel |
| 399 | Moderate restenosis managed medically |
| 543 | Small vessel, thought high risk of further restenosis, managed medically |

Note on Table 4-4: 1 further patient with restenosis included in the list above as having TLR (PAT ID 60, not included in the table) had additional ISR in a second stent in a previously treated vessel; this was managed medically as cardiac MRI scanning showed no viability in the territory supplied by this vessel.

Of the 46 patients with restenosis who underwent further successful PCI to the target lesion, 4 are known to have developed recurrent target lesion ISR. Of these, 1 patient died of IHD without undergoing any further revascularisation attempts, 1 went on to have a successful CABG (alive), 1 had a third PCI to the target lesion (successful, alive) and 1 had further (successful) PCI to the target lesion but went on to die of ischaemic cardiomyopathy.

### 4.5.5. Further revascularisation, restenosis and death rates

The need for further unplanned revascularisation for restenosis did not appear to have a substantial impact on mortality in the short to medium term, in contrast to further revascularisation for other indications, illustrated in Figure 4-5. Of the 6 deaths which occurred in the non restenosis group within 1 year of the further revascularisation procedure, 4 occurred in patients with definite stent thrombosis.

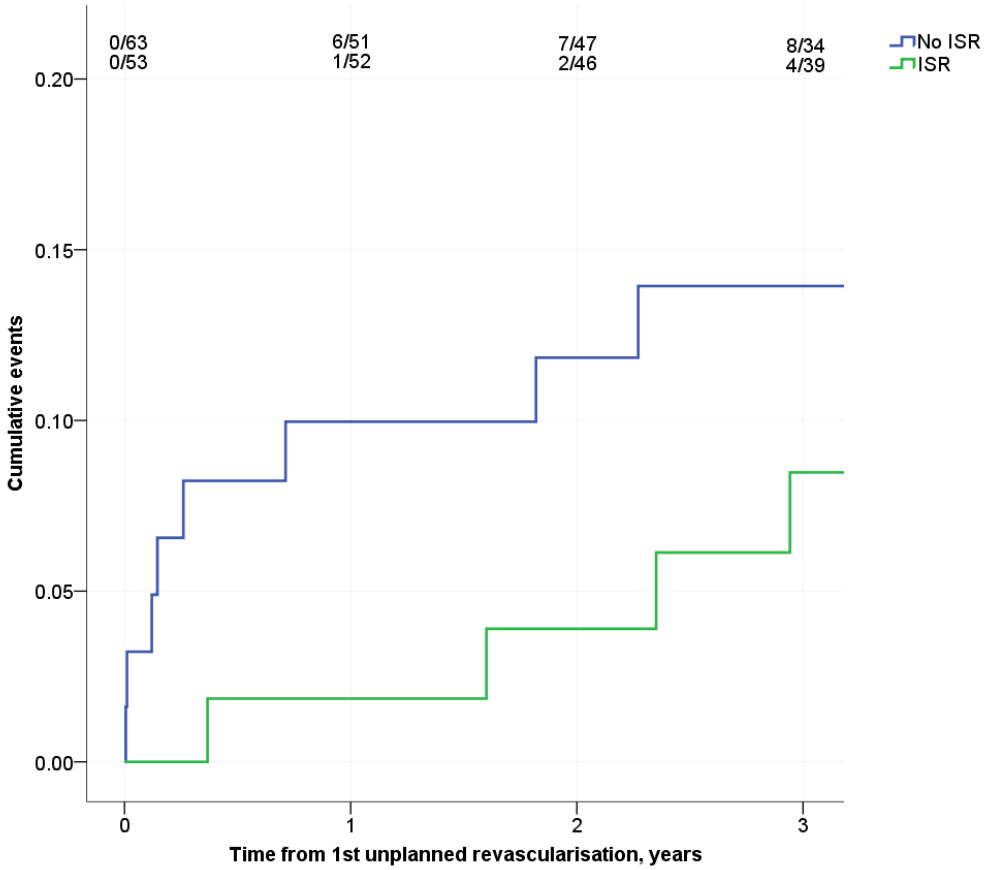



**Figure 4-5 Kaplan-Meier estimates of all-cause mortality rates following further unplanned revascularisation in 118 patients, stratified by presence or absence of binary in-stent restenosis**

## 4.6. Chapter 4 summary points and discussion

- Procedural success rate was high at 97.5% and only 1 procedural death occurred.
- Repeat angiography was mostly symptom-driven and occurred in 20%.
- Unplanned further revascularisation took place in 12.2% altogether (rate of 6.9% at 1 year) mainly by repeat PCI and often for recurrent ACS.
- Rates of target lesion revascularisation (TLR) and non-target lesion revascularisation (non-TLR) were very similar, both at 12 months and at 3.3 years, with higher rates over the 1st year for both.
- Certain baseline characteristics were more common in patients requiring further revascularisation including presentation with UA, previous IHD, Type C lesions and longer stent length.
- Further revascularisation occurred less often in STEMI patients receiving convalescent PCI; and in those with Xience stents.
- The 1 year ISR rate was only 2% in those with only with Xience stents compared to 4.8% with all other stent types.
- Baseline SYNTAX score was directly proportional to the rate of unplanned further revascularisation. Roughly, patients with a SYNTAX score of 4 had a further revascularisation rate of 10%; SYNTAX 16 had a 15% rate and SYNTAX 27 had a 20% rate over long-term follow-up.
- Angiographic complications such as localised dissection or trapped side branches occurred in 13.2%, but there was little to suggest that this type of complication had a significant impact on subsequent clinical outcome.
- 60 patients were found to have definite binary in-stent restenosis (ISR).
- ISR rates were not different in patients with DES and BMS.
- Most patients with ISR were treated by repeat target lesion revascularisation with insertion of drug-eluting stents.
- ISR did not appear to have any impact on mortality rates.

### 4.6.1. Comparison of OPERA study further revascularisation rates with contemporary data

The 2 large contemporary antiplatelet trials discussed in Chapter 3, PLATO Invasive and TRYTON-TIMI 38, do not report unplanned revascularisation as a separate endpoint. Neither is this data available from BCIS. However, Stolker et al have published unplanned revascularisation rates at 12 months from a large prospective unselected PCI registry based in the US, the EVENT registry. From July 2004 to June 2007 10144 patients were recruited from 55 PCI centres and were followed up by

telephone at 6 and 12 months. Of patients whose indication for PCI was non-STE ACS, 8.4% underwent unplanned further revascularisation at 12 months compared to 6.9% in OPERA. Of all cases of unplanned revascularisation, TLR took place in 47% in EVENT and 54% in OPERA. These rates are broadly similar and suggest that the problem of recurrent coronary ischaemia requiring unplanned revascularisation is common and widespread, with about half the cases due to problems with the original intervention and half due to untreated or new lesions.

### 4.6.2. Restenosis

Most cases of TLR in OPERA were due to severe ISR. Several changes in stent technology took place during the OPERA recruitment period which were aimed at reducing restenosis rates, namely the increase of DES and decrease of BMS use; and the development of '2$^{nd}$ generation' DES. These changes do appear to have had a beneficial effect, with lower restenosis rates seen in the 2$^{nd}$ recruitment phase compared to the 1st. In OPERA, 2$^{nd}$ generation DES such as Xience stents do appear to be considerably less prone to restenosis than earlier stents. However, the patients in OPERA who received BMS were no more likely to experience restenosis than those receiving DES, indicating that the use of BMS may still be a reasonable choice in selected ACS patients. BMS have the advantage of requiring a much shorter duration of DAPT and remain a valuable option in patients at high risk of bleeding.

### 4.6.3. The SYNTAX score

This angiographic score was a powerful predictor of further unplanned revascularisation in ACS patients in OPERA; an approximately linear relationship was observed. The SYNTAX score was developed to assess coronary disease severity for patients in the SYNTAX trial (Serruys et al), a study of clinical outcomes in patients with complex disease randomised to treatment with either PCI or CABG. In the SYNTAX trial, the score was found to be highly predictive of adverse outcome in those patients undergoing PCI. The patients in OPERA had considerably lower SYNTAX scores than these patients, but even in the lower range the score still seems remarkably consistent. The SYNTAX score has been criticised for having high inter-observer variability i.e. of being too subjective; and also for not containing any information pertaining to functional assessment of myocardial ischaemia. However, in OPERA the SYNTAX score at baseline was one of the best predictors of the need for both short and long term unplanned revascularisation (Figure 4-3) and would seem to be a highly valuable risk stratification tool despite any perceived shortcomings in the literature.

# Chapter 5 Recurrent ACS, stent thrombosis and cardiac death

## 5.1. Incidence of recurrent ACS during long-term follow-up

The Kaplan-Meier estimated cumulative rates of first episode of recurrent ACS (any type; excluding suspected non-cardiac symptoms) at 30 days, 1 year and 3.4 years were 1.4%, 6.7% and 11.2%. The rate of recurrent ACS was higher immediately following PCI and declined in the months that followed. After the first year, the rate remained stable at approximately 1.8% per year over the subsequent 3 years of follow-up, see Figure 5-1.

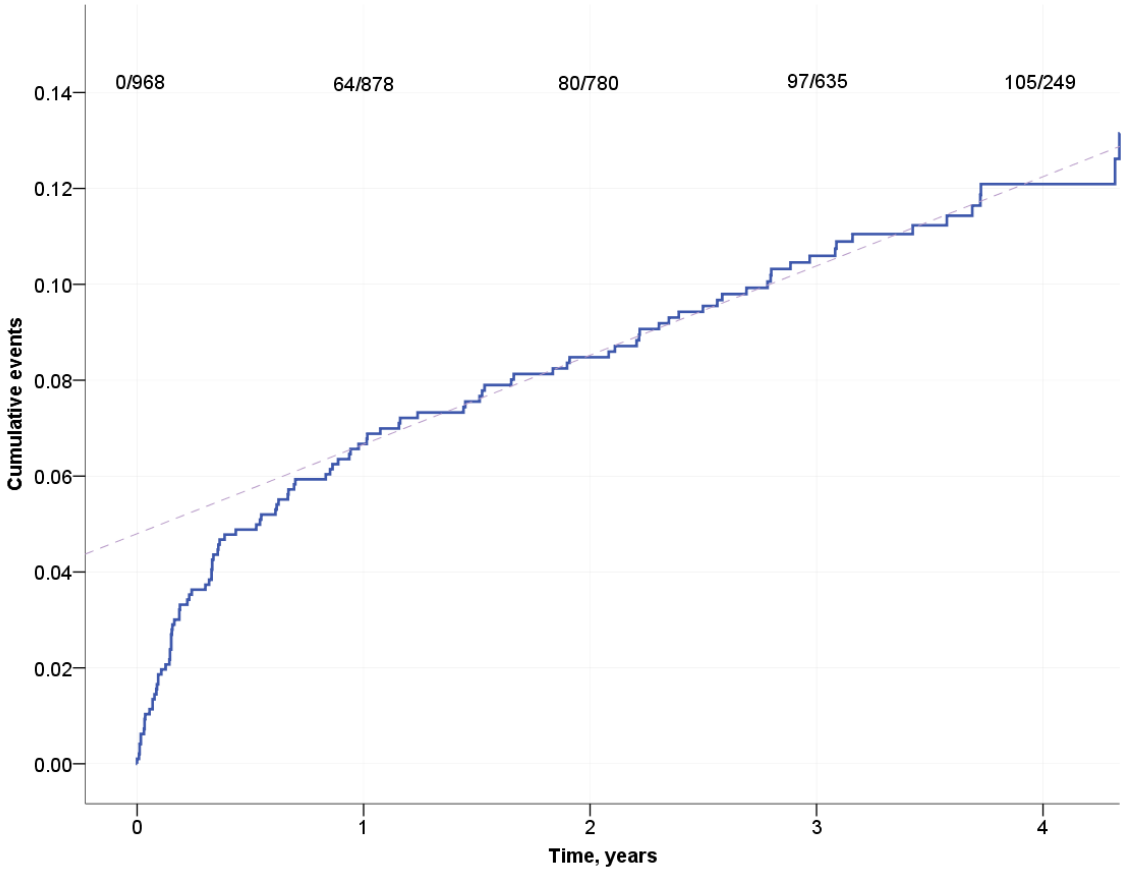



**Figure 5-1 KM curve showing cumulative incidence of recurrent ACS over median follow-up; perforated straight reference line added to highlight relatively steady rate after the 1 year point**

## 5.2. Sub-types of recurrent ACS

The following sub-types of recurrent/secondary ACS could be identified based on presentation, clinical impression and subsequent investigations:

- Unstable angina (troponin-negative but symptoms initially thought to be cardiac by admitting medical team)
- NSTEMI
- STEMI
- Out-of-hospital witnessed cardiac arrest

Includes cases of possible, probable or definite stent thrombosis

- Suspected ACS, unable to verify due to inadequate data
- Hospital admission with suspected ACS but thought non-cardiac by admitting medical team

NSTEMI was the commonest clinical syndrome by which patients presented with recurrent ACS, although admissions with NSTEMI were matched in frequency by admissions with chest pain thought (by the admitting medical team) to be of non-cardiac origin. The frequency of sub-types of recurrent ACS over the total follow-up period, including hospital attendances with atypical/suspected non-cardiac symptoms, is illustrated in Figure 5-2.

The event rates for the different sub-types of ACS and ARC-defined stent thrombosis at 30 days, 1 year, and 3.4 year (median follow-up) are shown in Table 5-1. Note that biomarker data is missing for 5 recurrent ACS patients.

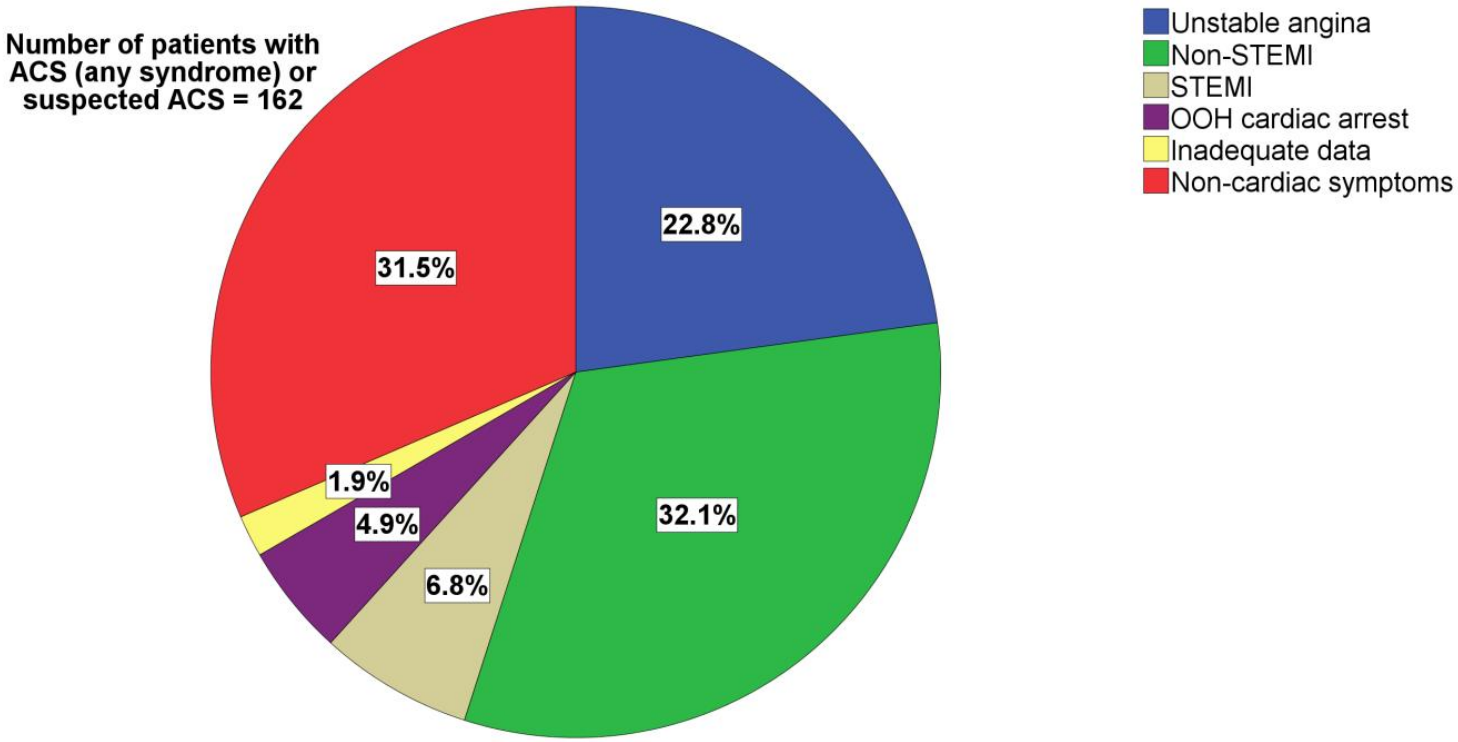



**Figure 5-2 Pie chart showing relative frequency of different sub-types of recurrent ACS over total follow-up; also included are hospital attendances with suspected non-cardiac symptoms**

Notes: (i) Hospital admissions with symptoms suggestive of possible ACS but which were troponin-negative, without dynamic ECG changes, and concluded to be of likely non-cardiac origin by the admitting medical team were not recorded as ACS in the database, nor were they included in any analyses of ACS presented here, although I have included them in this chart for completeness; (ii) hospital admissions in which the predominant clinical features were in keeping with a diagnosis of cardiac failure but without evidence of new acute coronary ischaemia, even if the troponin was mildly elevated, are not recorded as ACS in the database and are not included here; and (iii) although we had a low threshold for categorising troponin elevations as ACS when the clinical team had diagnosed it as such, troponin elevations in acutely ill patients thought secondary to haemodynamic instability from an acute non-coronary medical condition e.g. due to tachy/brady arrhythmias, sepsis, surgery, severe anaemia etc have not usually been recorded as ACS in the database; for example: PAT ID 339 (small troponin rise after presenting with severe bradycardia); PAT ID 135 (small troponin rise after an episode of fast AF following surgery for an ischaemic leg); and PAT ID 280 (small troponin rise after an episode of fast AF following elective hip surgery).

**Table 5-1 The KM estimated cumulative frequency of different sub-types of recurrent ACS at 30 days, 1 year and 3.4 years**

| **Type of ACS event** (events may be included in >1 category) | **30 days** | **1 year** | **3.4 years** |
|---|---|---|---|
| Unstable angina | 2 (0.2%) | 24 (2.5%) | 34 (3.9%) |
| Witnessed out-of-hospital cardiac arrest | 3 (0.3%) | 8 (0.8%) | 8 (0.9%) |
| STEMI | 5 (0.5%) | 8 (0.8%) | 11 (1.3%) |
| NSTEMI | 4 (0.4%) | 22 (2.3%) | 45 (5.3%) |
| Possible stent thrombosis | 0 | 3 (0.3%) | 3 (0.3%) |
| Probable stent thrombosis | 2 (0.2%) | 3 (0.3%) | 3 (0.3%) |
| Definite stent thrombosis | 6 (0.6%) | 8 (0.8%) | 12 (1.3%) |
| Definite or probable stent thrombosis | 8 (0.8%) | 11 (1.1%) | 15 (1.6%) |
| Definite, probable or possible stent thrombosis | 8 (0.8%) | 14 (1.5%) | 18 (2.0%) |
| Total (spontaneous) MI including stent thrombosis | 12 (1.2%) | 37 (3.8%) | 63 (7.3%) |
| All ACS (number = 111, 11.5% of total cohort) | 14 (1.4%) | 64 (6.7%) | 101 (11.2%) |

## 5.3. Aetiology of recurrent ACS

In most cases of recurrent ACS, analysis of the clinical features, the results of repeat coronary angiography, the expert opinion of the arbitration committee and occasionally post-mortem exam results allowed us to arrive at a probable specific cause for the adverse cardiac event. Over long term follow-up, 82 (73.9%) of the patients who experienced a recurrent ACS of any type, and 53 (75.7%) of the patients who had a recurrent MI, underwent at least 1 further coronary angiogram. Patients with recurrent ACS were often found to have further obstructive coronary disease at angiography, either in the index ‘target’ lesion or a ‘de novo’ lesion.

### 5.3.1. Recurrent ACS: Unstable angina

There were 37 cases of UA during follow-up (3.8% of the total cohort); 27 were investigated by coronary angiography. Of these, 24 were found to have obstructive coronary disease of which 11 patients had restenosis (PAT ID: 133, 273, 286, 353, 543, 551, 567, 584, 766, 906 and 1001) and 13 were found to have patent stents but severe non-target lesion disease (PAT ID: 69, 232, 235, 338, 585, 589, 645, 674, 748, 798, 850, 909 and 920). No significant focal disease was found in a further 3 patients (PAT

ID: 634, 883 and 907). Of the 10 patients who were admitted with suspected UA but did not undergo further angiography, 5 were subsequently re-classified as probable non-cardiac pain by a cardiologist, 3 after negative non-invasive functional testing (PAT ID: 622, 111 and 1036) and 2 without a functional test (PAT ID: 728, 1019). 2 patients were thought unsuitable for further invasive investigation, due in 1 case to multiple co-morbidities (PAT ID: 780) and in the other to chronic alcohol dependence (PAT ID 889); and in 3 the reason for no further angiogram is unclear or information is missing (PAT ID: 152, 428 and 562).

### 5.3.2. Recurrent ACS: NSTEMI

There were 52 patients (5.4% of the total cohort) who had a NSTEMI during follow-up of whom the majority, 42 patients, were investigated by invasive angiography. Of these, 22 (42.3% of patients with recurrent NSTEMI) were found to have severe restenosis of at least 1 stent which had been implanted at the index procedure (PAT ID: 60, 70, 102, 154, 162, 172, 184, 253, 311, 383, 399, 403, 436, 590, 613, 628, 677, 757, 831, 942, 993 and 1049). A further 10 patients (19.2%) were found to have patent stents but severe disease elsewhere: 9 of these had ‘de novo’ (new) disease (PAT ID: 14, 95, 264, 314, 336, 392, 662, 740 and 903) and 1 had restenosis of a previous non- study stent (PAT ID: 997). There was 1 patient who had angiographic evidence of a fractured stent without restenosis (PAT ID 254). In the remaining 9 patients with recurrent NSTEMI undergoing invasive investigation (17.3%), their stents were found to be patent and no other severe disease or other angiographic abnormality was identified (PAT ID: 42, 58, 161, 197, 282, 366, 483, 811 and 1029).

A further 10 patients (19.2% of patients with recurrent NSTEMI) were managed medically without undergoing angiography. Of these, 2 were thought unsuitable for invasive investigation due to severe co-morbidities (PAT ID: 171 (lung cancer) and 186 (bilateral subdural haematoma); 1 patient declined angiography (PAT ID: 595); 1 patient had a negative functional test (PAT ID: 665); 1 patient was critically ill on admission with metabolic acidosis and died shortly after (PAT ID: 864); and in 4 patients the reason for medical rather than invasive management was unclear from the medical records (PAT ID: 198, 275, 803 and 856). There was missing data for 1 patient due to inability to obtain medical records from the district general hospital (PAT ID: 115).

Of interest 1 patient (PAT ID: 154) had both a STEMI due to stent thrombosis and a NSTEMI due to restenosis at different times during follow-up. Another patient (PAT ID: 997) who had a NSTEMI during follow-up was found to have restenosis of a non-study stent and had a large thrombus aspirated from this stent during his repeat PCI

procedure, so has been classified as having both restenosis and definite stent thrombosis (none of the other patients with stent thrombosis were thought to have significant restenosis).

### 5.3.3. Recurrent ACS: STEMI

12 patients (1.2% of the total cohort) had a recurrent ACS presenting with ST segment elevation and all underwent emergent angiography with a view to PCI if required. Of these, 10 patients had a definite angiographic stent thrombosis treated by PPCI (PAT ID: 19, 154, 229, 278, 405, 447, 466, 563, 764 and 972). The other 2 patients had ST elevation due to thrombosis of a non-target vessel (PAT ID: 34 and 72). As mentioned in the previous section, 1 patient (PAT ID 154) also had a NSTEMI (at another time) due to restenosis.

### 5.3.4. Recurrent ACS: Out-of-hospital cardiac arrest

A witnessed unexpected out-of-hospital cardiac arrest occurred in 8 patients (0.8%), 6 of whom died within 24 hours. There was 1 case of definite stent thrombosis confirmed by post mortem (P.M.) examination, and 4 other fatal cases were classified as probable or possible stent thrombosis but P.M. examination had not been performed. 2 patients were found to have acute thrombosis of a non-target vessel (1 by P.M. examination and 1 by angiography), and the remaining patient had a new diagnosis of a (probably) non-ischaemic cardiomyopathy. All cases of cardiac arrest took place within the first year of PCI. The details are summarised in the Table 5-2.

**Table 5-2 Details of all episodes of out-of-hospital cardiac arrest**

| PAT ID | Details of cardiac arrest |
|---|---|
| 182 | Arrested and died unexpectedly at home 3 days post PCI. Coroner's P.M. conclusion: definite stent thrombosis. |
| 299 | VF arrest 81 days post PCI, survived; angiogram showed fully patent stent in circumflex, but a newly occluded diagonal branch of the LAD, managed medically. |
| 334 | Collapsed suddenly at home 10 months post PCI, died later the same day. No P.M. performed. Death certified as (I) IHD; (II) Chronic pulmonary embolic disease. OPERA arbitration committee conclusion: 'possible stent thrombosis'. |
| 412 | Collapsed suddenly in asystole 55 days post PCI, died later same day. Coroner P.M. concluded death due to acute MI: acute thrombotic occlusion of a non-study coronary vessel; the stent was patent. |
| 755 | Collapsed suddenly at home 10 months post PCI; died later the same day in hospital. Non-specific ECG changes. No P.M. performed. Death certified as IHD. OPERA arbitration committee conclusion: 'possible stent thrombosis'. |
| 839 | VF arrest 11 months post PCI, resuscitated and survived; angiogram showed stent widely patent; small troponin rise; found to have severe LV systolic dysfunction, thought to be some form of non-ischaemic cardiomyopathy. |
| 843 | Sudden collapse 1 month post PCI, CPR given but died before reaching hospital, no P.M. performed. OPERA arbitration committee conclusion: 'probable stent thrombosis'. |
| 890 | Collapsed with VF 12 days post PCI, died later the same day; no P.M. Death certified as 'acute left ventricular failure'. OPERA arbitration committee conclusion: 'probable stent thrombosis'. |

## 5.4. Baseline factors associated with recurrent ACS

Table 5-3 compares the frequencies of baseline characteristics in patients who experienced no further ACS with those who experienced any recurrent ACS (includes recurrent UA, NSTEMI, STEMI, out-of-hospital cardiac arrest and stent thrombosis, but excludes non-cardiac chest pain admissions); and with those who experienced a spontaneous MI (troponin-positive ACS and/or definite, probable or possible stent thrombosis and fatal MI) over long-term follow-up.

There was a trend for patients with recurrent ACS to be older than those without, and patients with a history of tobacco smoking were more likely to experience recurrent ACS than patients who never smoked. Recurrent ACS was more common in those with a prior history of IHD, a history of stroke, those with diabetes and in those with a history of hypertension. Haemoglobin concentration pre-PCI was lower in patients with recurrent ACS, and patients with recurrent MI were more likely to have poor renal function. SYNTAX scores and EuroSCOREs were both higher. (Note: SYNTAX score is also discussed in Section 3.5.14 and baseline clinical characeristics associated with SYNTAX score are listed in Table 3.4). Neither age nor sex were significantly associated with recurrent ACS.

The rates of recurrent ACS and recurrent MI were particularly low in patients presenting originally with STEMI treated by pharmacological thrombolysis followed by convalescent PCI relative to the other clinical syndromes at presentation. Patients originally presenting with NSTEMI had the highest rates. Recurrent ACS and MI rates at 1 year in patients originally presenting with STEMI treated by thrombolysis/convalescent PCI were 1.8% and 0.9%; in patients with unstable angina, 6.0% and 1.7%; in patients receiving PPCI for STEMI, 8.4% and 3.6%; and in patients presenting originally with NSTEMI, 8.2% and 5.2%. Of all recurrent MIs recorded in the study over total follow-up, 80% occurred in patients who originally presented with a NSTEMI.

PCI of more than 1 vessel, implantation of more than 1 stent, PCI of restenotic lesions and heavily calcified lesions and longer stent length were all associated with a greater incidence of recurrent ACS. Recurrent ACS was seen more often in those receiving PCI to the LMS and to the circumflex artery than to the LAD and the RCA. AHA lesion type was not significantly associated with the incidence of recurrent ACS, although there was a trend for more MIs in patients with type C lesions.

Regarding stent type, patients receiving ‘Taxus’ stents had higher rates of recurrent ACS (although note should be made that this was a small subgroup of only 34 patients) and those with ‘Xience’ stents had lower rates. There was no significant difference in the recurrent ACS rates between patients receiving exclusively BMS compared to those receiving exclusively DES.

Neither procedural angiographic nor procedural clinical complications were significantly associated with recurrent ACS. However, the rate of angiographic complications was numerically higher in patients with recurrent MI.

**Table 5-3 Comparison of baseline characteristics in patients experiencing and not experiencing recurrent ACS of any sub-type; and between patients experiencing recurrent MI vs. no recurrent ACS; all events over total follow-up period are included**

| **Characteristic**<br>Binary characteristics shown as Number (%) | **No recurrent ACS**<br>N = 857 | **ACS**<br>N = 111 | **P value** | **MI**<br>N = 71 | **P value** |
|---|---|---|---|---|---|
| *Demographics and clinical syndrome* | | | | | |
| Recruitment in first study phase | 640 (74.7) | 83 (74.8) | 0.98 | 55 (77.5) | 0.58 |
| Mean (SD) age, years | 62.0 (11.9) | 64.1 (12.8) | 0.08 | 65.0 (13.5) | 0.04 |
| Age ≥80 years | 59 (6.9) | 11 (9.9) | 0.25 | 9 (12.7) | 0.07 |
| Female | 217 (25.3) | 25 (22.5) | 0.52 | 19 (27.1) | 0.66 |
| Median (IQR) Body Mass Index | 28 (6) | 28 (6) | 0.56 | 27 (6) | 0.19 |
| Any history of smoking | 576 (67.5) | 91 (82.0) | < 0.01 | 57 (80.3) | 0.04 |
| Family history of IHD, | 427 (51.9) | 63 (60.0) | 0.12 | 37 (56.9) | 0.48 |
| Unstable angina | 107 (12.4) | 19 (17.1) | 0.16 | 8 (11.3) | 0.67 |
| Non-STEMI | 548 (63.9) | 79 (71.2) | 0.13 | 57 (80.3) | <0.01 |
| ST elevation MI: Convalescent PCI | 115 (13.4) | 6 (5.4) | 0.02 | 2 (2.8) | 0.01 |
| ST elevation MI: Primary PCI | 89 (10.4) | 7 (6.3) | 0.18 | 4 (5.6) | 0.21 |
| *Previous medical history* | | | | | |
| Angina | 357 (41.7) | 75 (67.6) | < 0.01 | 46 (64.8) | < 0.01 |
| MI | 183 (21.3) | 44 (39.6) | < 0.01 | 29 (40.8) | < 0.01 |
| PCI | 88 (10.3) | 24 (21.6) | < 0.01 | 12 (16.9) | 0.15 |
| CABG | 57 (6.6) | 15 (13.5) | < 0.01 | 9 (12.7) | 0.07 |
| Previous IHD, any | 384 (44.8) | 78 (70.3) | < 0.01 | 49 (69.0) | < 0.01 |
| Diabetes mellitus | 130 (15.2) | 29 (26.1) | < 0.01 | 19 (26.8) | 0.02 |
| Hypertension | 418 (48.7) | 71 (64) | < 0.01 | 43 (60.6) | 0.07 |
| Hyperlipidaemia | 478 (55.9) | 67 (60.9) | 0.32 | 43 (61.4) | 0.40 |
| Respiratory disease | 83 (9.7) | 20 (18.0) | < 0.01 | 13 (18.3) | 0.03 |
| Stroke | 38 (4.4) | 11 (9.9) | 0.01 | 6 (8.5) | 0.16 |
| Peripheral vascular disease | 38 (4.4) | 5 (4.5) | 0.97 | 3 (4.2) | 0.92 |

| | | | | | |
|---|---|---|---|---|---|
| Untreated aortic valve disease | 9 (1.0) | 3 (2.7) | 0.14 | 3 (4.2) | 0.02 |
| Heart failure | 11 (1.3) | 4 (3.6) | 0.06 | 3 (4.2) | 0.06 |
| | | | | | |
| *Blood results (pre-procedural)* | | | | | |
| Mean (SD) eGFR, ml/min/1.73m$^2$ | 75 (36) | 71 (37) | 0.21 | 70 (43) | 0.10 |
| eGFR < 60 ml/min/1.73m$^2$ | 253 (30.2) | 40 (37.0) | 0.15 | 30 (44.0) | 0.02 |
| Mean (SD) Hb concentration, g/dL | 14.0 (1.6) | 13.5 (1.9) | 0.05 | 13.3 (2.0) | 0.03 |
| Hb <10g/dL | 12 (1.5) | 5 (5.1) | 0.02 | 4 (6.1) | 0.01 |
| Mean (SD) platelet count, x10$^9$/L | 248 (86) | 249 (79) | 0.89 | 249 (114) | 0.84 |
| Mean white cell count, x10$^9$/L | 8.8 (2.9) | 9.2 (3.2) | 0.12 | 9.1 (2.7) | 0.17 |
| | | | | | |
| *Risk scores* | | | | | |
| Median (IQR) EuroSCORE I | 3.0 (4.4) | 4.8 (8.0) | < 0.01 | 5.1 (9.2) | < 0.01 |
| Lowest EuroSCORE quartile (≤1.9) | 228 (26.6) | 19 (17.1) | 0.03 | 11 (15.5) | 0.04 |
| Highest EuroSCORE quartile (≥6.4) | 204 (23.8) | 39 (35.1) | < 0.01 | 30 (42.3) | < 0.01 |
| Median (IQR) SYNTAX score | 8.0 (11.0) | 12.0 (12.1) | < 0.01 | 12.5 (12.5) | < 0.01 |
| Lowest SYNTAX score quartile (≤4.0) | 223 (26.6) | 13 (11.8) | < 0.01 | 8 (11.3) | < 0.01 |
| Highest SYNTAX score quartile (≥15.5) | 194 (23.1) | 40 (36.4) | < 0.01 | 27 (38.0) | < 0.01 |
| | | | | | |
| *Medication* | | | | | |
| Aspirin | 828 (96.8) | 110 (99.1) | 0.18 | 70 (98.6) | 0.44 |
| Clopidogrel | 807 (94.2) | 108 (97.3) | 0.17 | 69 (97.2) | 0.31 |
| Prasugrel | 52 (6.1) | 3 (2.7) | 0.15 | 2 (2.8) | 0.28 |
| Dual antiplatelet therapy | 827 (96.8) | 110 (99.1) | 0.18 | 70 (98.6) | 0.44 |
| $P2Y_{12}$ inhibitor <24 hours before PCI | 111 (12.9) | 13 (11.7) | 0.72 | 8 (11.3) | 0.68 |
| Heparin | 736 (86.1) | 98 (88.3) | 0.53 | 62 (87.3) | 0.80 |
| IIbIIIa inhibitor | 267 (31.1) | 45 (40.5) | 0.05 | 31 (43.7) | 0.03 |
| Bivalirudin | 132 (15.5) | 14 (12.7) | 0.45 | 9 (12.9) | 0.57 |
| Thrombolysis on admission | 72 (8.4) | 6 (5.4) | 0.28 | 2 (2.8) | 0.09 |

| | | | | | |
|---|---|---|---|---|---|
| *Procedural characteristics* | | | | | |
| Arterial access: radial route | 181 (21.1) | 24 (21.6) | 0.90 | 13 (18.3) | 0.53 |
| Worst lesion treated: Type A | 125 (14.7) | 14 (12.8) | 0.61 | 10 (14.5) | 1.0 |
| Worst lesion treated: Type B1 | 211 (24.9) | 22 (20.2) | 0.29 | 11 (15.9) | 0.09 |
| Worst lesion treated: Type B2 | 198 (23.2) | 24 (22.0) | 0.78 | 15 (21.7) | 0.78 |
| Worst lesion treated: Type C | 318 (37.3) | 49 (45.0) | 0.12 | 33 (47.8) | 0.08 |
| Number vessels treated: 1 | 686 (80.1) | 78 (70.3) | 0.02 | 51 (71.8) | 0.12 |
| Number vessels treated: 2 or 3 | 171 (19.9) | 33 (29.7) | 0.02 | 20 (28.2) | 0.12 |
| Number vessels treated: 3 | 12 (1.4) | 1 (0.9) | 0.67 | 1 (1.4) | 0.96 |
| LMS treated | 17 (2.0) | 8 (7.2) | < 0.01 | 6 (8.5) | < 0.01 |
| LAD treated | 415 (48.5) | 52 (46.8) | 0.75 | 31 (43.7) | 0.41 |
| Circumflex treated | 253 (29.5) | 45 (40.5) | 0.02 | 29 (40.8) | 0.06 |
| Right coronary artery | 356 (41.5) | 38 (34.2) | 0.14 | 24 (33.8) | 0.23 |
| Restenotic lesion | 27 (3.1) | 9 (8.1) | < 0.01 | 5 (7.0) | 0.13 |
| Bypass graft lesion | 28 (3.3) | 7 (6.3) | 0.12 | 3 (4.2) | 0.74 |
| Ostial lesion | 52 (6.1) | 8 (7.2) | 0.64 | 4 (5.6) | 0.84 |
| Bifurcation lesion | 322 (37.6) | 44 (39.6) | 0.67 | 30 (42.3) | 0.43 |
| Chronic total occlusion | 28 (3.3) | 1 (0.9) | 0.17 | 1 (1.4) | 0.41 |
| Heavily calcified lesion | 148 (17.2) | 34 (30.6) | < 0.01 | 22 (31.0) | < 0.01 |
| Lesion with angiographic thrombus | 217 (25.3) | 27 (24.3) | 0.83 | 15 (21.1) | 0.42 |
| Rotational atherectomy | 3 (0.3) | 1 (0.9) | 0.39 | 1 (1.4) | 0.18 |
| TIMI flow <III pre-PCI, any treated lesion | 39 (4.5) | 3 (2.7) | 0.37 | 1 (1.4) | 0.21 |
| Median (IQR) width of smallest stent, mm | 3.0 (0.8) | 3.0 (0.3) | 0.88 | 3.0 (0.3) | 0.47 |
| At least 1 small stent implanted (≤2.5mm) | 195 (23.2) | 25 (22.5) | 0.88 | 16 (22.5) | 0.90 |
| Median (IQR) total length all stents, mm | 24 (23) | 32 (22) | 0.04 | 32 (26) | 0.15 |
| 2 or more stents implanted | 231 (27.5) | 42 (37.8) | 0.02 | 26 (36.6) | 0.12 |
| DES only | 469 (54.8) | 58 (52.3) | 0.62 | 38 (53.5) | 0.88 |
| BMS only | 334 (38.9) | 46 (41.4) | 0.61 | 30 (42.3) | 0.60 |

| | | | | | |
|---|---|---|---|---|---|
| Combination of DES and BMS | 37 (4.3) | 7 (6.3) | 0.34 | 3 (4.2) | 0.89 |
| No stent, POBA only | 12 (1.4) | 0 | 0.21 | 0 | 0.33 |
| 1st generation DES only | 125 (14.9) | 20 (18.0) | 0.39 | 15 (21.1) | 0.15 |
| 2nd generation DES only | 330 (39.4) | 30 (27.0) | 0.01 | 20 (28.2) | 0.08 |
| *'Cypher'* DES only | 104 (12.1) | 13 (11.7) | 0.90 | 9 (12.7) | 0.88 |
| *'Taxus'* DES only | 21 (2.4) | 7 (6.3) | 0.02 | 6 (8.5) | < 0.01 |
| *'Xience'* DES only | 250 (29.3) | 20 (18.0) | 0.01 | 12 (16.9) | 0.03 |
| 'Endeavor' DES only | 80 (9.3) | 10 (9.0) | 0.91 | 8 (11.3) | 0.53 |
| Staged PCI | 22 (2.6) | 5 (4.5) | 0.24 | 3 (4.2) | 0.45 |
| | | | | | |
| *Procedural complications* | | | | | |
| Unsuccessful procedure | 25 (2.9) | 2 (1.8) | 0.50 | 2 (2.8) | 0.99 |
| Angiographic complication, any | 107 (12.5) | 15 (13.5) | 0.76 | 12 (16.9) | 0.26 |
| Angiographic localised dissection (UMS) | 38 (4.4) | 6 (5.4) | 0.64 | 4 (5.6) | 0.65 |
| Angiographic trapped side branch (UMS) | 39 (4.5) | 4 (3.6) | 0.65 | 3 (4.2) | 0.93 |
| Other procedural clinical complication (non-haemorrhagic) | 10 (1.2) | 2 (1.8) | 0.57 | 1 (1.4) | 0.90 |

## 5.5. Baseline characteristics independently associated with recurrent ACS

Baseline characteristics associated with recurrent ACS (as univariable characteristics) are shown in Table 5-4. In a model incorporating individual factors without risk scores, only a background of smoking and a previous history of IHD were associated with an increased rate of recurrent ACS; while use of second generation DES and index presentation with STEMI treated by convalescent PCI were associated with a lower rate of recurrent ACS (Table 5-5). In a second model incorporating SYNTAX score, the same results were found with the addition of an independent association of SYNTAX score with recurrent ACS (Table 5-6). In a third model incorporating the SYNTAX and EuroSCOREs, the same results were found again, with EuroSCORE providing only a trend towards an independent association with recurrent ACS (Table 5-7).

**Table 5-4 Characteristics individually associated with recurrent ACS; significance level of P ≤ 0.1**

| Characteristic | Hazard ratio (as univariate) | 95% C.I. | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Age, per year | 1.02 | 1.00 | 1.03 | 0.04 |
| History of tobacco smoking | 2.08 | 1.28 | 3.37 | < 0.01 |
| Presentation with STEMI: convalescent PCI | 0.36 | 0.16 | 0.82 | 0.02 |
| PMH of IHD | 2.77 | 1.84 | 4.16 | < 0.01 |
| PMH of Diabetes | 2.00 | 1.31 | 3.06 | < 0.01 |
| PMH of Hypertension | 1.82 | 1.23 | 2.68 | < 0.01 |
| PMH of respiratory disease | 1.94 | 1.19 | 3.14 | < 0.01 |
| PMH of stroke | 2.44 | 1.31 | 4.55 | < 0.01 |
| PMH of heart failure | 3.23 | 1.19 | 8.77 | 0.02 |
| Haemoglobin concentration pre-procedure, per g/dL | 0.85 | 0.76 | 0.95 | < 0.01 |
| PCI to more than 1 vessel | 1.68 | 1.12 | 2.53 | < 0.01 |
| PCI to left main stem | 4.03 | 1.96 | 8.28 | < 0.01 |
| PCI to circumflex artery | 1.56 | 1.09 | 2.32 | 0.02 |
| PCI to restenotic lesion | 2.58 | 1.30 | 5.10 | < 0.01 |

| | | | | |
|---|---|---|---|---|
| PCI to heavily calcified lesion | 2.04 | 1.37 | 3.06 | < 0.01 |
| 2 or more stents implanted | 1.58 | 1.07 | 2.31 | 0.02 |
| Only 2nd generation DES used | 0.66 | 0.44 | 1.01 | 0.06 |
| Only *Taxus* DES used | 1.98 | 0.92 | 4.27 | 0.08 |
| Risk scores | | | | |
| EuroSCORE, per point | 1.05 | 1.03 | 1.06 | < 0.01 |
| SYNTAX score, per point | 1.04 | 1.02 | 1.06 | < 0.01 |

**Table 5-5 Results of Cox regression analysis for characteristics independently associated with recurrent ACS; Model 1, without risk scores**

| Characteristic | Adjusted HR | 95% Confidence Interval | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Only 'second generation' DES inserted | 0.51 | 0.31 | 0.83 | < 0.01 |
| History of smoking | 2.31 | 1.38 | 3.87 | < 0.01 |
| PMH of IHD | 2.09 | 1.31 | 3.32 | < 0.01 |
| STEMI with convalescent PCI | 0.35 | 0.13 | 0.97 | 0.04 |
| PCI to LMS | 2.33 | 0.91 | 5.98 | 0.08 |
| Age per year | 1.00 | 0.98 | 1.02 | 0.85 |
| PMH of diabetes | 1.21 | 0.26 | 2.04 | 0.48 |
| PMH of hypertension | 1.16 | 0.75 | 1.79 | 0.52 |
| PMH of chronic airways disease | 1.17 | 0.65 | 2.08 | 0.61 |
| PMH of stroke | 1.63 | 0.81 | 3.30 | 0.17 |
| PMH of heart failure | 1.82 | 0.57 | 5.82 | 0.32 |
| Pre-procedural haemoglobin, per g/dL | 0.95 | 0.83 | 1.08 | 0.44 |
| PCI to more than 1 vessel | 0.92 | 0.44 | 1.96 | 0.84 |
| PCI to circumflex artery | 1.33 | 0.85 | 2.07 | 0.21 |
| PCI to restenotic lesion | 1.89 | 0.85 | 4.19 | 0.12 |
| PCI to heavily calcified lesion | 1.50 | 0.89 | 2.54 | 0.13 |
| More than 1 stent inserted | 1.17 | 0.62 | 2.21 | 0.64 |

**Table 5-6 Results of Cox regression analysis for characteristics independently associated with recurrent ACS; Model 2, incorporating SYNTAX score**

| Characteristic | Adjusted HR | 95% Confidence Interval | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| SYNTAX score, per point | 1.032 | 1.011 | 1.052 | < 0.01 |
| PMH of IHD | 2.026 | 1.275 | 3.218 | < 0.01 |
| History of smoking | 2.479 | 1.476 | 4.162 | < 0.01 |
| Only second generation DES inserted | 0.537 | 0.332 | 0.868 | 0.01 |
| STEMI with convalescent PCI | 0.340 | 0.123 | 0.936 | 0.04 |
| PMH of diabetes | 1.298 | 0.781 | 2.159 | 0.34 |
| PMH of hypertension | 1.239 | 0.803 | 1.911 | 0.33 |
| PMH of chronic airways disease | 1.327 | 0.761 | 2.316 | 0.32 |
| PMH of stroke | 1.724 | 0.845 | 3.515 | 0.13 |
| PMH of heart failure | 1.590 | 0.511 | 4.946 | 0.42 |
| Pre-procedural haemoglobin, per g/dL | 0.956 | 0.841 | 1.086 | 0.49 |
| Age per year | 0.993 | 0.974 | 1.013 | 0.50 |

**Table 5-7 Results of Cox regression analysis for characteristics independently associated with recurrent ACS; Model 3, incorporating SYNTAX score and EuroSCORE**

| Characteristic | Adjusted HR | 95% Confidence Interval | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| History of smoking | 2.513 | 1.5 | 4.21 | < 0.01 |
| Only second generation DES inserted | 0.527 | 0.326 | 0.852 | < 0.01 |
| PMH of IHD | 1.979 | 1.243 | 3.149 | < 0.01 |
| SYNTAX score, per point | 1.026 | 1.005 | 1.047 | 0.02 |
| STEMI with convalescent PCI | 0.35 | 0.127 | 0.962 | 0.04 |
| EuroSCORE, per point | 1.022 | 0.998 | 1.048 | 0.08 |
| PMH of diabetes | 1.347 | 0.823 | 2.206 | 0.24 |
| PMH of hypertension | 1.173 | 0.762 | 1.805 | 0.47 |
| Pre-procedural haemoglobin, per g/dL | 0.967 | 0.859 | 1.087 | 0.57 |

## 5.6. SYNTAX score and adverse events

There was a strong association between angiographic SYNTAX score and the frequency of recurrent ACS. Figure 5-3 shows Kaplan-Meier (unadjusted) estimates of the cumulative incidence of ACS in patients stratified by SYNTAX score quartile. Much of the difference in outcome between quartiles occurs within the first year, although the first and second quartiles continue to diverge from the third and fourth quartiles over the longer term. SYNTAX score was also strongly associated with rate of cardiac death long-term, shown in Figure 5-4. Figure 5-5 is a graph of the incidence of total combined adverse events over long-term follow-up by SYNTAX score, showing an approximately linear relationship.

Note that SYNTAX score was different in patients with a number of baseline clinical characteristics also associated with recurrent ACS. Details of these differences are found in Section 3-5-13 and Table 3-4.

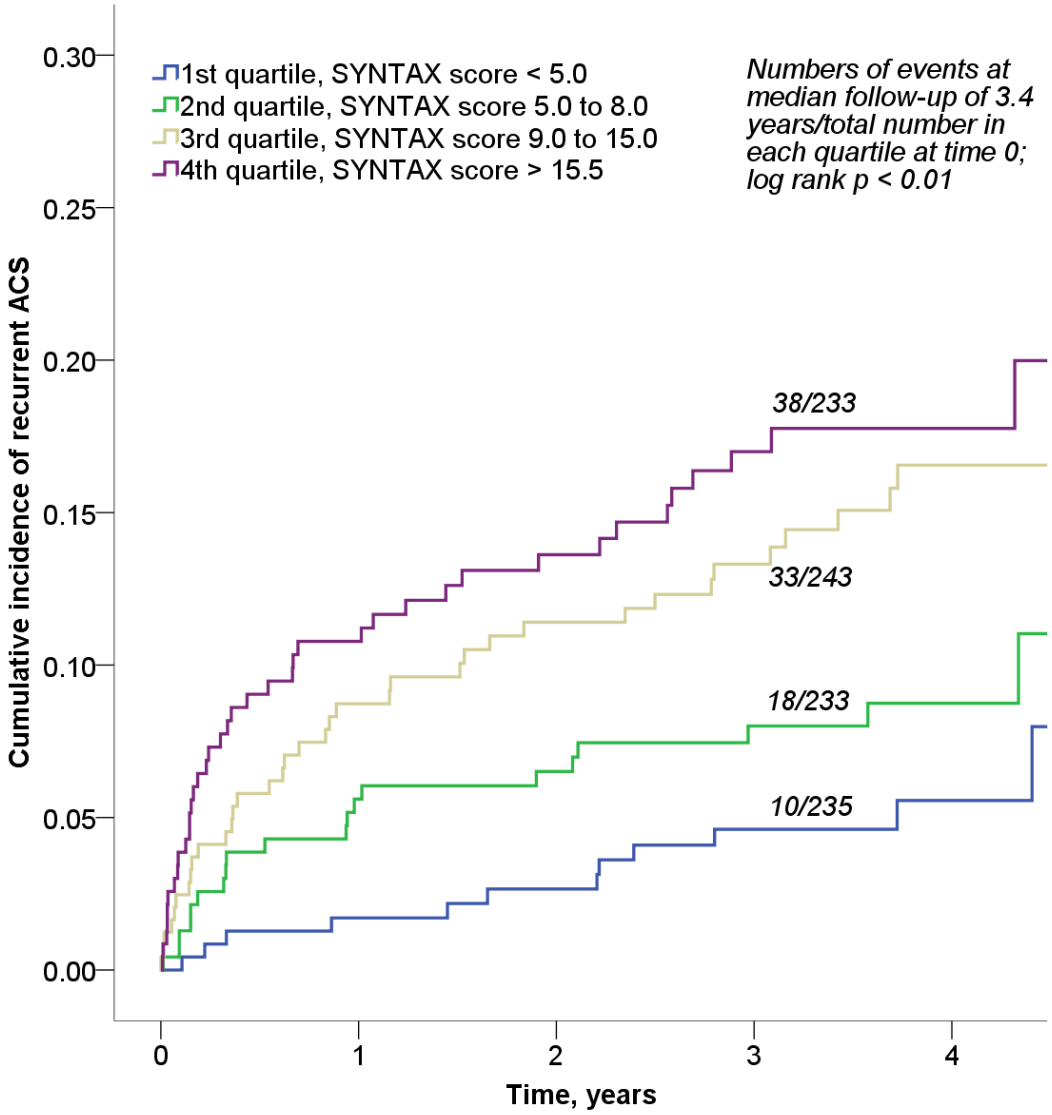



**Figure 5-3 Unadjusted cumulative incidence of recurrent ACS over median follow-up, stratified by SYNTAX score quartile**

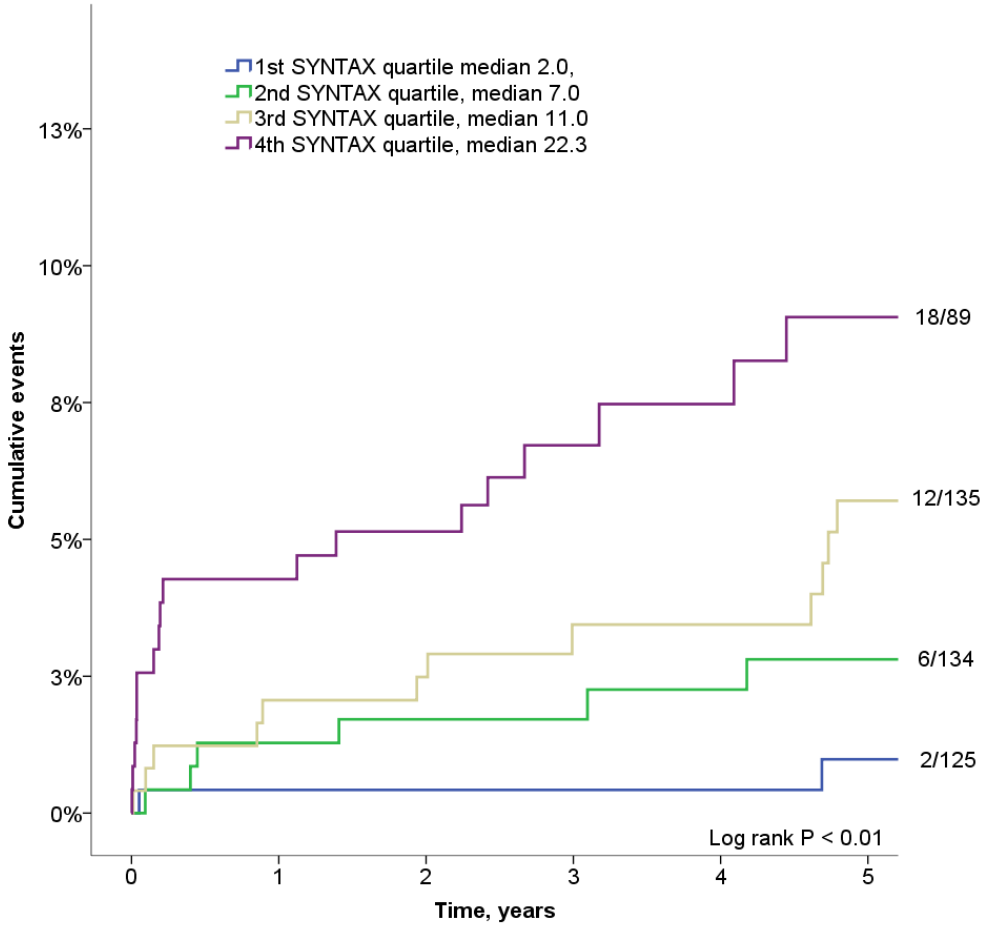



**Figure 5-4 KM estimates of cardiac death rate for the entire cohort over long-term follow-up, stratified by SYNTAX score quartile; event rates at 5.4 years (median follow-up) shown**

## 5.7. Recurrent ACS: stent thrombosis

Over long term follow-up there were 18 cases of stent thrombosis (12 definite, 3 probable and 3 possible). The median (IQR) time from PCI to ST was 40 (390) days, with a range of 1018 days, a minimum time of 3 days and a maximum time of 1021 days. Using the 'early (< 30 days), late (30 days to 1 year) and very late (> 1 year)' classification, there were 8 cases of early, 6 late and 4 very late ST. There was not a single case of acute (< 24 hours) stent thrombosis.

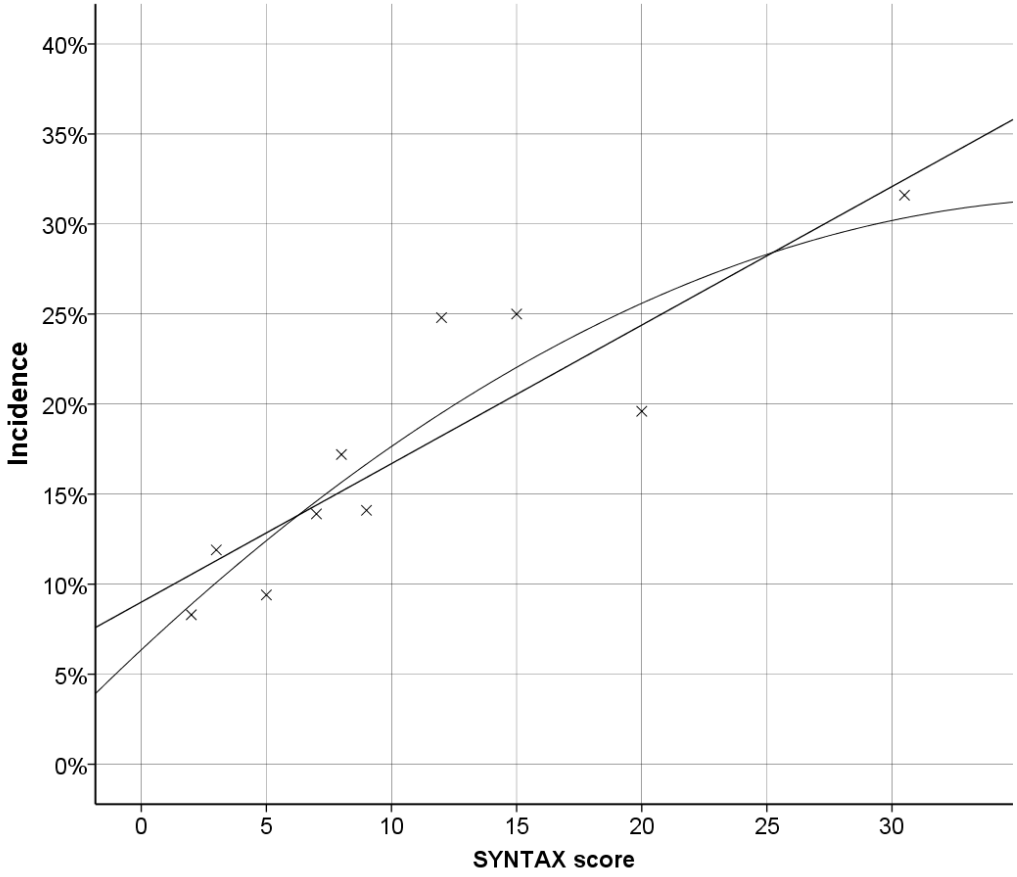



**Figure 5-5 Graph of incidence of combined adverse events (MI, further unplanned revascularisation or death) according to SYNTAX score (groups composed of deciles of SYNTAX score); automatic straight and quadrantic lines of best fit added**

### 5.7.1. Clinical presentation and subsequent outcome of stent thrombosis

Stent thrombosis (any type) presented as several different clinical syndromes: STEMI (9 cases), out-of-hospital cardiac arrest (5 cases) and NSTEMI (3 cases). In the remaining case of probable ST there was insufficient data to classify the clinical syndrome. 11 patients with ST (any type) underwent coronary angiography and further PCI; 7 did not undergo further angiography either because they collapsed and died out-of-hospital, or they survived to reach hospital but were too unwell for angiography. Only 1 of these 7 patients underwent a P.M. examination to confirm the ST; hence the others have been classified as ‘probable’ or ‘possible’. Table 5-8 summarises the clinical details of the patients with definite or suspected ST.

### 5.7.2. Associations of stent thrombosis with baseline characteristics

There was a trend for a higher rate of stent thrombosis (ST) in patients with a background of smoking (2.4% vs. 0.7%, p = 0.07) and with DM (3.8% vs. 1.5%, p = 0.05). There were no significant differences in age (64.1 years vs. 62.2 years, p = 0.51) or gender (22.2% vs. 25.1% female) between patients with and without ST. Regarding clinical syndrome at index presentation, 15 of the ST cases occurred in patients originally presenting with NSTEMI, 2 with STEMI and 1 with UA, a non-significant difference. There was no change over time in the rate of ST: there were 12 (1.7%) cases in the first recruitment phase and 6 (2.4%) in the second, p = 0.43.

Regarding angiographic characteristics, ST occurred in patients whose worst AHA classification lesion type was A, B1, B2 and C in 2 (1.4%), 4 (1.7%), 4 (1.8%) and 8 (2.2%) cases respectively, a non-significant difference. Median (IQR) SYNTAX scores were higher in those with ST (12.0 (10.6) vs. 8.5 (11.0), p = 0.04). ST was more common in patients with heavily calcified lesions (8 cases, 4.4% vs. 1.3%, p < 0.01) and there was a trend for more ST in patients having PCI of thrombotic lesions (8 cases, 3.3% vs. 1.4%, p = 0.06). There was no difference in the rate of ST in patients with and without bifurcation lesions however (7 cases, 1.9% vs. 1.8%, p = 0.92).

Regarding procedural characteristics, ST was more common following a procedural angiographic complication (6 cases, 4.9% vs. 1.4%, p < 0.01), 3 in procedures complicated by localised dissection (6.8% vs. 1.6%, p = 0.01) and 3 with angiographic no-reflow (13.0% vs. 1.6%, p < 0.01). Trapped side branches and non-angiographic procedural complications were not associated with ST. Note that in most cases of ST there was no obvious angiographic complication: in 12 of the 18 cases of ST there were apparently good angiographic results. Median total stent length was non-significantly greater in those with ST, 33mm vs. 24mm, p = 0.3. There was no difference in the rate of ST between patients with BMS vs. DES, or between patients with first vs. second generation DES.

**Table 5-8 Table showing clinical details of each case of stent thrombosis**

| PAT ID | Age at index PCI | ARC class | Stent type | Vessel | Procedural details | Time from PCI to ST, days | Clinical presentation of ST | Further PCI | Treatment and outcome | Time from ST to death, days |
|---|---|---|---|---|---|---|---|---|---|---|
| 19 | 46 | Definite | 3 Cypher stents (3mm x 23mm; 3mm x 13mm; 3mm x 13mm) | LAD | Long calcified bifurcation lesion; proximal dissection covered with extra stent | 4 (early ST) | STEMI | Yes | Anterior STEMI, given thrombolysis with TNK followed by 'rescue' angioplasty; cardiogenic shock; transferred to a heart transplant centre for assessment but died after a cardiac arrest. | 4 |
| 154 | 66 | Definite | Cypher (3.5mm x 23mm) | RCA | Heavily calcified lesion; no procedural complications. Had LAD PCI at same sitting. | 982 (very late ST) | STEMI | Yes | ST was the second ACS experienced by this patient since OPERA study enrolment. ST presented with inferior STEMI 2 weeks after a fem-pop bypass; RCA occluded by thrombus, aspirated and further stent inserted | Alive |
| 182 | 80 | Definite | BMS (3mm x 30mm; 2.75mm x 8mm; 3mm x 9mm) | LAD | Complicated by local dissection, required 3 layers of stent to seal; had PCI to Cx and RCA at same sitting. | 3 (early ST) | OOH cardiac arrest | No | Cardiac arrest and death at home, coroner's P.M. confirmed acute thrombosis of LAD stent. | 0 |
| 229 | 57 | Definite | Cypher (2.25mm x 23mm) | Diagonal | Uncomplicated; had PCI to RCA at same sitting. | 529 (very late ST) | STEMI | Yes | PPCI, treated by POBA, no further problems. | Alive |
| 278 | 38 | Definite | Endeavor (3mm x 15mm) | LAD | Uncomplicated | 68 (late ST) | STEMI | Yes | PPCI; treated by BMS deployed within thrombosed stent. Died suddenly after a cardiac arrest 3 months later; cause of death: recurrent ST in the same stent diagnosed at P.M. | 95 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 405 | 68 | Definite | BMS (3.5mm x 20mm; 3.5mm x 24mm) | SVG to OM | Initially slow flow in SVG but had improved by the end of the procedure | 6 (early ST) | STEMI | Yes | PPCI; had thrombus aspiration followed by additional BMS; no further problems. | Alive |
| 447 | 72 | Definite | BMS (4.0mm x 12mm to ostium; 3mm x 24mm and 3mm x 12mm to mid vessel) | RCA | Uncomplicated; ostium and mid RCA bifurcation treated. | 12 (early ST) | STEMI | Yes | PPCI complicated by catheter dissection to LMS during the diagnostic angiogram which required PCI; and then had thrombus aspiration to thrombosed RCA stent. Referred for CABG but fell and broke leg while awaiting surgery; died of E. coli sepsis. | 260 |
| 466 | 63 | Definite | BMS (2.75mm x 14mm) | Cx | Vessel completely occluded at start, TIMI 3 at finish; uncomplicated. | 357 (late ST) | STEMI | Yes | PPCI, new Xience stents to occluded Cx, good result and no further problems. | Alive |
| 563 | 49 | Definite | Cypher (2.5mm x 23mm) | Cx | Index lesion was a possible ST, possible restenosis. Thrombus visible, TIMI 2 at start, good flow at finish. Uncomplicated. | 1021 (very late ST) | STEMI | Yes | Had PCI to Cx with thrombus aspiration and new Cypher stent, good result. | Alive |
| 764 | 63 | Definite | BMS (2.25mm x 18mm) | RCA | Uncomplicated, Type A lesion | 4 (early ST) | STEMI | Yes | PPCI, further stent to RCA. Went on to have CABG for 3 vessel disease. Subsequently has had further PCI to a degenerated SVG. | Alive |
| 972 | 61 | Definite | Xience (2.25mm x 12mm; 2.25mm x 23mm) | Cx | Complex bifurcation lesion, heavy calcification, TIMI 2 flow at start, good flow at finish, uncomplicated | 11 (early ST) | STEMI | Yes | Delayed presentation after 48 hours of chest pain. Presented in pulmonary oedema, had delayed PCI to re-open blocked stent but never recovered. | 2 |
| 997 | 61 | Definite | Xience (3mm x 8mm; 3mm x 8mm) | LAD | Bifurcation lesion, uncomplicated | 670 (very late ST) | NSTEMI | Yes | Presented with a NSTEMI, had severe in-segment (but not in-stent) restenosis of LAD proximal to the stent, with a large clot within the LAD stent which was removed by | Alive |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | aspiration. Further stent to proximal LAD, no further problems. | |
| 826 | 86 | Probable | BMS (4.0mm x 15mm; 3mm x 18mm) | LAD | Proximal and mid-vessel lesions treated, both uncomplicated | 46 (late ST) | New LBBB | No | Presented to hospital critically ill with new LBBB, no cardiac biomarkers were checked and she died shortly after with no P.M. | 25 |
| 843 | 45 | Probable | Xience (4mm x 12mm LAD; 3mm x 18mm Cx) | LAD and/or Cx | Type A lesion in LAD, uncomplicated. C x occluded at start of procedure, good flow at finish, uncomplicated. | 13 (early ST) | OOH cardiac arrest | No | Sudden collapse and death OOH; no P.M. performed. | 0 |
| 890 | 66 | Probable | Xience | LAD | Heavily calcified lesion; uncomplicated. | 12 (early ST) | OOH cardiac arrest | No | Sudden collapse at home, CPR, VF, shocked to asystole. No P.M. performed. | 0 |
| 334 | 83 | Possible | BMS (3mm x 15mm RCA; 3mm x 18mm LAD) | RCA and LAD | RCA lesion heavily calcified, uncomplicated procedure. Had staged PCI to type A LAD lesion 1 week later, uncomplicated. | 311 (late ST) | OOH cardiac arrest | No | Collapsed at home, found by son, CPR, and died before reaching hospital. No P.M. performed. Patient also had pulmonary hypertension secondary to chronic pulmonary embolic disease. | 0 |
| 755 | 75 | Possible | BMS (3.5mm x 18mm) | Cx | Heavy calcification; uncomplicated procedure. | 324 (late ST) | OOH cardiac arrest | No | Collapsed and died suddenly at home, no P.M. performed. | 1 |
| 864 | 74 | Possible | Xience (3mm x 15mm; 3mm x 18mm) | Cx | Occluded vessel, complicated by no-reflow, TIMI 3 at finish | 34 (late ST) | NSTEMI | No | Admitted with acute LVF and metabolic acidosis, died shortly after, no angiogram or P.M. | 0 |

## 5.8. Recurrent ACS and death

The 1 year all-cause mortality and ARC-defined cardiac death rates for the whole cohort were 2.9% and 2.0%. The Kaplan-Meier estimated all-cause and cardiac death rates at median follow-up (5.4 years) were 13.2% and 4.8%. Mortality rate declined over the first 6 months and became fairly constant after 6 months, illustrated in Figure 5-4. Mortality rates stratified by clinical syndrome at presentation are shown in Figure 5-6. Over the first 6 months MI patients have a higher mortality rate than UA patients. Over the longer term, STEMI patients have a lower mortality rate than either NSTEMI or UA patients.

Although patients overall were more likely to die of a non-cardiac than cardiac cause, recurrent ACS was an important cause of death in patients in the OPERA study particularly in the months immediately following PCI. Cardiac death as a proportion of total all-cause mortality before and after 1 year was 68% and 26%. Of note there were 10 deaths directly attributable to possible, probable or definite stent thrombosis, all of which occurred in the first year following PCI. Furthermore, there were 9 deaths certified - usually in the community by a general practitioner - as being caused by 'IHD', but only 1 of these underwent a post-mortem examination (the post mortem showed coronary atherosclerosis but no stent thrombosis). Only 2 of the deaths caused by recurrent MI were confirmed to be due to a non-index lesion. In marked contrast with stent thrombosis, in-stent restenosis, while frequently presenting with recurrent ACS, did not appear to be the direct cause of death in any patient.

The cause of death by ARC category (Cutlip et al, 2007) and specific cause is shown in Table 5-9. Classification was complicated by the frequent co-existence multiple chronic illnesses and the post-mortem rate was low. Furthermore, death was often certified as 'cardiac' when in fact there had been gradual deterioration due to advanced age or a non-cardiac severe illness such as chronic respiratory disease, motor neurone disease or renal failure. Nevertheless I have used the certified cause of death to guide the following categorisation, except in 1 patient who was certified as dying of cardiac failure when in fact the cause of death was more likely end stage pulmonary disease (he had a firm diagnosis of end-stage pulmonary fibrosis and good LV systolic function by echocardiography).

**Table 5-9 Frequency of ARC-defined and specific cause of death**

| **Cause of death (ARC definitions)** Details/specific cause or mode of death | **Number of cases** | **Proportion of total deaths** | **Proportion of total cohort** |
|---|---|---|---|
| Cardiac | 41 | 35.0% | 4.2% |
| Heart failure, any cause | 17 | 14.5% | |
| 'Pump failure', non-valvular cause | 13 | 11.1% | |
| 'Pump failure' due to valvular disease | 4 | 3.4% | |
| Acute MI, total | 21 | 17.9% | |
| MI, stent thrombosis (any type) | 10 | 8.5% | |
| Possible stent thrombosis | 3 | 2.6% | |
| Probable stent thrombosis | 3 | 2.6% | |
| Definite stent thrombosis | 4 | 3.4% | |
| MI, confirmed non-target vessel | 2 | 1.7% | |
| IHD - unspecified | 9 | 7.7% | |
| Upper GI bleeding while on dual antiplatelet | 1 | 0.9% | |
| Contrast nephropathy | 1 | 0.9% | |
| Vascular access site bleeding | 1 | 0.9% | |
| Vascular | 16 | 13.7% | 1.7% |
| Cerebral infarction | 9 | 7.7% | |
| Aortic dissection/aneurysm rupture | 2 | 1.7% | |
| Pulmonary embolism | 2 | 1.7% | |
| Peripheral vascular disease/limb ischaemia | 2 | 1.7% | |
| Intracranial haemorrhage (off dual | 1 | 0.9% | |
| Non-cardiovascular | 57 | 48.7% | 5.9% |
| Metastatic/terminal cancer | 31 | 26.5% | |
| Sepsis | 15 | 12.8% | |
| Advanced lung disease | 2 | 1.7% | |
| End stage renal failure | 2 | 1.7% | |
| End stage liver disease | 2 | 1.7% | |
| Suicide/trauma | 3 | 2.6% | |
| Bowel obstruction | 1 | 0.9% | |
| Dementia | 1 | 0.9% | |
| Data unavailable | 2 | 1.7% | 0.2% |
| Total deaths, all cause | 117 | 100% | 10.3% |

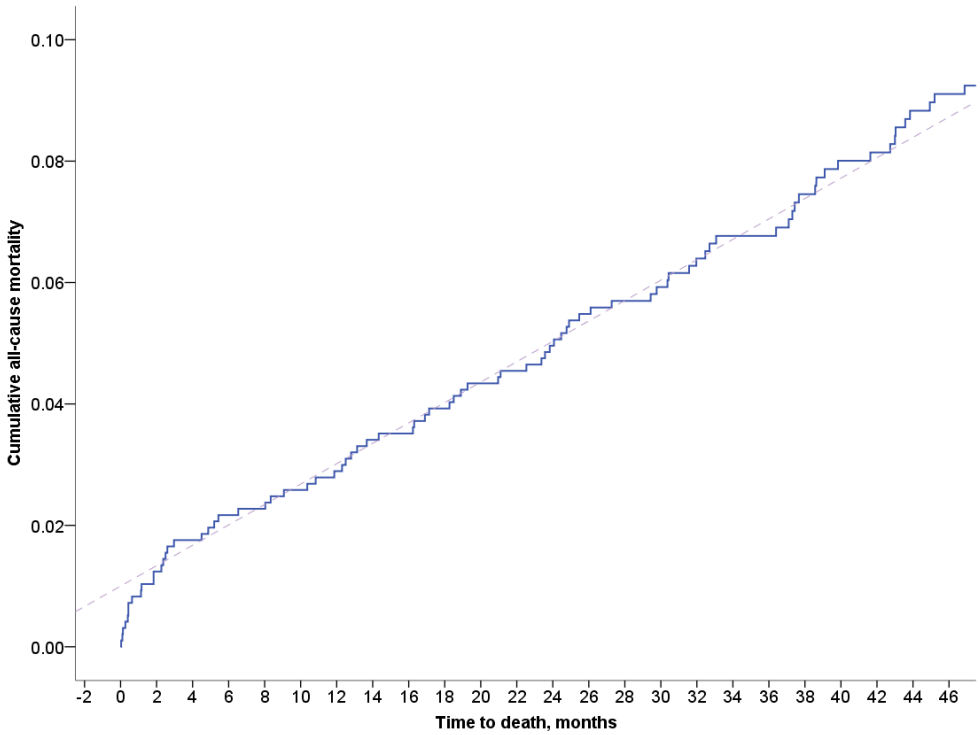



**Figure 5-6 Cumulative all-cause mortality estimate for OPERA patients; a straight (dashed) line added to illustrate constant rate**

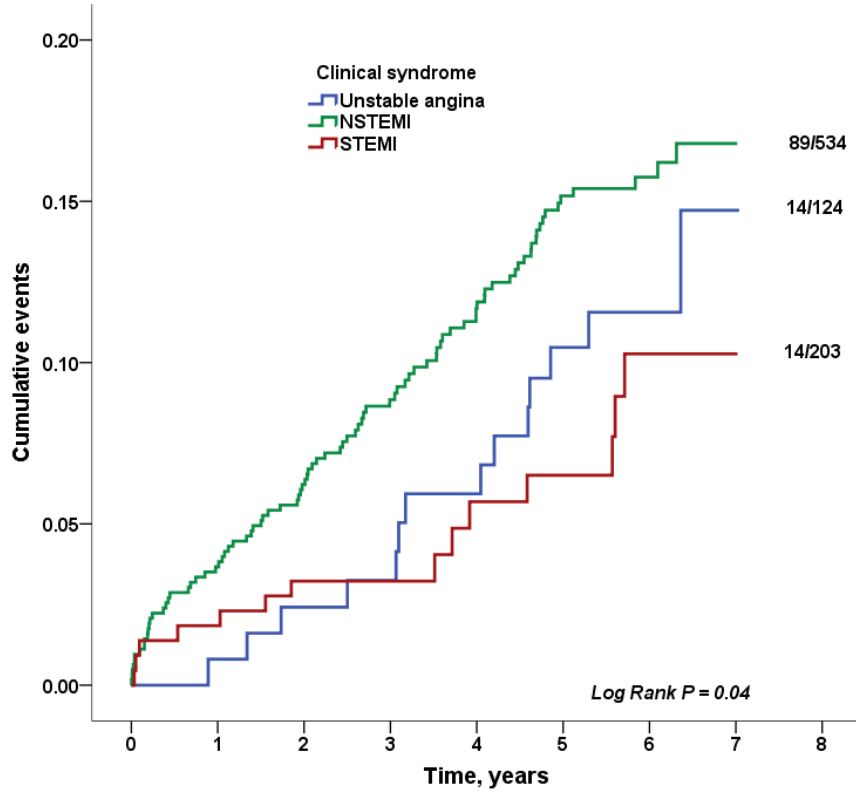



**Figure 5-7 Cumulative all-cause mortality estimates for OPERA patients, stratified by clinical syndrome at presentation**

## 5.9. Summary points and discussion for Chapter 5

- The rate of recurrent ACS was 6.7% at 1 year and then 1.8% per year thereafter.
- The rates of recurrent MI and of definite or probable stent thrombosis at 1 year were 3.8% and 1.1%.
- The rate of recurrent ACS did not appear to decrease over time
- The most common clinical syndrome of recurrent ACS overall was NSTEMI.
- Re-admission to hospital with non-cardiac chest pain was as common as NSTEMI.
- Patients with recurrent ACS presenting either as UA or NSTEMI were usually found to have obstructive disease at repeat angiography, in more than half the cases due to restenosis.
- Patients with recurrent ACS presenting as STEMI or cardiac arrest was usually due to stent thrombosis.
- Stent thrombosis was rare but carried a high risk of death; of the total 18 cases of definite, probable or possible stent thrombosis, only 7 were still alive at the last mortality check.
- Multiple baseline characteristics were associated with recurrent ACS; a history of smoking, prior IHD and having a stent other than 2$^{nd}$ generation DES were independent predictors.
- SYNTAX score was also an independent predictor of recurrent MI; patients with SYNTAX > 8 were at particularly high risk.
- Within 1 year of PCI, deaths were usually of cardiac cause; but after the first year, non-cardiac death was more common.

### 5.9.1. Comparison of recurrent ACS rates with published data

The rate of recurrent ACS is not given in either the PLATO Invasive or TRYTON TIMI 38 studies. The rate of non-fatal MI at 12 months in PLATO Invasive was 6.6% with clopidogrel (5.3% with ticagrelor); and the rate of MI in TRYTON TIMI 38 at 15 months was 9.7% with clopidogrel (7.4% with prasugrel). In both studies the MI rates seem rather high in comparison to OPERA, in which the Kaplan Meier estimated total MI rate at 12 months was only 3.8%, including fatal and non-fatal MI and stent thrombosis. It is possible that this discrepancy is due to differences in the definition of MI. Both former studies were permitted to include MI events based on cardiac enzyme elevation following PCI and neither reported ‘clinical MI’ alone as a study endpoint. In contrast, all OPERA ACS events required clinical judgement of symptoms, ECG

changes, biomarker levels, coronary angiography, functional imaging or post mortem examination; biomarker elevation alone was not considered an MI.

Stent thrombosis rates in OPERA were also low compared to some contemporary data. Definite or probable stent thrombosis rate at 12 months was 1.1% in OPERA compared with 3.0% in the clopidogrel-treated arm of PLATO Invasive (2.2% with ticagrelor). The 15 months rate in TRYTON-TIMI 38 was 2.4% in the clopidogrel arm (1.1% with prasugrel). While this may not seem to be a large difference, stent thrombosis has a very high mortality rate: in OPERA 61% of all deaths at 12 months were in patients who had definite, probable or possible stent thrombosis. Published stent thrombosis rates 2 to 3 times higher than in our study therefore become much more important. The rate in OPERA is much more similar to that from another contemporary study of PCI in ACS patients, the ACUITY trial (Stone et al, 2007), of 1.4%. The relatively high rates in PLATO Invasive and TRYTON TIMI 38 were not commented upon by the authors and are difficult to explain.

### 5.9.2. Change in rate of recurrent ACS over time

As discussed in Chapter 4, improvements in stent technology over the course of OPERA recruitment appeared to lead to a reduction in the need for target lesion revascularisation. However, recurrent ACS rates did not follow the same trend. The rate of ACS within 12 months was 5.5% in the 1$^{st}$ recruitment phase and 9.8% in the 2$^{nd}$, $p = 0.02$. This was seen in patients presenting with all clinical syndromes and may be explained by the increased baseline risk of the cohort over the years, demonstrated by the increase in both median SYNTAX score (8.0 vs. 11.0, $p < 0.01$) and median EuroSCORE (2.8 vs. 4.4, $p < 0.01$) between the 1$^{st}$ and 2$^{nd}$ phases. In patients in the lowest EuroSCORE quartile (EuroSCORE <2.0) there was no significant difference in recurrent ACS rates between the 1$^{st}$ and 2$^{nd}$ phases.

The most likely explanation for the increasing risk profile of the patients in OPERA is that, previously, those deemed to be high risk would often have been managed with medical therapy and not been listed for invasive investigation, particularly the elderly. For example, the proportion of patients aged over 80 years in OPERA was 5.8% in the 1$^{st}$ recruitment phase vs. 11.4% in the 2$^{nd}$; $p < 0.01$. Clearly age and co-morbidities were perceived as less of a barrier to invasive investigation as time went on. The lowering of the threshold for invasive investigation and PCI is illustrated by the BCIS national audit data which shows an uninterrupted year-on-year rise in the number of PCI cases taking place in the UK: approximately 10,000 PCI cases took place in 1991 compared to almost 100,000 in 2014. The number of cases of CABG

by contrast has remained completely static over this time; there is currently a ratio of 5 PCI cases to 1 CABG cases in the UK.

### 5.9.3. Causes of recurrent ACS in OPERA

In the OPERA study patients with recurrent UA and STEMI were usually found to have obstructive coronary disease on repeat angiography, often due to restenosis of a stent. Patients with the less common clinical syndromes of STEMI, out-of-hospital arrest and sudden death were often found to have had a stent thrombosis. As discussed in the previous chapter, patients who required TLR for restenosis had a low subsequent mortality rate; ISR appears to be a very benign entity in comparison with stent thrombosis, at least over the first few years after PCI. The rates of both cardiac death and stent thrombosis decline steeply after the first few months of PCI.

The small number of cases of stent thrombosis hampers the search for associations with baseline characteristics and possible causative factors. Baring this in mind, however, stent thrombosis did appear to occur with greater frequency in patients with more complex disease such as Type C or calcified lesions; and also in patients with the angiographic complications of no-reflow and localised dissection (although most patients with ST did *not* have an angiographic complication). These ‘mechanical’ factors are among many characteristics found by previous investigators to be associated with stent thrombosis, summarised in a 2008 review article by Lemesle et al. Stent thrombosis is also known to be strongly associated with low levels of platelet inhibition as measured by platelet function tests, explored further in Chapter 7.

# Chapter 6 Bleeding and antiplatelet drugs

## 6.1. Bleeding rates and severity

Bleeding from any site, of any severity, was recorded in 85 patients over long-term follow-up, 8.8% of the whole OPERA ACS cohort of 968 patients. The estimated rate of 'clinically significant' bleeding (BARC 2 to 5) at 3.5 years was 5.7%. In 59 patients there was a distinct bleeding event in which bleeding occurred at a well-defined time-point. However, 26 patients reported chronic bleeding, particularly epistaxis. For distinct bleeding events with a recorded date only, cumulative bleeding rates at 48 hours, 30 days, 1 year and 3.5 years (K.M. estimates) are shown in Table 6-1. Figure 6-1 shows the incidence of bleeding by clinical severity (BARC classification) for patients who reported bleeding from any site at any time over long-term follow-up. If patients had more than 1 episode of bleeding the worst episode is shown.

The bleeding classification definitions are summarized here:

**BARC 1** bleeding: not actionable; does not cause the patient to seek any medical input

**BARC 2**: bleeding which requires medical intervention, hospitalization or increased level of care

**BARC 3a**: bleeding with a Hb drop of 3 to 5 g/dL; or any drop requiring transfusion

**BARC 3b**: bleeding with Hb drop of > 5 g/dL, cardiac tamponade, requires surgical intervention or requires use of inotropes

**BARC 3c**: intracranial haemorrhage

**BARC 4**: CABG-related bleeding

**BARC 5**: fatal bleeding (Type 5a probable; type 5b definite)

**Table 6-1 Cumulative incidence of bleeding events stratified by BARC classification of clinical severity**

| Severity of bleed | 0 to 48 hours | 0 to 30 days | 0 to 1 year | 0 to 3.5 years |
|---|---|---|---|---|
| BARC 1 only | 1 (0.1%) | 1 (0.1%) | 2 (0.2%) | 6 (0.9%) |
| BARC 2 only | 14 (1.5%) | 14 (1.5%) | 24 (2.6%) | 31 (3.6%) |
| BARC 3 only | 3 (0.3%) | 3 (0.3%) | 9 (1.0%) | 15 (1.9%) |
| BARC 4 only | 0 | 0 | 0 | 0 |
| BARC 5 only | 1 (0.1%) | 2 (0.2%) | 3 (0.3%) | 4 (0.5%) |
| BARC 2 to 5 | 18 (1.9%) | 19 (2.0%) | 35 (3.8%) | 49 (5.7%) |
| BARC 3 to 5 | 4 (0.4%) | 5 (0.5%) | 12 (1.3%) | 19 (2.3%) |
| All bleeding | 19 (2.0%) | 20 (2.1%) | 37 (4.0%) | 55 (6.5%) |

NB Most BARC 1 bleeds (21 of 30 BARC 1 cases) and some BARC 2 bleeds (5 of 36 BARC 2 cases) were chronic or recurrent without a date of event and are not included in this table

The rate of bleeding decreased over time from PCI in 3 distinct stages. There was a high peri-procedural (< 48 hours after PCI) rate; a lower rate up to approximately 400 days; and a still lower rate from 400 days onwards. This is illustrated in Figure 6-2. The rate change at around 400 days occurs shortly after the majority of patients cease dual antiplatelet therapy (median (IQR) time on DAPT 366 (74) days). Peri-procedural bleeding was high mainly due to arterial access site bleeding: of the 19 patients who had a bleeding event within 48 hours of the procedure there were 15 cases of femoral puncture site bleeding, 1 case of radial puncture site bleeding, 1 of GI bleeding and 2 cases of epistaxis. After this point there were no obvious time-dependent differences either in site or severity of bleeding.

The 1 year bleeding rate was higher in the second phase of recruitment than in the first: 15 cases (6.1%) in the second phase vs. 22 cases (3.0%), $p = 0.03$. This was not obviously due to bleeding from any one particular site.

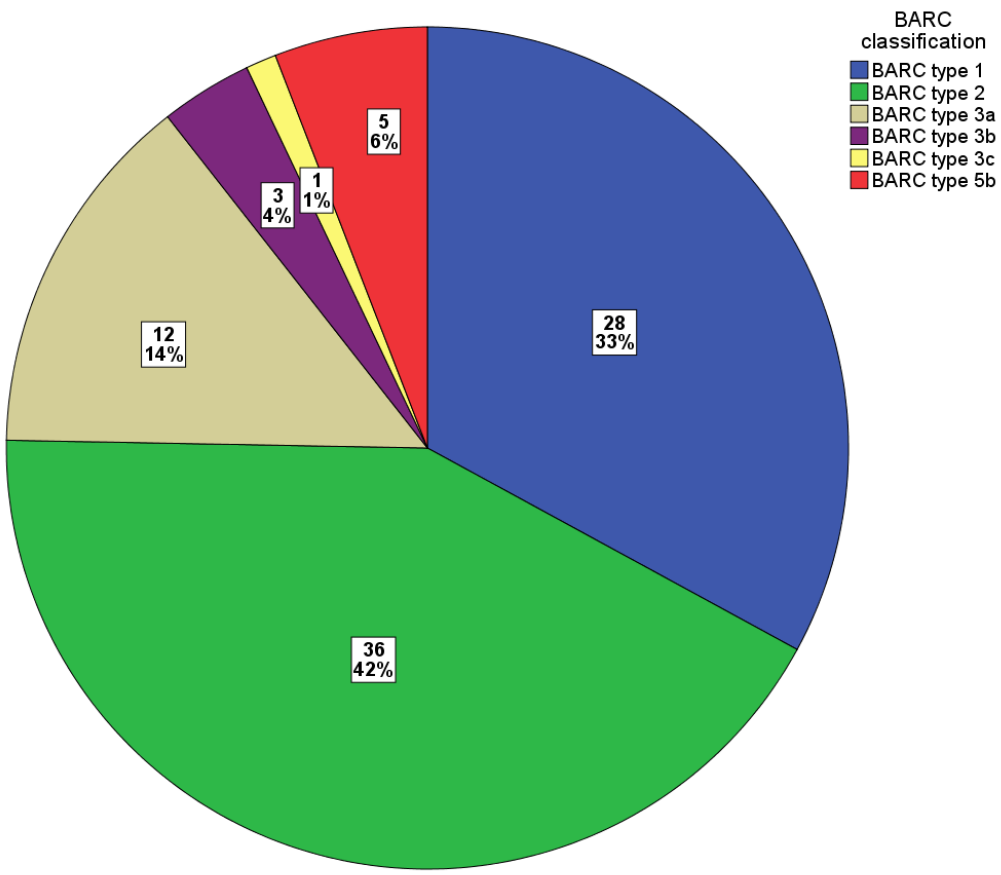



**Figure 6-1 Pie chart showing relative proportions of total bleeding episodes classified by clinical severity using BARC categorisation** ***NB: There were no recorded BARC Type 4 (related to CABG) bleeds; however, the details of CABG operations during follow-up were not carefully examined for this complication***

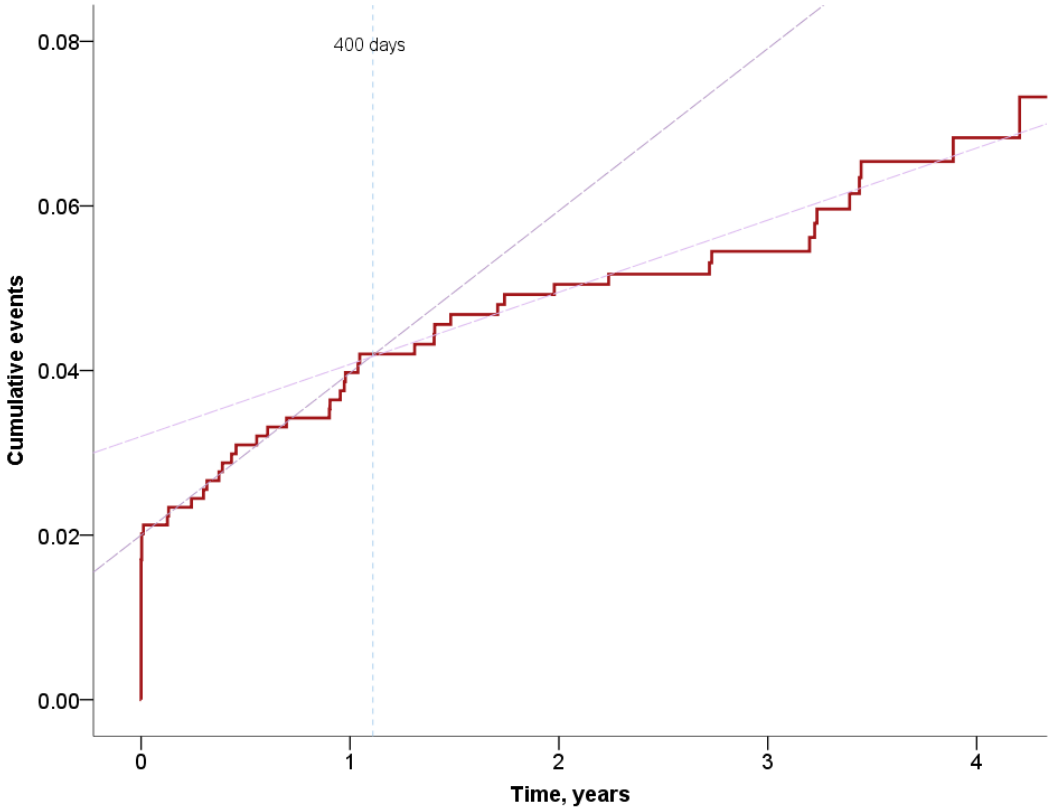



**Figure 6-2 Cumulative incidence of all distinct bleeding events for which a date was recorded; straight reference lines added to highlight rate changes (long dash); vertical dashed line at point of change in rate at approximately 400 days**

### 6.1.1. Fatal bleeding

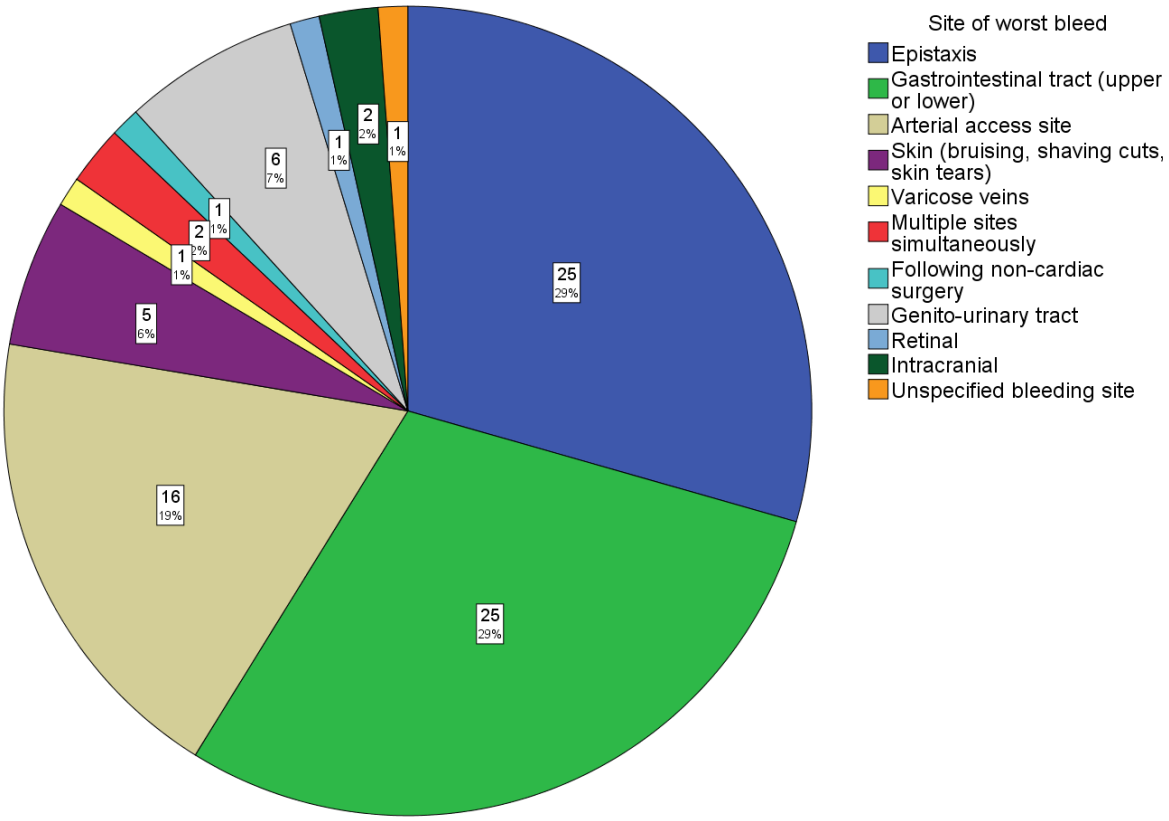
Although most cases of bleeding were trivial or had only minor clinical consequences (57 cases - 73.1% of bleeds, or 5.9% of the total cohort - were either BARC 1 or 2), bleeding was the direct cause of death in 5 patients (6.4% of bleeds; 0.5% of the total cohort), see Table 6-2. Only 1 of these haemorrhagic deaths (PAT ID 951, femoral access site haemorrhage) could be said to be directly related the procedure.

**Table 6-2 Table listing details of the 5 patients who died due to bleeding**

| PAT ID | Time from PCI to death | Cause of death |
|---|---|---|
| 38 | 4 days | Upper GI bleed, exact source unconfirmed |
| 186 | 221 days | Spontaneous subdural haematomas |
| 317 | 1635 days | Bleeding oesophageal varices – alcoholic liver disease |
| 353 | 722 days | Bleeding from colo-vaginal fistula – unconfirmed cause |
| 951 | 1 day | Bleeding from femoral puncture site |

## 6.2. Bleeding from specific sites

Figure 6-3 shows the frequency of bleeding by site of bleeding for 85 patients who reported bleeding of any severity. 1 patient reported ‘minor bleeding’ but no site was recorded. Epistaxis and gastrointestinal tract bleeding were the 2 most common types of haemorrhagic adverse event overall, occurring with equal frequency and accounting for 58% of all bleeding events.




**6-3 Pie chart showing site of bleeding for whole cohort over total follow-up**

### 6.2.1. Epistaxis

The nasal mucosa was the commonest bleeding site. Most of the 25 documented cases of epistaxis had no, or only minor, immediate clinical consequences: 18 (72% of cases of epistaxis) were classified as BARC 1 and 23 (92%) were either BARC 1 or 2. The other 2 patients with epistaxis had rather more severe bleeding: PAT ID 856 required multiple episodes of cauterisation and a 3 unit blood transfusion; and PAT ID 36 required admission to hospital, cauterisation and had a 3g/dL drop in haemoglobin but no transfusion.

More often than not, patients reported chronic recurrent epistaxis rather than 1 or more distinct episodes (14 (56%) cases were chronic). The vast majority of cases of

epistaxis occurred during longer term follow up (median (IQR) time to epistaxis: 382 (1102) days), with only 2 cases recorded during the index inpatient stay.

Epistaxis was more common in older patients: mean age 67.1 (12.9) vs. 62.1 (12.0), p = 0.04; and there was a trend for more epistaxis in patients with a history of hypertension (3.5% vs. 1.7%, p = 0.08) and in those with a history of renal failure (8.7% vs. 2.4%, p = 0.06).

### 6.2.2. Gastro-intestinal tract haemorrhage

There were 25 cases of reported gastro-intestinal blood loss of any type or severity during long-term follow-up. These adverse events are shown in greater detail in Table 6-3.

**Table 6-3 Table detailing the frequency of different types of gastro-intestinal bleeding**

| Type/cause of GI bleed | Number of patients (% of GI bleeds) | PAT ID |
|---|---|---|
| Fresh PR bleed; haemorrhoids | 10 (40.0) | 59, 170, 187, 192, 240, 576, 740, 753, 859, 904 |
| Upper GI bleed (haematemesis and/or melaena); peptic ulcer disease or oesophagitis on OGD | 10 (40.0) | 38, 232, 431, 455, 466, 507, 567, 657, 841, 901 |
| Gastric varices/chronic liver disease | 2 (8.0) | 29, 317 |
| Positive faecal occult blood, anaemia: diverticulosis on colonoscopy | 1 (4.0) | 629 |
| Iron deficiency anaemia, blood transfusion: bowel cancer on colonoscopy | 1 (4.0) | 1001 |
| Unstable angina, anaemia, blood transfusion: erosive gastritis on OGD | 1 (4.0) | 16 |

The median (IQR) time from PCI to GI bleed was 512 (1053) days, with only 1 case occurring during the index hospital stay (following PCI). There were 5 patients with chronic recurrent bleeding. GI bleeding was classified as BARC 1 in 4 (16%) cases, BARC 2 in 11 (44%), BARC 3a in 6 (24%) and 3b in 2 (8%). In the remaining 2 patients, GI bleeding was the direct cause of death (BARC 5b): PAT ID 38 died of a massive upper GI bleed presenting with hypovolaemic shock 2 days after being discharged from hospital from her index admission; and PAT ID 317 had alcohol dependence and died of bleeding oesophageal varices more than 5 years after his index event.

The baseline characteristics associated with GI bleeding were: older age (mean 67.7 (10.6) years vs. 62.1 (12.1) years, p = 0.03); a history of COPD (5.8% vs. 2.1%, p = 0.02); a history of renal failure (8.7% vs. 2.3%, p = 0.05); and a pre-procedural haemoglobin level of < 10g/dL (17.6% vs. 2.3%, p < 0.01). There was no significant difference in the rate of GI bleeding between men and women (2.3% vs. 2.9%, p = 0.63), or between the 2 phases of recruitment.

The use of pre-procedural ACE-inhibitors and the use of beta-blockers were associated with less GI bleeding (1.8% vs. 4.4%, p = 0.02 and 2.0% vs. 4.5%, p = 0.05 respectively). There was no difference in the rate of GI bleeding in patients taking or not taking proton pump inhibitors at PCI (2.7% vs. 2.4%, p = 0.75); nor was there a difference in GI bleeding rates in patients receiving or not receiving IIbIIIa inhibitors at PCI (2.6% vs. 2.4%, p = 0.91).

### 6.2.3. Arterial access site bleeding

Arterial access site bleeding was the third most common form of bleeding. There were 16 arterial access site bleeds, 1 radial and 15 femoral, all recorded within 24 hours of PCI. 13 patients had simple haematomas (12 femoral, 1 radial) which required prolonged compression and/or increased length of hospital stay (BARC 2); and 1 of these patients required a blood transfusion (BARC 3a). There were 2 episodes of more serious access site bleeding: 1 patient (PAT ID 545) had a retroperitoneal bleed following PCI which required surgical repair, classified as BARC 3b (she survived this to die of stroke disease several years later); and 1 patient (PAT ID 951) died following a massive bleed from his femoral puncture site despite efforts to repair it surgically, BARC 5b (the femoral puncture in this case had been into a femoral graft rather than the native femoral artery due to lack of alternative vascular access sites).

Analysis of arterial access site bleeding in relation to baseline characteristics was limited by the small event numbers; however, it was more common in patients over the age of 80 (7.1% vs. 1.2%, p < 0.01), in females (4.1% vs. 0.8%, p < 0.01) and in patients with a previous history of IHD (2.8% vs. 0.6%, p < 0.01). There was a trend for less access site bleeding in patients taking ACE-inhibitors (1.1% vs. 2.7%, p = 0.08) and in patients with a history of smoking (1.2% vs. 2.7%, p = 0.09). There was numerically more access site bleeding in patients with a history of hypertension (2.2% vs. 1.0%, p = 0.14). There was no significant difference in arterial access site bleeding rates between patients receiving and not receiving IIbIIIa inhibitors (1.0% vs. 0.9%, p = 0.94).

### 6.2.4. Intracranial bleeding

There were only 2 cases of intracranial bleeding during long-term follow-up: 1 patient had haemorrhagic transformation of a cerebral infarction (PAT ID 1049); and 1 patient developed bilateral subdural haematomas leading to her death (PAT ID 186).

## 6.3. Bleeding and baseline clinical characteristics

Table 6-4 shows the baseline clinical characteristics in patients who experienced any bleeding and BARC 2 or more bleeding at any point during follow-up.

Many baseline characteristics were associated with a higher incidence of bleeding. These included age, female sex, low body weight and low BMI, a previous history of IHD, heart failure, chronic lung disease and hypertension. Bleeding rate increased with increasing EuroSCORE. Patients with anaemia or renal dysfunction pre-procedure were also more likely to have bleeding problems, as were patients who underwent PCI to Type C lesions and heavily calcified lesions. There was a non-significant trend for higher bleeding rates with increasing SYNTAX score.

Characteristics associated with a lower rate of bleeding included presentation with STEMI, a history of tobacco smoking and treatment with an ACE-inhibitor. Patients with bleeding of any sort, but not BARC 2 to 5 bleeding, were less likely to have received aspirin at the time of PCI.

The univariable characteristics associated with BARC 2 to 5 bleeding are shown in 6-5. After adjustment (Cox regression), the main factors independently associated with BARC 2 to 5 bleeding were age and pre-procedural haemoglobin level, whereas treatment with ACE inhibitors conferred a lower risk of bleeding. In a model with just these 3 variables, the adjusted hazard ratios (95% C.I.) were: for haemoglobin (Hb), 0.73 (0.62 to 0.87) per gram/dL, $p < 0.01$; for age, 1.04 (1.01 to 1.07) per year, $p < 0.01$; and ACE-inhibitor use, 0.46 (0.26 to 0.81), $p = 0.01$.

**Table 6-4 Table comparing baseline characteristics in patients with and without bleeding of any severity; and in patients with BARC 2 to 5 bleeding compared with no bleeding**

| Baseline characteristic | No bleeding N = 883 | Any bleed N = 85 | P value | Type 2 to 5 bleed N = 57 | P value |
|---|---|---|---|---|---|
| *Demographics and clinical syndrome* | | | | | |
| Mean (SD) Age, years | 61.6 (11.9) | 68.5 (11.8) | <0.01 | 69.7 (11.8) | <0.01 |
| Age over 80 years | 52 (5.9%) | 18 (21.2%) | <0.01 | 13 (22.8%) | <0.01 |
| Female | 206 (23.3%) | 36 (42.4%) | <0.01 | 25 (43.9%) | <0.01 |
| Median weight (IQR), kg | 81.2 (19.8) | 77.3 (18.7) | 0.01 | 79.1 (20.0) | 0.09 |
| Median (IQR) BMI | 28.1 (6.0) | 26.6 (5.0) | 0.01 | 26.4 (4.0) | 0.02 |
| Any history of smoking | 618 (70.3%) | 48 (57.1%) | 0.01 | 31 (54.4%) | 0.01 |
| Presented with unstable angina | 109 (12.3%) | 15 (17.6%) | 0.16 | 10 (17.5%) | 0.27 |
| Presented with NSTEMI | 566 (64.1%) | 61 (71.8%) | 0.16 | 43 (75.4%) | 0.08 |
| Presented with STEMI | 208 (23.6%) | 9 (10.6%) | <0.01 | 4 (7.0%) | <0.01 |
| STEMI, convalescent PCI | 117 (13.3%) | 4 (4.7%) | 0.02 | 1 (1.8%) | 0.01 |
| STEMI, Primary PCI | 91 (10.3%) | 5 (5.9%) | 0.19 | 3 (5.3%) | 0.23 |
| *Risk scores* | | | | | |
| Median (IQR) EuroSCORE | 5.3 (4.2) | 8.6 (10.1) | <0.01 | 9.6 (11.5) | <0.01 |
| EuroSCORE 1st quartile (0 to 1.9) | 236 (26.7%) | 11 (12.9%) | <0.01 | 6 (10.5%) | <0.01 |
| EuroSCORE 2nd quartile (2.0 to 3.0) | 212 (24.0%) | 15 (17.6%) | | 11 (19.3%) | |
| EuroSCORE 3rd quartile (3.1 to 6.3) | 229 (25.9%) | 22 (25.9%) | | 13 (22.8%) | |
| EuroSCORE 4th quartile (6.4 to 62.0) | 206 (23.3%) | 37 (43.5%) | | 27 (47.4%) | |
| SYNTAX score | 8.0 (11.0) | 11.0 (14.3) | 0.05 | 11.0 (16.3) | 0.05 |
| SYNTAX score 1st quartile (1.0 to 4.0) | 222 (25.7%) | 14 (16.5%) | 0.15 | 10 (17.5%) | 0.25 |
| SYNTAX score 2nd quartile (5.0 to 8.0) | 212 (24.6%) | 22 (25.9%) | | 13 (22.8%) | |
| SYNTAX score 3rd quartile (9.0 to 15.0) | 223 (25.8%) | 21 (24.7%) | | 14 (24.6%) | |

| | | | | | |
|---|---|---|---|---|---|
| SYNTAX score 4th quartile (15.5 to 48.0) | 206 (23.9%) | 28 (32.9%) | | 20 (35.1%) | |
| *Past medical history* | | | | | |
| Prior coronary revascularisation, any type | 152 (17.2%) | 14 (16.5%) | 0.86 | 10 (17.5%) | 0.94 |
| Previously known IHD, any syndrome | 410 (46.4%) | 52 (61.2%) | <0.01 | 37 (64.9%) | <0.01 |
| Heart failure | 10 (1.1%) | 5 (5.9%) | <0.01 | 4 (7.0%) | <0.01 |
| Diabetes mellitus | 146 (16.6%) | 13 (15.3%) | 0.76 | 10 (17.5%) | 0.82 |
| Hypertension | 438 (49.6%) | 51 (60.0%) | 0.07 | 36 (63.2%) | 0.05 |
| Hyperlipidaemia | 493 (56.1%) | 52 (61.2%) | 0.37 | 34 (59.6%) | 0.63 |
| Peripheral vascular disease | 39 (4.4%) | 4 (4.7%) | 0.90 | 3 (5.3%) | 0.76 |
| Chronic lung disease | 87 (9.9%) | 16 (18.8%) | 0.01 | 10 (17.5%) | 0.08 |
| Stroke | 44 (5.0%) | 5 (5.9%) | 0.72 | 2 (3.5%) | 0.58 |
| *Blood results* | | | | | |
| Median (IQR) pre-procedural Cr, µmol/L | 94 (23) | 96 (29) | 0.31 | 100 (29) | 0.12 |
| Renal impairment, Cr >200 µmol/L | 16 (1.8%) | 7 (8.2%) | <0.01 | 5 (8.8%) | <0.01 |
| Renal impairment, eGFR < 60 ml/min/1.73m$^2$ | 252 (29.2%) | 41 (49.4%) | <0.01 | 30 (54.5%) | <0.01 |
| Median (IQR) pre-procedural Hb, g/dL | 14.1 (2.1) | 12.9 (3.0) | <0.01 | 12.9 (3.1) | <0.01 |
| Hb < 10.0 g/dL | 11 (1.4%) | 6 (7.5%) | <0.01 | 5 (9.1%) | <0.01 |
| Median (IQR) platelet count x $10^9$ | 247 (87) | 258 (89) | 0.30 | 248 (103) | 0.75 |
| *Medications at PCI* | | | | | |
| Aspirin | 858 (97.5%) | 79 (92.9%) | 0.02 | 55 (96.5%) | 0.79 |
| Clopidogrel | 832 (94.2%) | 83 (97.6%) | 0.19 | 56 (98.2%) | 0.20 |
| Prasugrel | 52 (5.9%) | 3 (3.5%) | 0.37 | 2 (3.5%) | 0.47 |
| Heparin | 757 (85.9%) | 77 (90.6%) | 0.23 | 50 (87.7%) | 0.75 |

| | | | | | |
|---|---|---|---|---|---|
| Bivalirudin | 138 (15.7%) | 8 (9.6%) | 0.14 | 7 (12.7%) | 0.60 |
| IIbIIIa inhibitor | 283 (32.0%) | 29 (34.1%) | 0.70 | 20 (35.1%) | 0.63 |
| ACE inhibitor | 574 (68.9%) | 43 (53.8%) | <0.01 | 28 (50.9%) | <0.01 |
| Beta-blocker | 634 (75.9%) | 58 (71.6%) | 0.39 | 39 (70.9%) | 0.41 |
| HMG-CoA reductase inhibitor | 734 (83.1%) | 70 (82.4%) | 0.86 | 50 (87.7%) | 0.33 |
| Proton pump inhibitor | 263 (29.8%) | 31 (36.5%) | 0.20 | 21 (36.8%) | 0.27 |
| Warfarin (before admission with index event) | 17 (1.9%) | 2 (2.4%) | 0.79 | 2 (3.5%) | 0.39 |
| *Procedural characteristics* | | | | | |
| Arterial access route: femoral | 693 (78.6%) | 69 (81.2%) | 0.58 | 45 (78.9%) | 0.98 |
| Arterial access route: radial | 189 (21.4%) | 16 (18.8%) | | 12 (21.1%) | |
| More than 1 stent implanted | 250 (28.9%) | 23 (27.1%) | 0.73 | 15 (26.3%) | 0.68 |
| Drug eluting stent, any vessel | 522 (59.1%) | 49 (57.6%) | 0.79 | 32 (56.1%) | 0.65 |
| Staged PCI | 27 (3.1%) | 0 | 0.10 | 0 | 0.19 |
| Worst lesion type: | | | | | |
| Type A | 131 (15.0%) | 8 (9.4%) | <0.01 | 7 (12.3%) | 0.05 |
| Type B1 | 216 (24.7%) | 17 (20.0%) | | 14 (24.6%) | |
| Type B2 | 209 (23.9%) | 13 (15.3%) | | 6 (10.5%) | |
| Type C | 320 (36.5%) | 47 (55.3%) | | 30 (52.6%) | |
| Calcified lesion | 156 (17.7%) | 26 (30.6%) | <0.01 | 17 (29.8%) | 0.03 |
| Thrombotic lesion | 224 (25.4%) | 20 (23.5%) | 0.71 | 13 (22.8%) | 0.67 |
| Non-haemorrhagic procedural complication | 112 (12.7%) | 10 (11.8%) | 0.81 | 6 (10.5%) | 0.63 |

**Table 6-5 Table showing characteristics associated with BARC 2 to 5 bleeding for entire OPERA cohort (off or on treatment with P2Y12 inhibitors)**

| Characteristic | Hazard Ration (as univariate) | 95% C.I. | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Haemoglobin (per g/dL) | 0.65 | 0.56 | 0.75 | < 0.01 |
| ACE inhibitor | 0.45 | 0.26 | 0.78 | < 0.01 |
| History of heart failure | 8.29 | 2.97 | 23.16 | < 0.01 |
| Type C lesion | 2.15 | 1.25 | 3.72 | < 0.01 |
| Age (per year) | 1.07 | 1.04 | 1.10 | < 0.01 |
| Female sex | 2.52 | 1.46 | 4.35 | < 0.01 |
| Body Mass Index | 0.93 | 0.88 | 1.00 | 0.03 |
| Presentation with STEMI | 0.30 | 0.11 | 0.83 | 0.02 |
| Previous IHD | 2.53 | 1.40 | 4.55 | < 0.01 |
| Chronic lung disease | 1.88 | 0.92 | 3.85 | 0.07 |
| Hypertension | 1.76 | 1.00 | 3.09 | 0.05 |
| Renal dysfunction, eGFR low | 2.99 | 1.71 | 5.22 | < 0.01 |
| Smoking | 0.48 | 0.28 | 0.82 | < 0.01 |

### 6.3.1. Bleeding and pre-procedural haemoglobin concentration

Bleeding rates were higher in patients who had low pre-procedural Hb concentration. This is illustrated in Figure 6-5 and explored further in Chapter 7.

### 6.3.2. Bleeding and age

Figure 6-4 shows the proportion of BARC 2 to 5 bleeding over long-term follow-up for patients by decade of age at time of PCI, showing a steady increase in bleeding rate with age up to a threshold of approximately 80 years. Beyond 80 years the rate increases more sharply, with much of the excess bleeding occurring in the immediate post-PCI period; 30% of 30 day bleeding and 30% of access site bleeding occurred in the relatively small group of over-80s (70 patients) in the OPERA study.

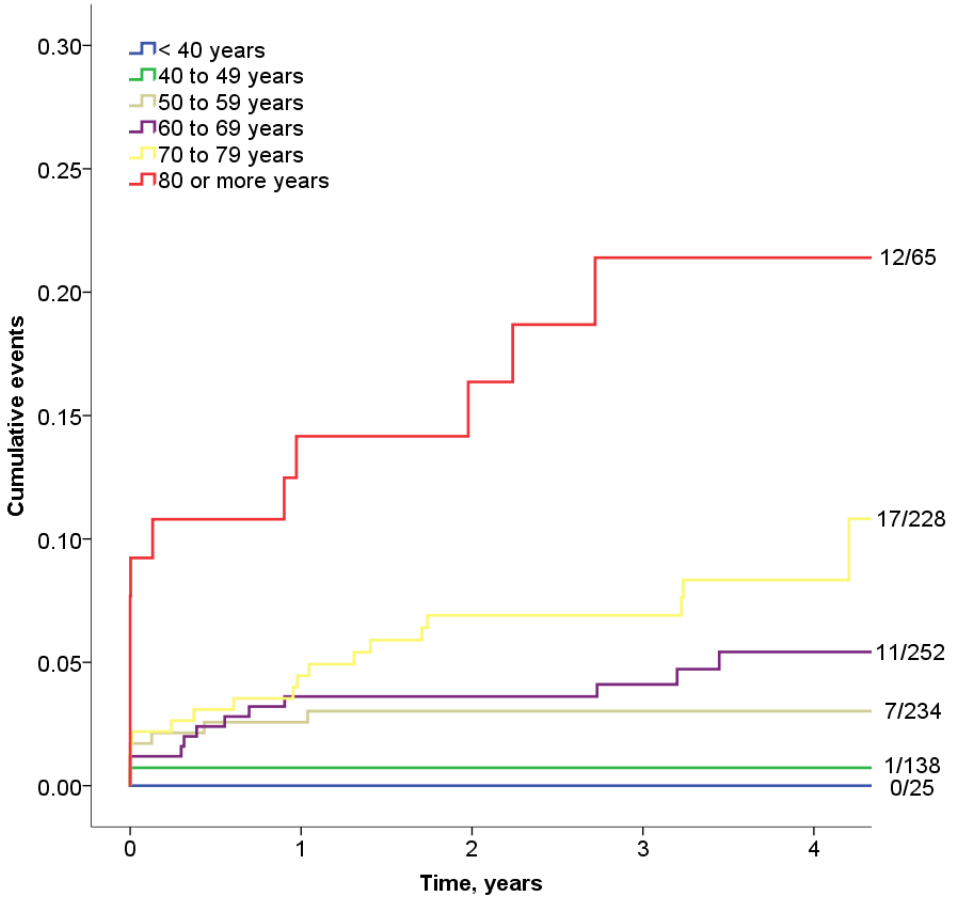



**Figure 6-4 KM estimates of BARC 2 to 5 bleeding during long-term follow-up stratified by age (decades); number of events at median follow-up (3.4 years)/number in starting group shown**

### 6.3.3. Risk of bleeding from combination of age and anaemia

Figure 6-5 shows the rates of BARC 2 to 5 bleeding stratified by the 2 main baseline risk factors, advanced age and anaemia. Age > 70 has been chosen to increase the group size; anaemia is defined as Hb < 12 g/dl.

### 6.3.4. Bleeding and ACE-inhibitor use pre-PCI

Figure 6-6 shows the unadjusted KM estimates of BARC 2 to 5 bleeding up to the median follow-up of 1273 days for patients stratified into groups in which ACE inhibitors were used at PCI or not. Most of the excess bleeding episodes in the group not on ACE-inhibitors were peri-procedural from the arterial access site.

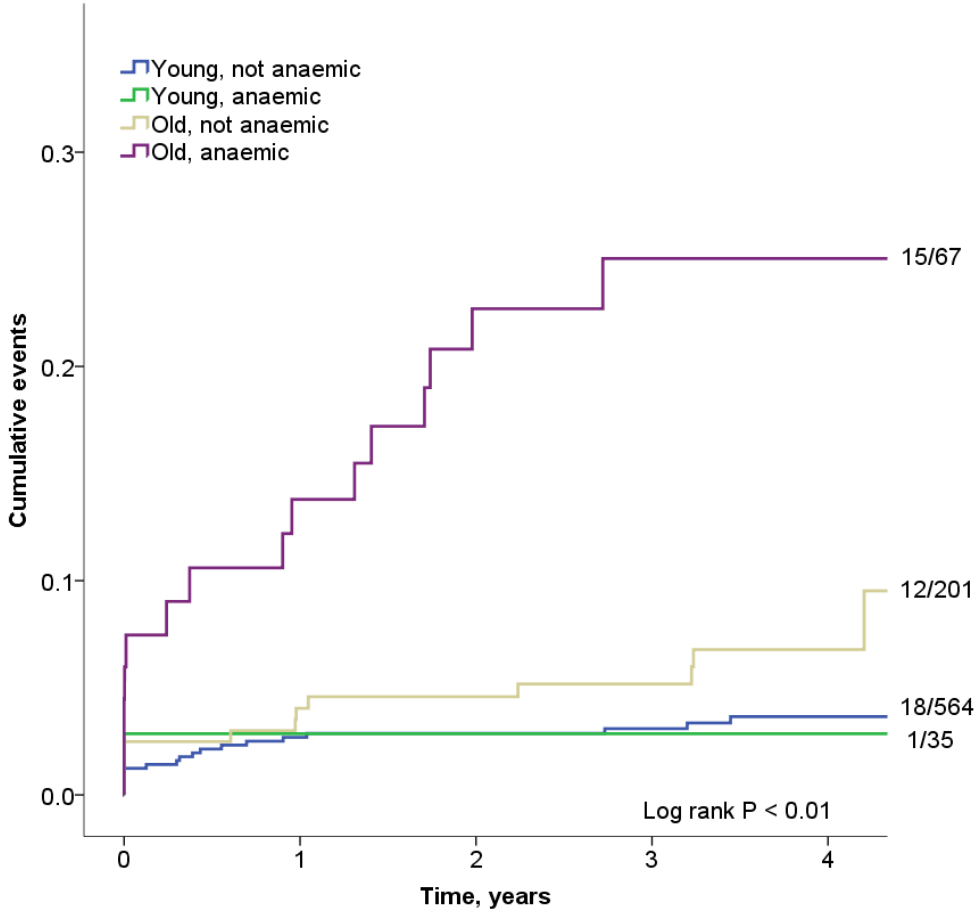



**Figure 6-5 KM estimates of BARC 2 to 5 bleeding rates in patients stratified into 4 groups based on age below and above 70 years and baseline Hb below and above 12 g/dl; number of events/number in group at start shown**

The association of ACE-inhibitor use with lower bleeding rates was not constant over time of patient recruitment: the rate of BARC 2 to 5 bleeding in the first recruitment phase was 3.9% with ACE-inhibitors vs. 9.9% without, $p < 0.01$; and in the second phase, 6.0% vs. 7.7%, $p = 0.82$. This change is not simply due to higher femoral access route in the first phase compared to the second: BARC 2 to 5 bleeding rates in femoral access cases were 4.7% with ACE-inhibitors vs. 9.0% without, $p = 0.04$; and in radial access cases, 3.7% with ACE-inhibitors vs. 9.5% without, $p = 0.10$. The association of ACE-inhibitor use with reduced bleeding rates was more prominent when IIbIIIa inhibitors were used at PCI: BARC 2 to 5 bleeding rates were 4.8% vs. 7.5% with and without ACE-inhibitors in those not receiving IIbIIIa inhibitors ($p = 0.17$); but 4.0% vs. 12.4% in those receiving IIbIIIa inhibitors, $p < 0.01$. It is possible that the difference over time may be partly explained by the lower rate of IIbIIIa inhibitor use in the second recruitment phase compared to the first (35.5% vs. 22.4%, $p < 0.01$).

There also seems to be an important difference in the effect of ACE-inhibitors on bleeding rate between men and women: BARC 2 to 5 bleeding rates in men were 2.9% vs. 8.9% with and without ACE-inhibitors ($p < 0.01$), but in women these rates were no different: 10.5% vs. 9.6% ($p = 0.82$).

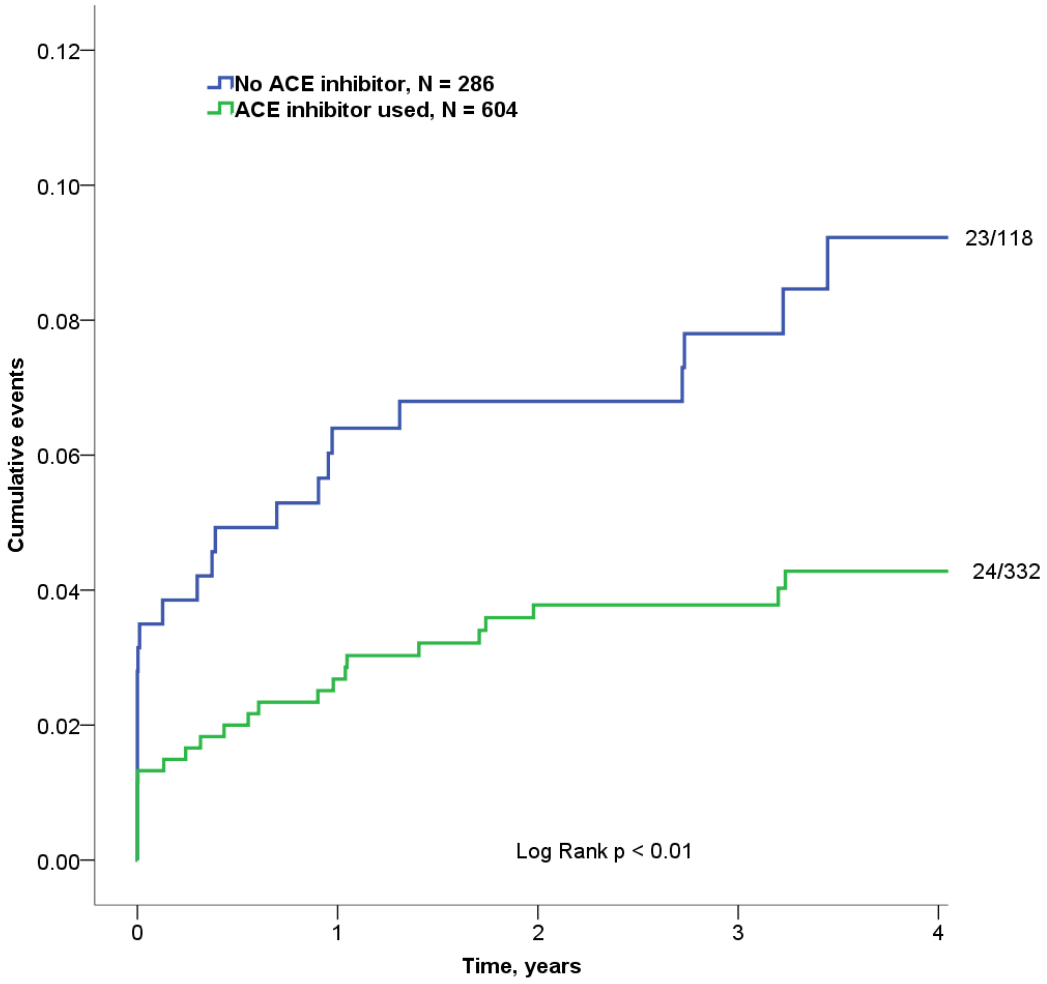



**Figure 6-6 KM estimates of cumulative incidence of BARC 2 to 5 bleeding in patients stratified by use or no use of ACE-inhibitors at PCI**

## 6.4. Bleeding and antiplatelet compliance

### 6.4.1. Aspirin compliance

Aspirin was given before PCI in nearly all patients (963, 99.5%) and was relatively well tolerated with the majority, 89%, continuing to take it long-term or until death. Aspirin was discontinued by 102 (10.5%) patients, only 12 of whom reported having to stop aspirin specifically due to bleeding problems, see Figure 6-7.

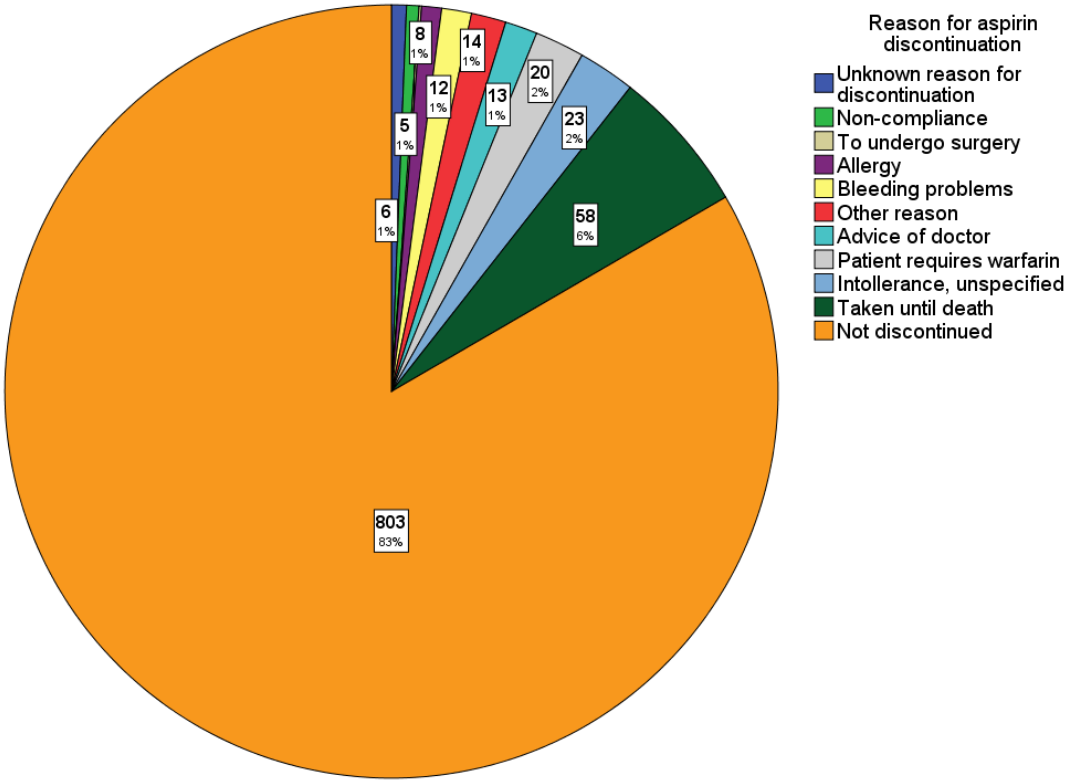



**Figure 6-7 Pie chart showing reasons for discontinuation of aspirin in OPERA patients**

### 6.4.2. Clopidogrel compliance

Clopidogrel was received by 915 patients before PCI and a further 24 patients received prasugrel at PCI but were changed to clopidogrel shortly afterwards. Of these 939 patients, clopidogrel was discontinued after 12 months according to guidelines for treatment of ACS in 677 (72.1%). Only 6 (0.6%) patients discontinued clopidogrel because of bleeding problems. Figure 6-8 illustrates the reasons for clopidogrel discontinuation.

Clopidogrel was never discontinued by 105 (11.2%) patients. In 11 of these, clopidogrel was continued long-term as a single antiplatelet due to aspirin intolerance. In the other 94 patients, DAPT with aspirin and clopidogrel was continued indefinitely for unclear reasons, which could include patient or physician preference or simple oversight.

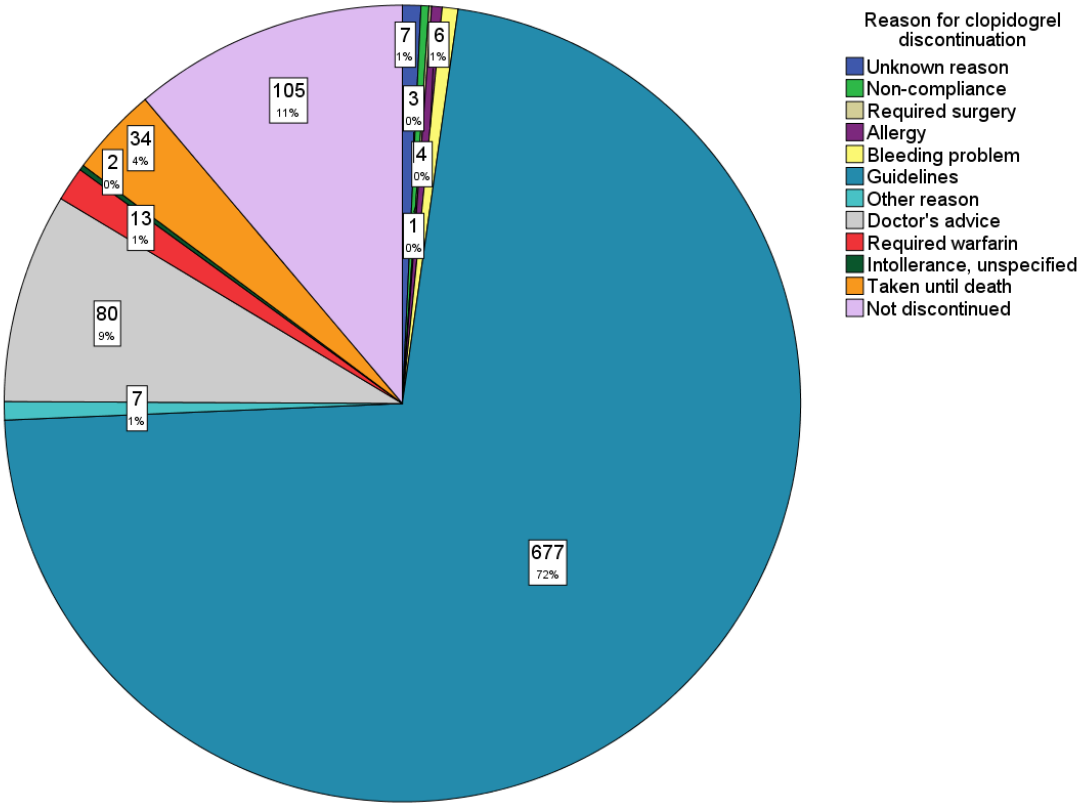



**Figure 6-8 Pie chart showing reasons for discontinuation of clopidogrel in OPERA patients**

### 6.4.3. Prasugrel compliance

Prasugrel was received by 53 patients before PCI; 48 of these were PPCI cases (as was the local protocol at the time of recruitment). In 20 cases, only prasugrel loading dose was given and the patient was switched to clopidogrel following PCI. There were 21 patients who took the recommended 12 months of prasugrel; 9 others continued indefinitely, 1 patient stopped due to allergy, 1 patient died while still on prasugrel, and 1 further patient discontinued the drug for unclear reasons. No patient had to stop the drug because of bleeding problems.

## 6.5. The association of bleeding with mortality

BARC 2 to 5 bleeding was associated with a higher rate of all-cause, cardiac and non-cardiovascular death during long-term follow-up. The crude death rates in patients with and without BARC 2 to 5 bleeding were: for all-cause mortality, 31.6% vs. 10.9%, $p < 0.01$; for cardiac death, 10.5% vs. 3.8%, $p = 0.02$; and for non-cardiovascular death, 17.5% vs. 5.3%, $p < 0.01$. Even discounting the 5 patients who died as a direct result of bleeding, patients with BARC 2 to 5 bleeding still appeared to have greater all-cause mortality (25.0%, $p < 0.01$) and non-cardiovascular mortality (15.4%, $p < 0.01$) when compared to patients without bleeding (the rate of cardiac death was no longer different). However, after adjustment for age (Cox regression), the association of non-

fatal BARC 2 to 5 bleeding with all-cause mortality lost statistical significance: for age, aHR (95% CI) 1.090 (1.07 to 1.11) per year, $p < 0.01$; for BARC 2 to 5 bleeding, aHR 1.80 (1.0 to 3.23), $p = 0.05$

The median (IQR) time from non-fatal BARC 2 to 5 bleed to death was 766 (1218) days. The specific causes of death in the 18 deceased patients with BARC 2 to 5 bleeding can be divided into those deaths directly related to bleeding and those without a clear relationship with bleeding, as follows:

Cause of death directly related to bleeding (5 patients):

- Femoral artery bleed: 1 patient
- Bilateral chronic subdural haematoma: 1 patient
- Upper GI bleed: 1 patient
- Bleeding oesophageal varices: 1 patient
- Bleeding from malignant colo-vaginal fistula: 1 patient

Cause of death not directly related to bleeding (13 patients):

- Terminal cancer: 4 patients
- CCF/valvular heart disease: 2 patients
- CCF/ischaemic cardiomyopathy: 2 patients
- Cerebral infarction: 2 patients
- COPD and breast cancer: 1 patient
- Cirrhosis: 1 patient
- Pneumonia and chronic pancreatitis: 1 patient

The relationship between bleeding and subsequent adverse clinical events is explored further in Chapter 7.

## 6.6. Association of BARC 2 to 5 bleeding with antiplatelet and anticoagulant medication

A record was made where possible of antiplatelet and anticoagulant medication taken at the time of BARC 2 to 5 bleeding events. In most cases (43 of 57 patients with BARC 2 to 5 bleeding), bleeding occurred while still receiving DAPT with aspirin and the index $P2Y_{12}$ inhibitor. In 2 further cases a bleeding event took place while the patient was on DAPT but with a different $P2Y_{12}$ inhibitor to that given at the index PCI (PAT ID 1018 and 1049). 6 patients had a BARC 2 to 5 bleed while on no $P2Y_{12}$ inhibitor, 5 of these while on aspirin alone (PAT ID 280, 629, 682, 740 and 317) and 1 while on Warfarin alone (PAT ID 289). A further patient had a BARC 2 to 5 bleed on

warfarin and clopidogrel (PAT ID 657). In 5 cases it was not possible to verify whether bleeding had taken place while taking the index $P2Y_{12}$ inhibitor (PAT ID 378, 582, 507, 576 and 753).

Of 53 patients given prasugrel prior to PCI, on-prasugrel BARC 2 to 5 bleeding occurred in 2 (3.8%) – but note that there was a high rate of switching from prasugrel to clopidogrel immediately following PCI. Of 910 patients given clopidogrel prior to PCI, on-clopidogrel BARC 2 to 5 bleeding occurred in 43 (4.7%).

## 6.7. Chapter 6 summary points and discussion

- Bleeding was relatively common, reported in about 9% of the population over long-term follow-up.
- Bleeding was often chronic and recurrent.
- The commonest types of bleeding were epistaxis and GI tract bleeding.
- Peri-procedural bleeding rate was high mainly due to femoral artery access site haematomas.
- Bleeding rate clearly decreased after about 12 to 13 months (the duration of DAPT).
- Most bleeding episodes were of minor clinical severity. Fatal bleeding was rare with only 5 cases throughout long-term follow up.
- The main baseline characteristics associated with bleeding were low pre-procedural Hb level and increasing age.
- ACE-inhibitors appeared to be protective against peri-procedural arterial access site bleeding – but only in men.
- Aspirin discontinuation was relatively common, seen in 10%, usually due to GI tract disturbance rather than bleeding. Clopidogrel discontinuation before 12 months was rare and many (11%) continued clopidogrel beyond 12 months.
- Bleeding was strongly associated with subsequent mortality, although this association was lost after adjustment for age. None of the deaths in patients who had bleeding were overtly haemorrhagic in nature.

### 6.7.1. Bleeding rates in comparison with published data

The total bleeding rate (BARC 1 to 5) at 12 months in OPERA was 4.0%. In TRYTON TIMI 38 the rate was very similar: 3.8% with clopidogrel (5.0% with prasugrel) at 15 months (TIMI definitions). In PLATO Invasive the rate of non-CABG related bleeding was comparatively high with PLATO definitions: 7.1% with clopidogrel (8.9% with ticagrelor) at 12 months. However, with TIMI definitions total

non CABG related bleeding was similar: 3.9% with clopidogrel (4.6% with ticagrelor). Note that patients in both the PLATO and TRYTON TIMI 38 studies were selected as being at low risk of bleeding, so perhaps the bleeding rates in OPERA are a little lower than one would expect in an all-comers population.

### 6.7.2. Risk factors for bleeding

In OPERA the main baseline characteristics associated with bleeding were advanced age and anaemia: bleeding was very uncommon in those under 70 years old and only 1 patient under 50 years reported any bleeding at all over long-term follow-up. In contrast, the rate of bleeding in the over-80s approached 25% by 3.5 years of follow-up. The rate of bleeding was noticeably higher during the first 13 months after PCI, corresponding to the duration of DAPT. This leads us to a treatment dilemma in that while older patients have the worst coronary disease and highest rates of recurrent ACS, they are least able to tolerate the side effects of DAPT. This will surely become more of a problem in a patient population which continues to age.

### 6.7.3. Bleeding and mortality

There has been a great deal of concern in the recent published literature regarding an apparent strong association with bleeding events and subsequent all-cause mortality in PCI patients, such as the 2011 paper by Mehran, Pocock et al. However, unlike these studies, in the OPERA study an effort was made to investigate the cause of all deaths individually in detail, especially in patients who had reported bleeding problems during follow up. While we also found an apparent association between bleeding and mortality, on closer inspection we found that deaths directly related to bleeding were very rare and the association was most likely due to confounding factors such as advanced age, present in both those with bleeding problems and those who died.

Therefore, while bleeding problems in older people following PCI are frequently encountered, a fear of bleeding should not necessarily preclude these patients from being offered PCI when found to have severe coronary disease. Certain manoeuvres could lower the risk of bleeding in the elderly however, such as the use of proton pump inhibitors to lower the chance of peptic ulceration; or shortening the duration of DAPT. Also we observed in our population a considerably lower rate of peri-procedural bleeding (mainly femoral access site) in men taking ACE-inhibitors. This was presumably due to lower blood pressure in patients on these drugs, which could not be demonstrated as blood pressure in the cath lab or in recovery was not recorded in the study database. The effect good blood pressure control may have on bleeding rates in patients on DAPT has not been studied to the best of our knowledge.

# Chapter 7 Platelet function testing

In this chapter the association of $P2Y_{12}$ receptor inhibition, measured using the VerifyNow bedside method, on the incidence of ischaemic and haemorrhagic events following PCI is studied. To improve the ability to detect associations we have analysed clinical adverse events which occurred while the affected patient was currently taking the same P2Y12 inhibitor they were on when the platelet function test was performed ('on-treatment events') with censorship at cessation of treatment. The dataset of 789 patients used for analyses in Chapter 7 incorporates all patients from the OPERA ACS cohort (presented in Chapters 1 to 6) who had a VerifyNow test result and who did not have emergent PCI (all PPCI patients excluded).

Note: the VerifyNow Base value represents the degree of thrombin receptor (PAR)-mediated platelet reactivity and is independent of the effect of ADP P2Y12 receptor inhibition; the PRU value represents ADP P2Y12 receptor-mediated reactivity; and the *%inhibition* value is derived from the difference in platelet reactivity between the Base and PRU and gives an indication of the magnitude of effect of the antiplatelet drug.

## 7.1. VerifyNow platelet function test results

Of 872 patients presenting with ACS with the exception of PPCI patients, 789 patients (90%) had a VerifyNow platelet function test result. PPCI patients who had a test result recorded were excluded from the analysis as we could not be confident of an accurate test given the short time from P2Y12 inhibitor administration and testing. Most of the patients with no test result had been pre-treated with a IIbIIIa inhibitor; this drug makes the VerifyNow P2Y12 test invalid.

### 7.1.1. Distribution of Base, % inhibition and PRU values

In these 789 patients the median (IQR) PRU was 242 (126) and the median *%inhibition* value was 20% (39%). The median Base value was 308 (85). The distributions of the PRU, *% inhibition* and Base values are shown in Figures 7-1, 7-2 and 7-3. The following observations can be made:

- All 3 platelet reactivity measurements showed a very high degree of variability between patients
- Most (78%) patients had some degree of platelet inhibition with clopidogrel, but the %inhibition range was very wide, from 0% to 98%

- 177 (22%) patients had 0% inhibition; i.e. in more than 1 fifth of patients, the VerifyNow device measured no effect of clopidogrel on platelet reactivity
- The Base value (platelet reactivity in response to thrombin receptor activation) had a normal distribution (skewness < 0.01; kurtosis 0.02)
- 95 (12.0%) patients had Baseline reactivity values which were low enough to fall into the 'responder' PRU range (< 238); these patients had particularly low PRU levels, median 161 (133)
- PRU was not normally distributed (skewness -0.32; kurtosis -0.35)
- PRU was strongly linked to the Base value – if Baseline reactivity was high, PRU was generally high also. This is highlighted in Figure 7-4

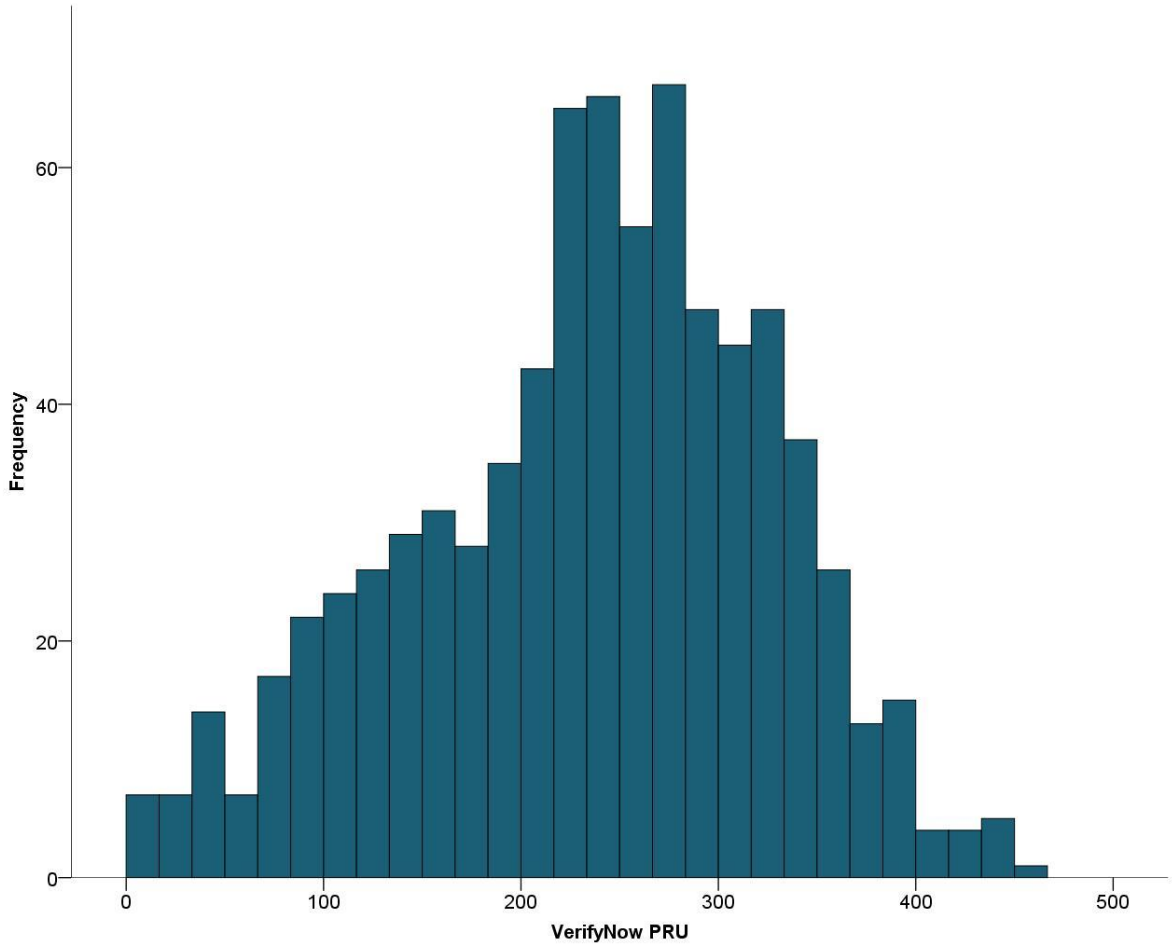



**Figure 7-1 Histogram showing distribution of VerifyNow PRU in 789 patients, PPCI patients excluded**

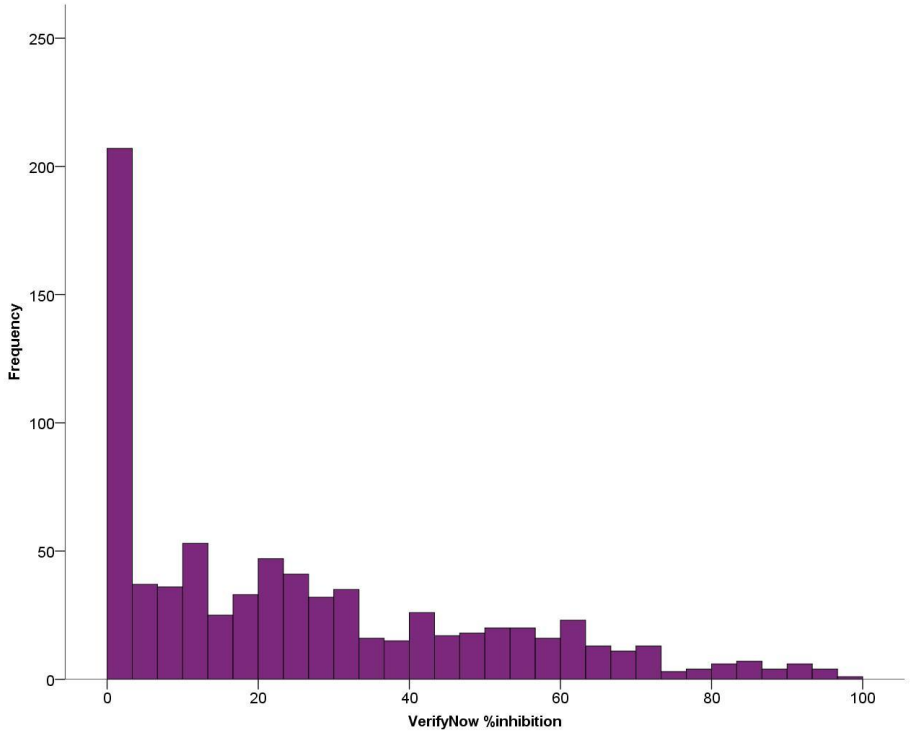



**Figure 7-2 Histogram showing distribution of VerifyNow *%inhibition* in 789 patients, PPCI patients excluded**

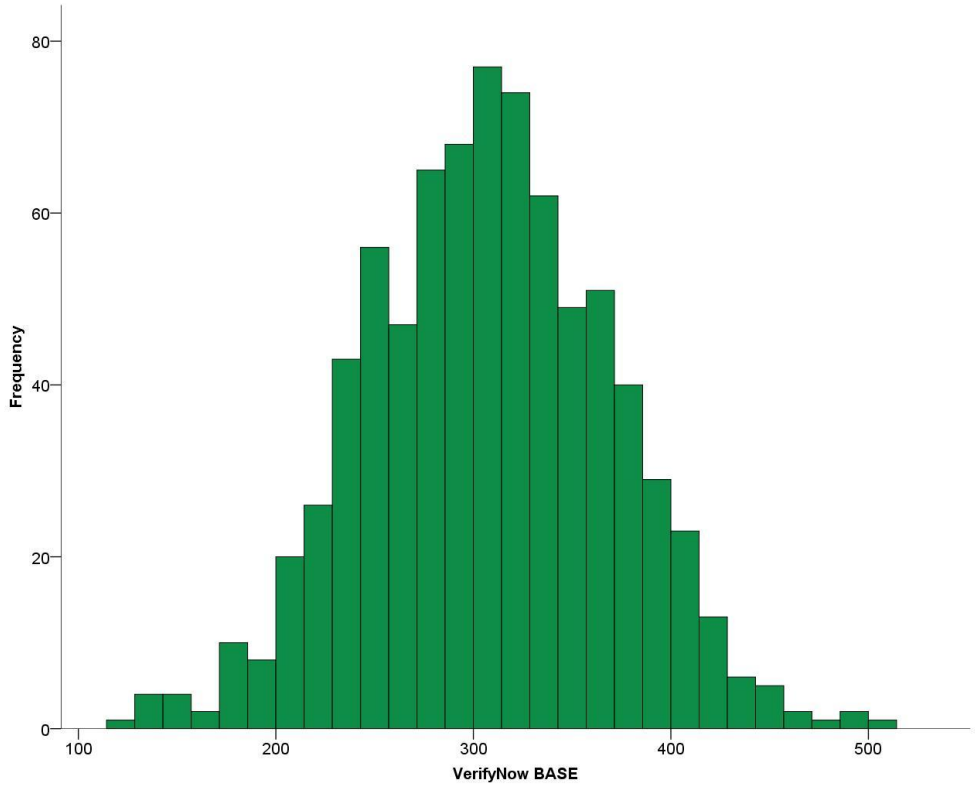



**Figure 7-3 Histogram showing the distribution of the VerifyNow 'Base' value in 789 patients, PPCI patients excluded**

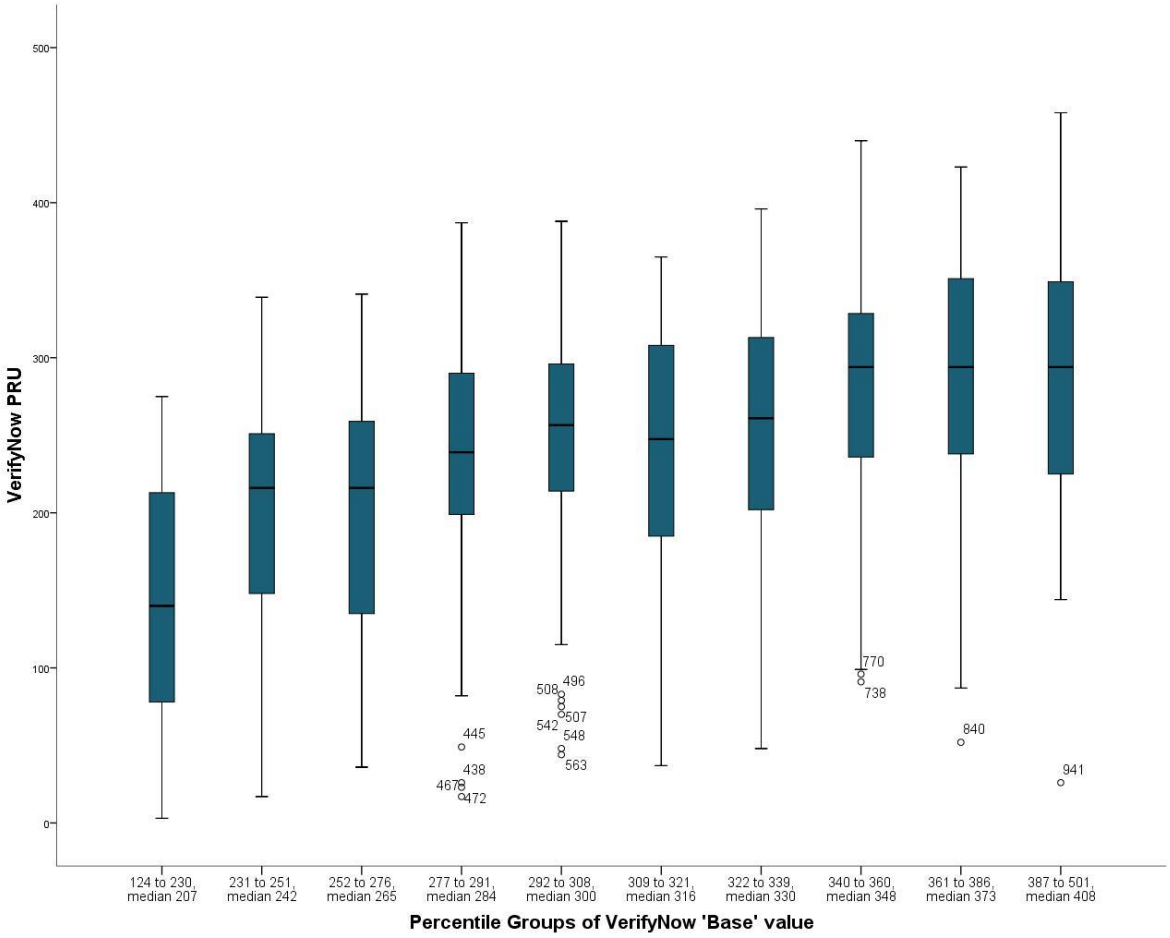



**Figure 7-4 Box and whisker plots showing the PRU of 789 patients ranked into 10 'Base value' percentile groups**

### 7.1.2. VerifyNow values and baseline characteristics

Platelet reactivity was different in patients with and without certain baseline characteristics. VerifyNow Base, PRU and %inhibition for patients with and without selected dichotomous baseline characteristics are shown in Table 7-1. The single factor with the greatest apparent association with platelet reactivity was pre-procedural Hb level. In addition, age, gender, diabetes, renal function, pre-procedural platelet count and BMI all appeared to correlate with platelet reactivity.

#### 7.1.2.1. Age and platelet reactivity

VerifyNow Base platelet reactivity increased with age (2-tailed Spearman's rho = 0.278, $p < 0.01$). However, elderly patients tended to have lower Hb levels; the mean Hb in patients under and over the age of 80 years was 14.0 vs. 12.1 g/dl, $p < 0.01$. The variation in Base reactivity with age disappeared when Hb level was taken into account.

PRU was weakly correlated with age (rho = 0.129, p < 0.01) but this seemed to be confined to those with the very highest PRU values only. There was no correlation between P2Y12 *%inhibition* and age; see Figure 7-5.

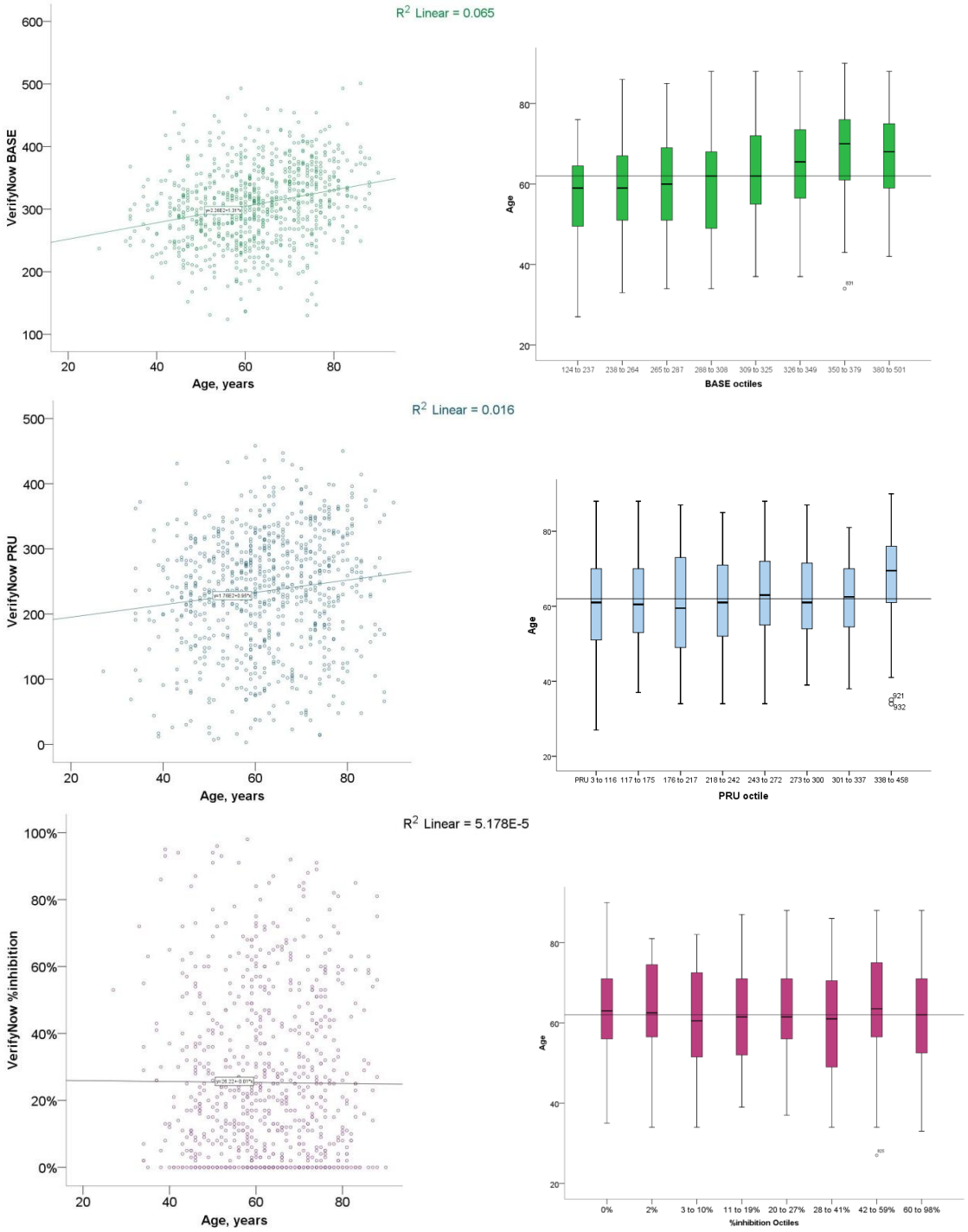



**Figure 7-5 Scatter graphs (with line of best fit) and box-and-whisker plots (with line at median value) showing the relationship between age and VerifyNow Base, PRU and *% inhibition* values**

#### 7.1.2.2. Pre-procedural haemoglobin concentration and platelet reactivity

Haemoglobin (Hb) concentration was the baseline characteristic with the greatest impact on platelet reactivity: generally, anaemia was associated with high reactivity and polycythaemia with low reactivity. The interactions between Hb level and the 3 VerifyNow platelet function test parameters are illustrated in Figs. 7-6 to 7-11. There was a moderately strong inverse correlation (Spearman's Rho) between baseline reactivity and Hb: -0.55, $p < 0.01$. This relationship was approximately linear throughout the range of Hb.

There was a similar inverse relationship between PRU and Hb, but the correlation was weaker due to the wide range of PRU values. Furthermore the relationship was non-linear, with lower PRU values seen at both extremes of Hb. In patients with Hb levels above 12 g/dL there was no correlation between the degree of platelet inhibition (%inhibition) and Hb, but intense inhibition was more often seen in anaemic patients: median %inhibition levels in these 2 groups were 35 and 18, $p \leq 0.01$.

#### 7.1.2.3. Gender and platelet reactivity

Female patients overall had higher VerifyNow Base reactivity than males (see Table 7-1). However, women had lower Hb levels than men (mean 12.8 vs. 14.2 g/dL, $p < 0.01$). Base reactivity was no different between males and females when stratified into groups with similar haemoglobin concentration. Women had greater clopidogrel-mediated platelet inhibition (i.e. higher % inhibition values), although this may have been partially related to having a lower BMI than men. PRU was not different overall between men and women.

#### 7.1.2.4. Renal function and platelet reactivity

Baseline platelet reactivity was higher in patients with poor renal function: there was a modest negative correlation between eGFR and the Base value (Spearman's rho = -0.278, $p < 0.01$); Table 7-1. However, patients with renal dysfunction were often anaemic. After allowance for baseline Hb level, patients with renal dysfunction had the same Base platelet reactivity as those with good renal function.

There was greater inhibitory effect from clopidogrel in patients with poor renal function: correlation between eGFR and %inhibition was -0.104, $P < 0.01$. Patients with very poor renal function (eGFR < 30) in particular had markedly higher %inhibition: 20 vs. 45 ($p < 0.01$). These patients had lower PRU despite higher baseline reactivity.

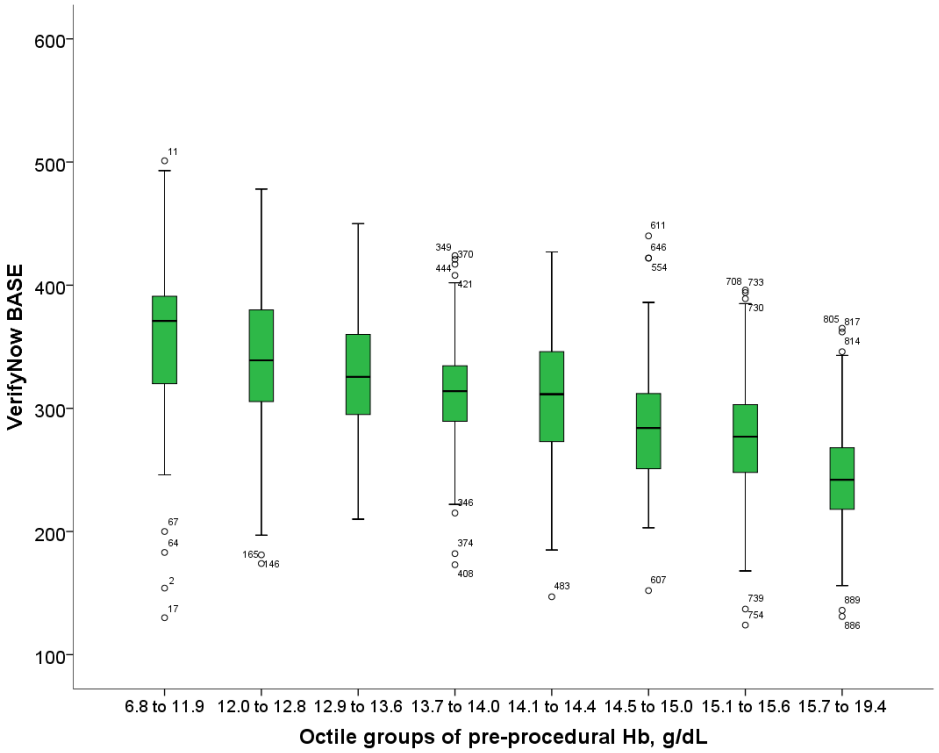



**Figure 7-6 Box and whisker plot of *VerifyNow BASE* in patients stratified by pre-procedural haemoglobin level**

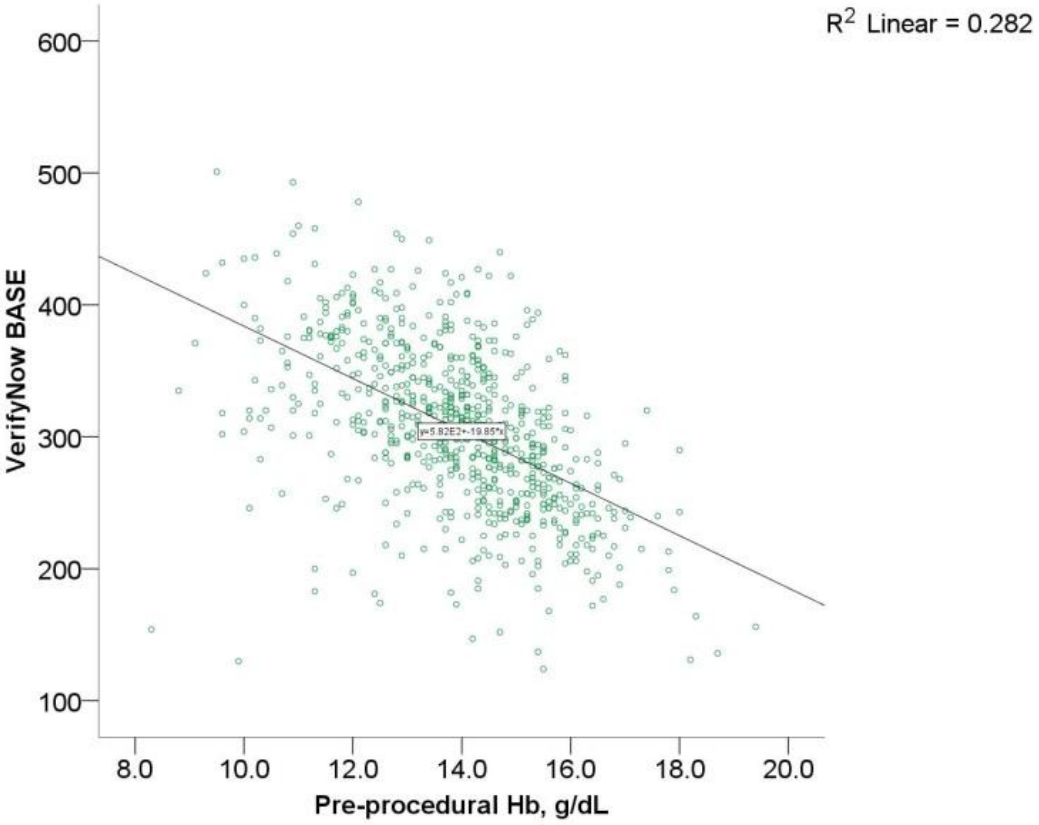



**Figure 7-7 Scatter plot of *VerifyNow BASE* levels against pre-procedural Hb in g/dL with automatic line of best fit**

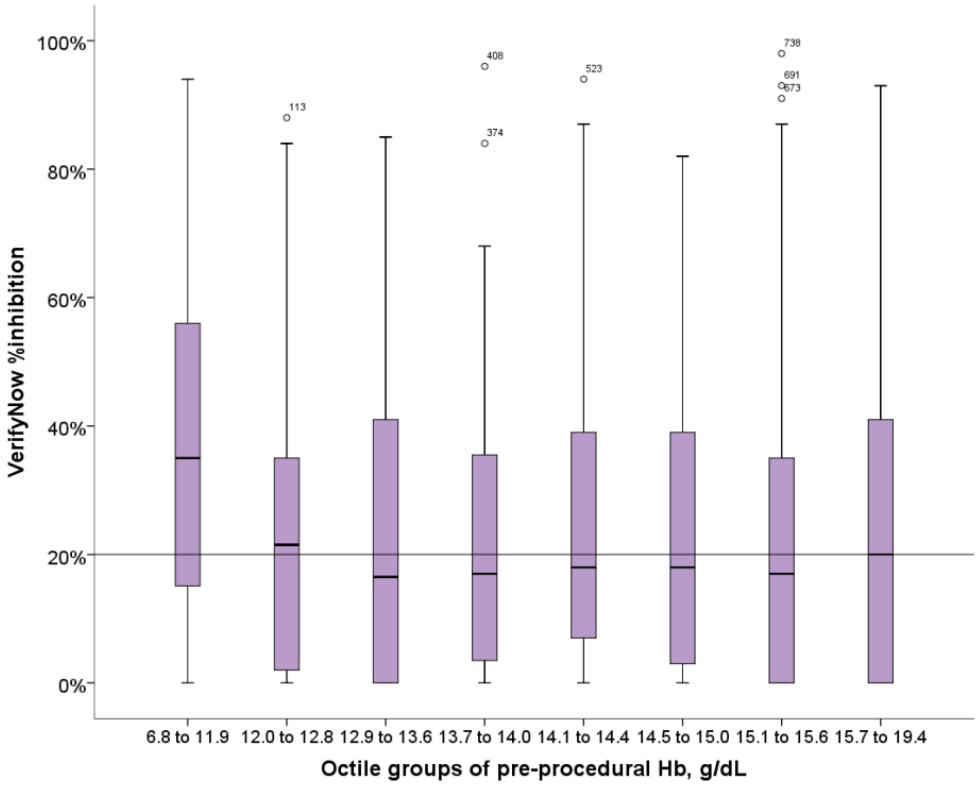



**Figure 7-8 Box and whisker plot of *VerifyNow %Inhibition* in patients stratified by pre-procedural haemoglobin level; horizontal line at median value**

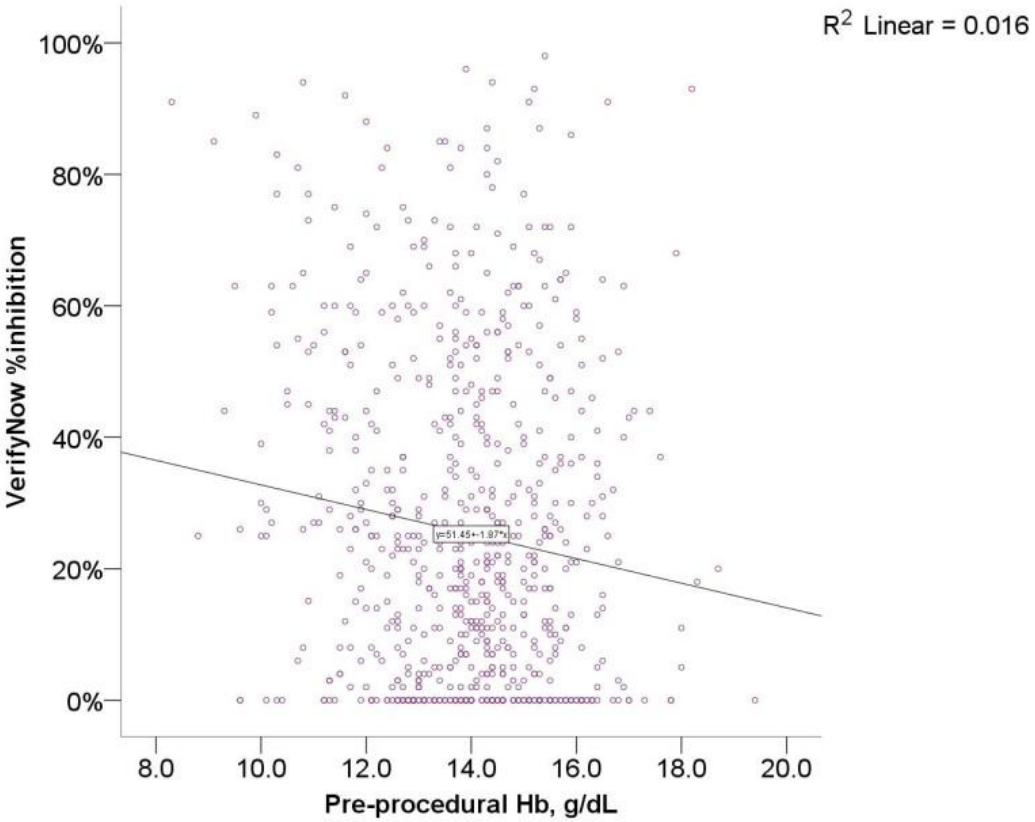



**Figure 7-9 Scatter plot of *VerifyNow %inhibition* levels against pre-procedural Hb in g/dL with automatic line of best fit**

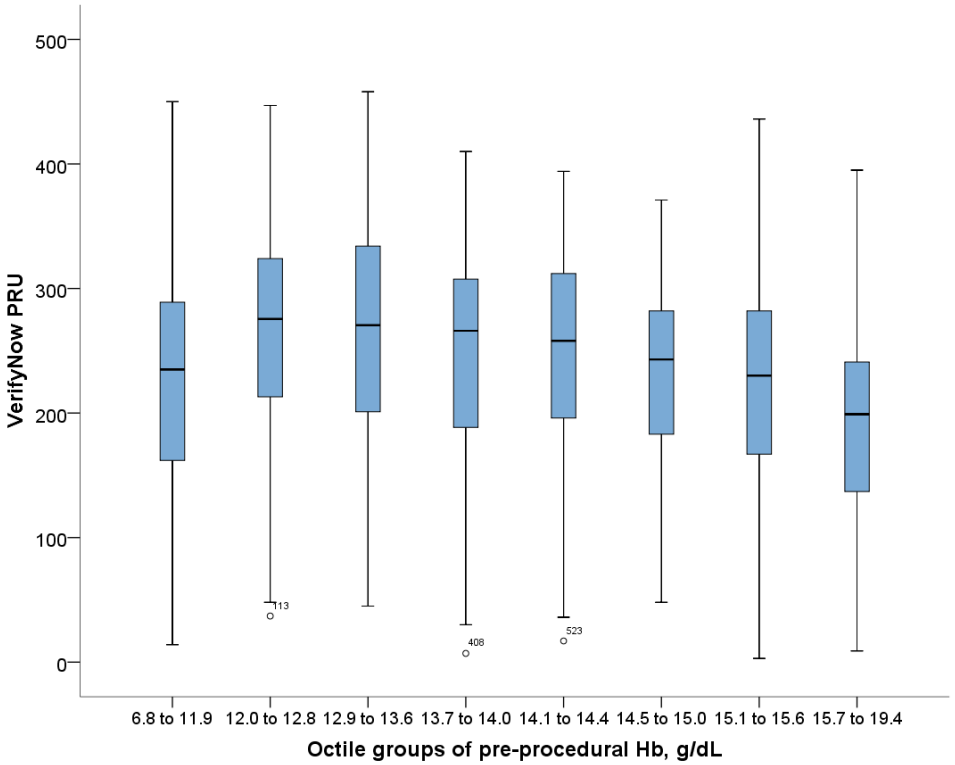



**Figure 7-10 Box and whisker plot of *VerifyNow PRU* in patients stratified by pre-procedural haemoglobin level**

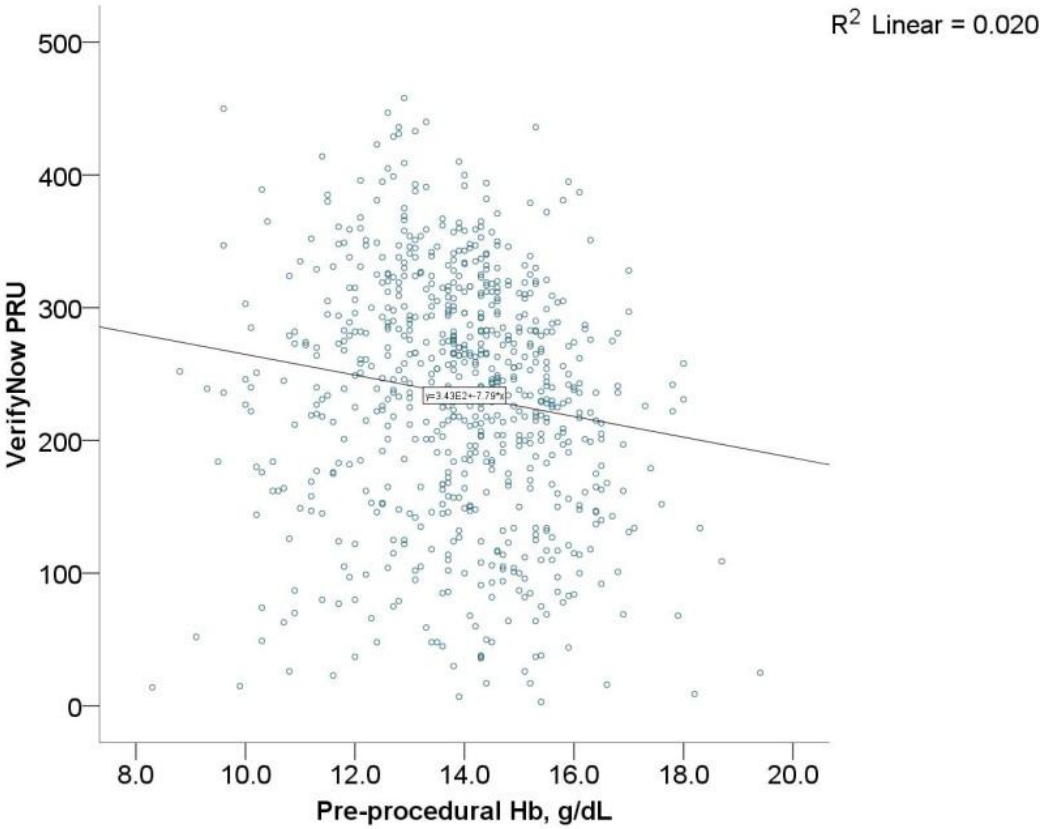



**Figure 7-11 Scatter plot showing the correlation with *VerifyNow* PRU and values with pre-procedural haemoglobin concentration, automatic line of best fit**

#### 7.1.2.5. Platelet count and platelet reactivity

Pre-procedural platelet count was weakly associated with baseline reactivity (rho = 0.132, $p < 0.01$ between Base and platelet count). The degree of platelet inhibition also increased with platelet count (rho = 0.195, $p < 0.01$ between %inhibition and platelet count), but there was little correlation between PRU and platelet count (rho = -0.089, $p = 0.02$).

#### 7.1.2.6. Body Mass Index and platelet reactivity

There was no significant correlation between BMI and the Base value. However, PRU and %inhibition were both weakly correlated with BMI (0.117, $p < 0.01$; and -0.158, $p < 0.01$), indicating less antiplatelet effect in larger patients.

#### 7.1.2.7. Blood pressure and heart rate

There was no correlation between platelet reactivity and either pre-procedural blood pressure or heart rate.

#### 7.1.2.8. SYNTAX score and platelet reactivity

There was no correlation between the angiographic severity/extent of coronary disease (SYNTAX score) and platelet reactivity.

#### 7.1.2.9. Diabetes and platelet reactivity

Patients with diabetes (DM) had higher platelet reactivity (both Base and PRU) and less %inhibition than those without. However, when baseline Hb level was taken into account, Base was not different between those with and without DM. Those with DM did seem to have higher %inhibition even when corrected for baseline Hb. However, patients with DM had worse renal function than those without: an eGFR <60 occurred in 40.7% with DM vs. 30.6% in those without, $p = 0.02$. Renal dysfunction tended to increase the effect of clopidogrel (see Section 7.1.2.4).Therefore, although platelet reactivity values did appear different in those with DM, this was likely due to differing renal function and Hb levels.

#### 7.1.2.10. Pre-procedural medication and platelet reactivity

In patients receiving proton pump inhibitors (PPI), platelet reactivity (both the Base and PRU values) was higher than in those not receiving PPIs, with no difference in level of inhibition. Note that anaemic (Hb < 12.0 g/dL) patients were more likely to be taking a PPI: 47.3% vs. 29.0%, $p < 0.01$, suggesting that, as with the examples above, any apparent association of PPI use and platelet reactivity may be confounded by the effect of Hb level on platelet reactivity.

There was no difference in platelet reactivity between patients receiving or not receiving ACE-inhibitors, ‘statins’ or beta-blockers.

#### 7.1.2.11. Patients with angiographically thrombotic lesions

Patients with angiographic thrombus had slightly lower baseline reactivity, and non-significantly higher PRU, with less platelet inhibition than those with non-thrombotic lesions.

## 7.2. Independent predictors of adequate response to clopidogrel

The binary logistic regression analysis of characteristics associated with PRU <238 are listed in Table 7-2. The only 2 independent predictors were pre-procedural Hb level and the VerifyNow Base value.

**Table 7-1 Baseline characteristics associated with adequate response to clopidogrel**

| Characteristic | Odds ratio | 95% confidence interval | P value |
|---|---|---|---|
| Use of proton pump inhibitor | 1.29 | 0.92 to 1.82 | 0.15 |
| Age over 80 years | 1.78 | 0.90 to 1.82 | 0.10 |
| VerifyNow Base value, per point | 0.98 | 0.98 to 0.99 | < 0.01 |
| PMH of diabetes | 1.34 | 0.89 to 2.17 | 0.15 |
| Pre-procedural Hb level, per g/dL | 0.82 | 0.72 to 0.93 | < 0.01 |

**Table 7-2 Base, PRU and *% inhibition* values in patients with and without selected dichotomous baseline characteristics**

| Characteristic | Median (IQR) Base value without characteristic | Median (IQR) Base value with characteristic | P value | Median (IQR) PRU without characteristic | Median (IQR) PRU with characteristic | P value | Median (IQR) *% inhibition* without characteristic | Median (IQR) *% inhibition* with characteristic | P value |
|---|---|---|---|---|---|---|---|---|---|
| Age ≥ 80 years | 305 (85) | 336 (72) | < 0.01 | 240 (125) | 265 (131) | 0.02 | 20 (39) | 18 (43) | 0.79 |
| Female | 296 (79) | 343 (69) | < 0.01 | 241 (117) | 246 (154) | 0.74 | 17 (35) | 28 (47) | < 0.01 |
| Diabetes | 303 (83) | 326 (92) | < 0.01 | 239 (130) | 271 (130) | < 0.01 | 20 (39) | 16 (32) | 0.02 |
| Smoker | 308 (96) | 309 (81) | 0.822 | 241 (137) | 244 (122) | 0.679 | 21 (45) | 19 (37) | 0.762 |
| 2nd phase of recruitment | 306 (88) | 318 (75) | 0.46 | 241 (129) | 254 (117) | 0.07 | 20 (39) | 17 (33) | 0.07 |
| Presented with UA | 308 (83) | 317 (94) | 0.12 | 241 (121) | 254 (154) | 0.48 | 20 (39) | 21 (41) | 0.71 |
| Presented with NSTEMI | 306 (95) | 309 (80) | 0.85 | 243 (150) | 241 (116) | 0.74 | 19 (40) | 20 (39) | 0.98 |
| Presented with STEMI | 309 (83) | 296 (103) | 0.06 | 243 (122) | 239 (148) | 0.78 | 20 (39) | 18 (42) | 0.73 |
| Previous IHD | 299 (83) | 314 (91) | < 0.01 | 241 (129) | 241 (126) | 0.90 | 20 (37) | 20 (42) | 0.45 |
| History of lung disease | 305 (84) | 318 (102) | 0.33 | 243 (123) | 229 (144) | 0.29 | 19 (40) | 26 (42) | 0.06 |
| eGFR < 30 | 305 (84) | 332 (100) | 0.05 | 243 (125) | 220 (177) | 0.03 | 20 (38) | 45 (51) | < 0.01 |
| PVD | 307 (86) | 305 (73) | 0.42 | 241 (130) | 255 (97) | 0.20 | 20 (40) | 15 (26) | 0.25 |
| Hb < 10 g/dL | 306 (84) | 335 (200) | 0.22 | 242 (126) | 236 (266) | 0.36 | 20 (39) | 44 (75) | 0.08 |
| PPI use | 299 (81) | 320 (85) | < 0.01 | 235 (133) | 254 (119) | < 0.01 | 20 (40) | 19 (30) | 0.27 |
| Statin use | 300 (102) | 306 (82) | 0.88 | 266 (132) | 240 (126) | 0.75 | 17 (32) | 20 (40) | 0.83 |
| Thrombotic lesion | 310 (62) | 294 (60) | 0.01 | 241 (124) | 256 (146) | 0.65 | 21 (39) | 14 (31) | 0.01 |

## 7.3. Recurrent MI and platelet reactivity

The median (IQR) PRU in patients with recurrent troponin-positive ACS, fatal MI or stent thrombosis (on-treatment events only) was higher than those patients without: 284 (129) vs. 241 (123), p = 0.04; see Fig. 7-12. The Base value was also higher in those with than without recurrent MI (325 (83) vs. 308 (84), p = 0.02). The *% inhibition* value was not significantly different (p = 0.27).

The rate of recurrent on-treatment MI in patients in the non-responder category ('ischaemic threshold' of PRU 238) was more than twice that seen in responders, shown in Fig. 7-13, although this was of borderline statistical significance. Note that nearly all the recurrent ACS in non-responders occurred within the first 5 months following PCI. These results are the same when an alternative ischaemic PRU threshold of 208 (ADEPT-DES registry) is used.

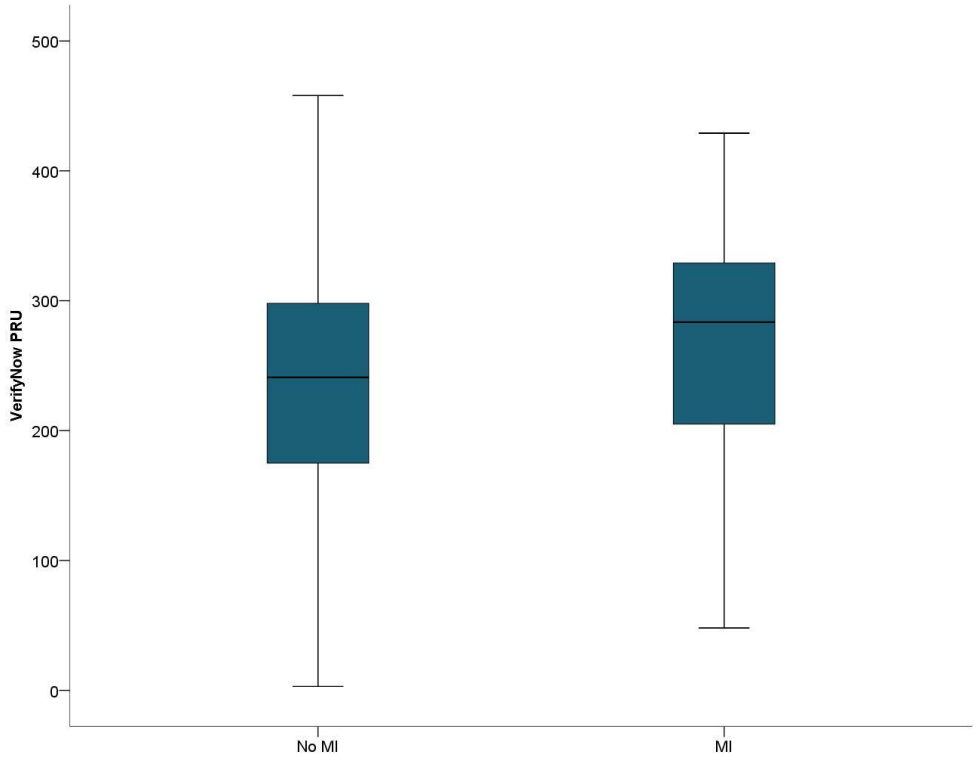



**Figure 7-12 Box-and-whisker plot showing the difference in PRU in patients with and without recurrent MI; on-treatment events only**

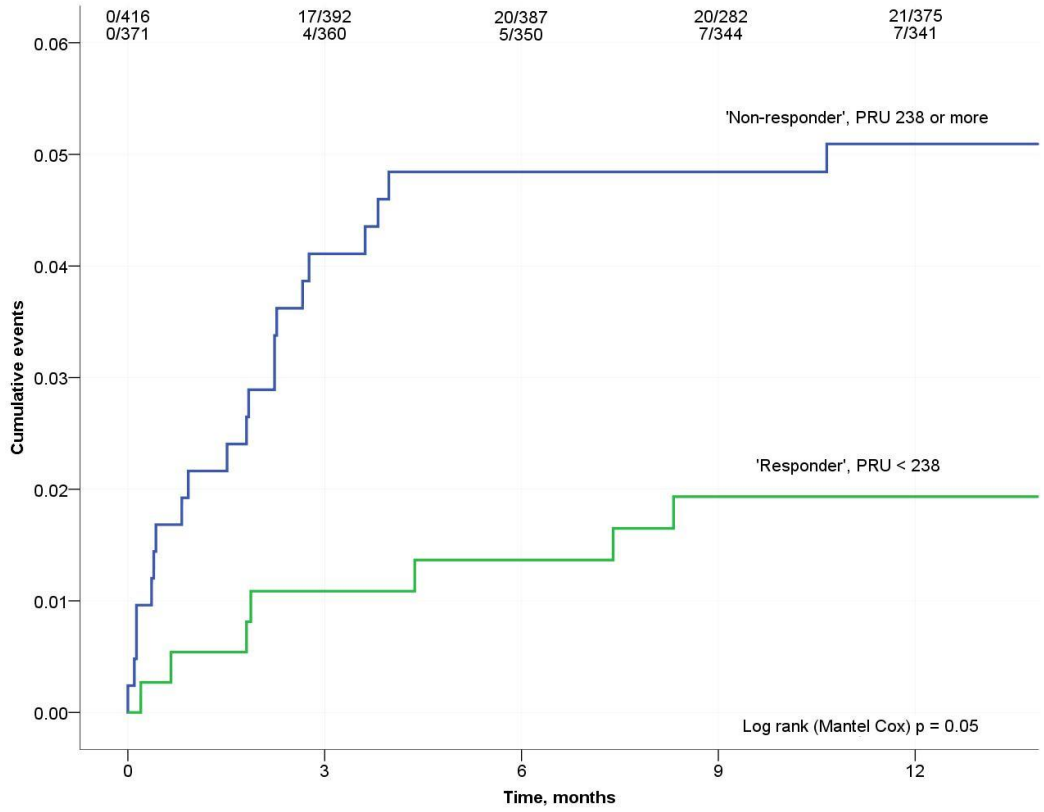



**Figure 7-13 Kaplan-Meier rates of recurrent MI in patients above and below an 'ischaemic PRU threshold' of 238; on-treatment events only**

The rate of on-treatment recurrent MI over long-term follow up by groups of PRU octiles can be seen in figure 7-14. Patients with PRU values in the upper ranges have the highest rates of recurrent MI. However, the relationship between PRU and recurrent MI is not linear: there is no apparent incremental benefit in terms of further reduction in MI rate in patients with PRU under a value of about 200.

PRU octiles were generated by automatic ranking; the 8 groups each contain approximately 100 subjects. Regarding baseline characteristics, these groups were fairly similar except for the highest octile, PRU 338 to 458. This group was generally higher risk for adverse events; for example they were older, had worse renal function, lower Hb and higher EuroSCORE.

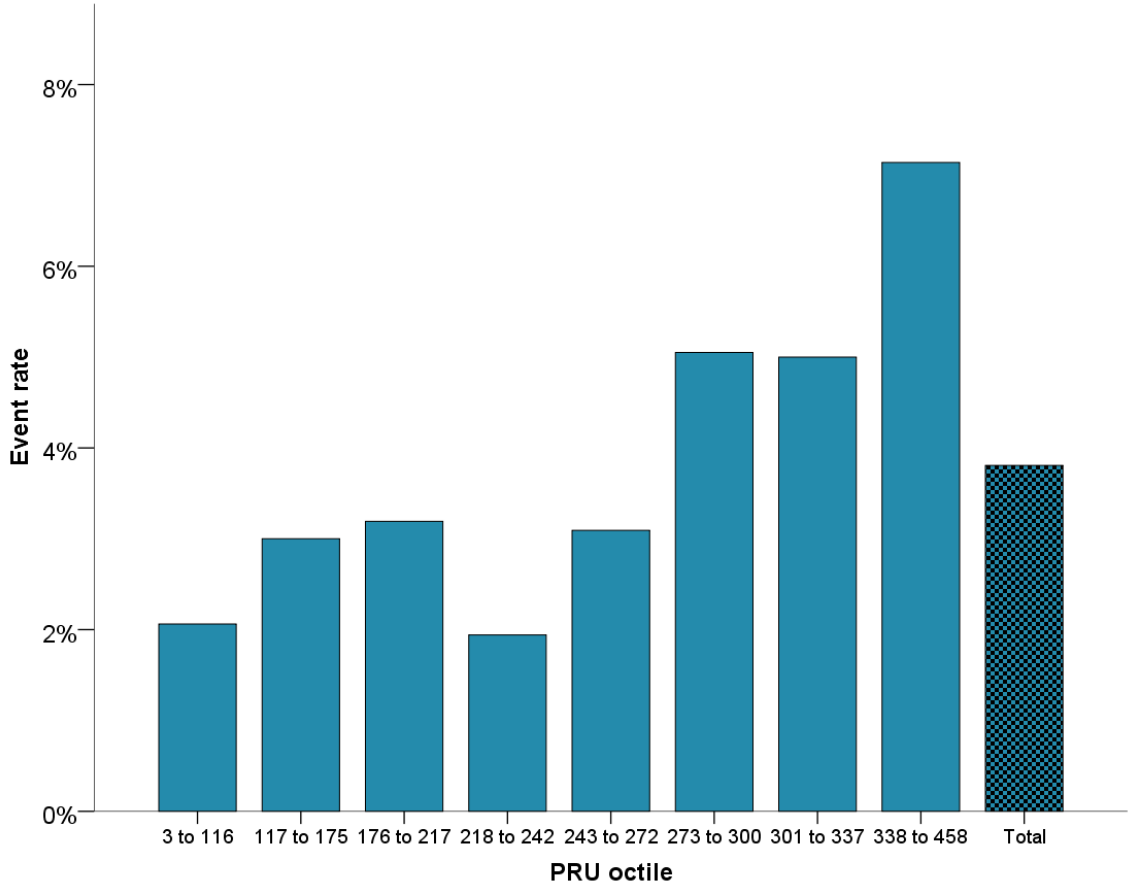



**Figure 7-14 Bar chart showing the rate of recurrent MI (troponin positive ACS, fatal MI and stent thrombosis) while on treatment with index P2Y12 inhibitor over long-term follow up**

Individual characteristics associated with recurrent troponin-positive ACS while on treatment with the index P2Y12 inhibitor are shown in Table 7-2. There was no association between recurrent MI and age, sex, BMI, clinical syndrome at presentation or stent type. The association of PRU and recurrent MI was of only borderline significance. After adjustment, SYNTAX score and diabetes were the 2 factors which independently predicted recurrent MI: aHR 1.05 (1.01 to 1.08, $p < 0.01$) per SYNTAX point and 2.70 (1.29 to 5.65, $p < 0.01$) if diabetic.

The apparent prognostic effect of SYNTAX score on recurrent MI rate was considerably stronger in clopidogrel resistant patients; the effect was still present in responders although event rate was approximately halved. This is shown in Figure 7-15.

**Table 7-3 Baseline characteristics associated with on-treatment recurrent MI in patients with platelet function test results**

| Characteristic | Hazard Ratio (unadjusted) | 95% C.I. | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| 2nd Phase of recruitment | 2.80 | 1.24 | 6.31 | 0.01 |
| Haemoglobin, per g/dL | 0.77 | 0.62 | 0.95 | 0.01 |
| Haemoglobin < 12 g/dL | 2.50 | 1.06 | 5.92 | 0.04 |
| History of heart failure | 4.90 | 1.17 | 20.64 | 0.03 |
| Previous revascularisation | 2.91 | 1.41 | 6.00 | < 0.01 |
| Previous IHD | 4.23 | 1.73 | 10.33 | < 0.01 |
| Diabetes | 3.21 | 1.56 | 6.61 | < 0.01 |
| Renal dysfunction, eGFR < 60 | 2.55 | 1.24 | 5.23 | 0.01 |
| PRU < 238 | 0.47 | 0.26 | 1.03 | 0.06 |
| PCI to LMS | 5.88 | 2.05 | 16.82 | < 0.01 |
| PCI to restenotic lesion | 4.02 | 1.40 | 11.49 | 0.01 |
| PCI to calcified lesion | 2.92 | 1.41 | 6.03 | < 0.01 |
| EuroSCORE, per point | 1.04 | 1.01 | 1.07 | < 0.01 |
| SYNTAX score, per point | 1.06 | 10.3 | 1.09 | < 0.01 |

### 7.3.1. Stent thrombosis and platelet reactivity

There were only 11 cases of stent thrombosis (any type) which occurred on the index P2Y12 inhibitor in the non-PPCI subgroup of 789 patients; all occurred within 1 year of PCI. The median (IQR) PRU in patients with and without stent thrombosis was 322 (64) vs. 241 (123), $p < 0.01$. The Base value was not different between patients with and without stent thrombosis ($p = 0.59$).

Regarding the ‘ischaemic threshold’, only 1 of the 11 patients with a stent thrombosis had a PRU under 238. This patient (PAT ID 405) had a PRU of 153 and suffered a subacute thrombosis of a saphenous vein graft stent at day 6 post PCI, treated successfully by thrombus aspiration and implantation of a further stent.

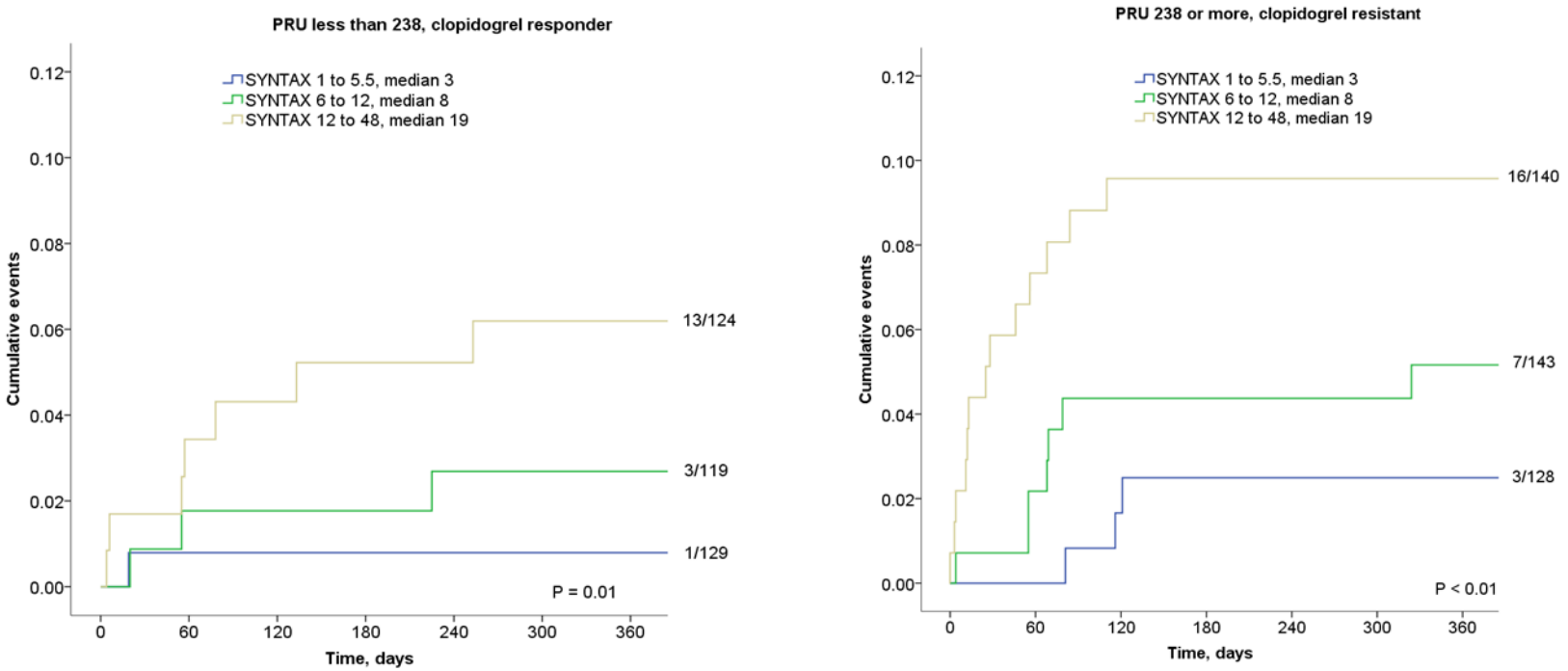



**Figure 7-15 KM estimates of recurrent MI (including fatal MI and stent thrombosis) stratified by SYNTAX tertile; population is divided into clopidogrel responders (left hand side graph) and non-responders (right hand side graph); patients were censored at cessation of index P2Y12 inhibitor so only on-treatment events shown; PPCI patients excluded**

### 7.3.2. Death and platelet reactivity

Of the 789 patients in this subgroup analysis, there were 34 all-cause and 19 cardiac on-treatment deaths over long term follow-up. Although on-treatment cardiac death rates were low (1.3% in responders vs. 2.4% and in non-responders at 1 year), there was a trend for a lower cardiac death rate in clopidogrel responders; log rank p = 0.06. The relationship between death rate and PRU was not clearly linear. All-cause and cardiac on-treatment death rates tended to be lowest in patients with PRU in the mid range, with higher rates in both patients with very high and very low PRU values, shown in Figures 7-15 and 7-16. There were no cases of on-treatment cardiac death during long-term follow up in patients with PRU within the range 132 to 246.

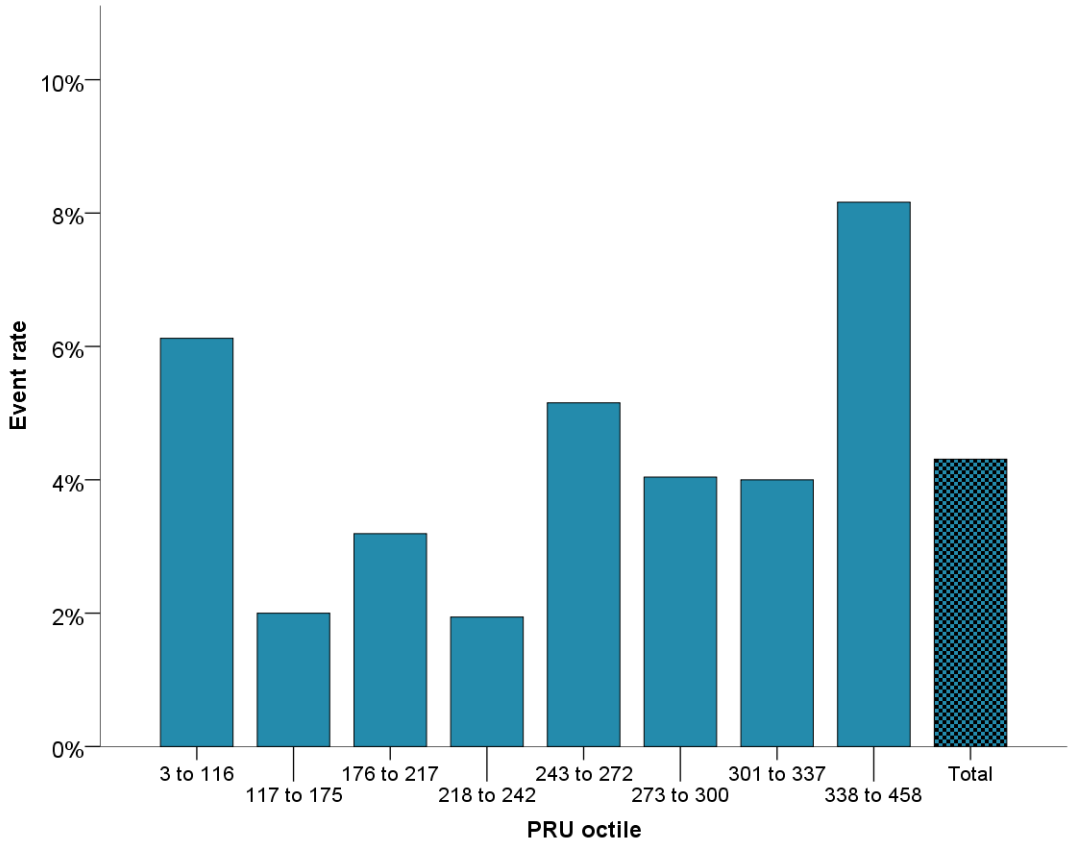



**Figure 7-16 Bar chart showing the rate of all-cause death while on treatment with index P2Y12 inhibitor over long-term follow up**

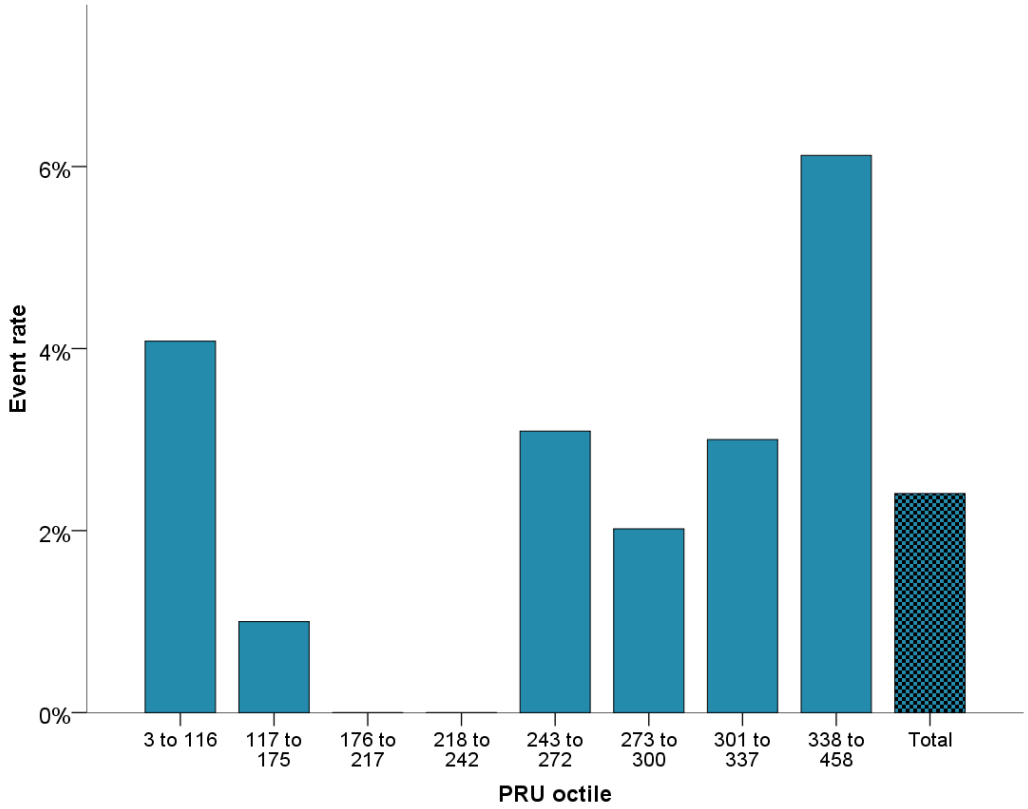



**Figure 7-17 Bar chart showing the rate of cardiac death while on treatment with index P2Y12 inhibitor over long-term follow up**

Regarding the 5 patients in the lower 4 PRU octiles who suffered a cardiac on-treatment death, the specific causes of death were:

- PAT ID 811: recurrent NSTEMIs, COPD exacerbation and heart failure
- PAT ID 611: contrast nephropathy leading to renal failure and pneumonia
- PAT ID 15: MI, heart failure
- PAT ID 38: massive gastro-intestinal bleed and MI
- PAT ID 406: sudden death at home; coroner ruled 'coronary atherosclerosis' as cause of death

Regarding the 8 patients in the lower 4 octiles who suffered a non-cardiac on-treatment death, specific causes were:

- PAT ID 27: cerebral infarction
- PAT ID 353: cancer
- PAT ID 224: cancer
- PAT ID 33: cancer
- PAT ID 892: cancer
- PAT ID 131: cancer

- PAT ID 165: hanging
- PAT ID 414: cancer

There seemed to be a higher rate of death from cancer in patients with intense $P2Y_{12}$ platelet inhibition, with the lowest rates in those with mild levels of inhibition; Figure 7-18.

### 7.3.3. Combined endpoint of cardiac death and recurrent MI

The rate of the combined endpoint of on-treatment stent thrombosis, recurrent MI and cardiac death according to PRU is shown in Figure 7-19. The lowest event rate occurs in patients with PRU within the range 176 to 242, which corresponds very closely with the ARMYDA-PROVE optimal range. Patients with very high platelet reactivity while on DAPT, with PRU within the range 338 to 458, had the worst prognosis. However, the association between this combined adverse endpoint and optimal PRU did not quite reach statistical significance: HR 0.4 (0.14 to 1.13, p = 0.08).

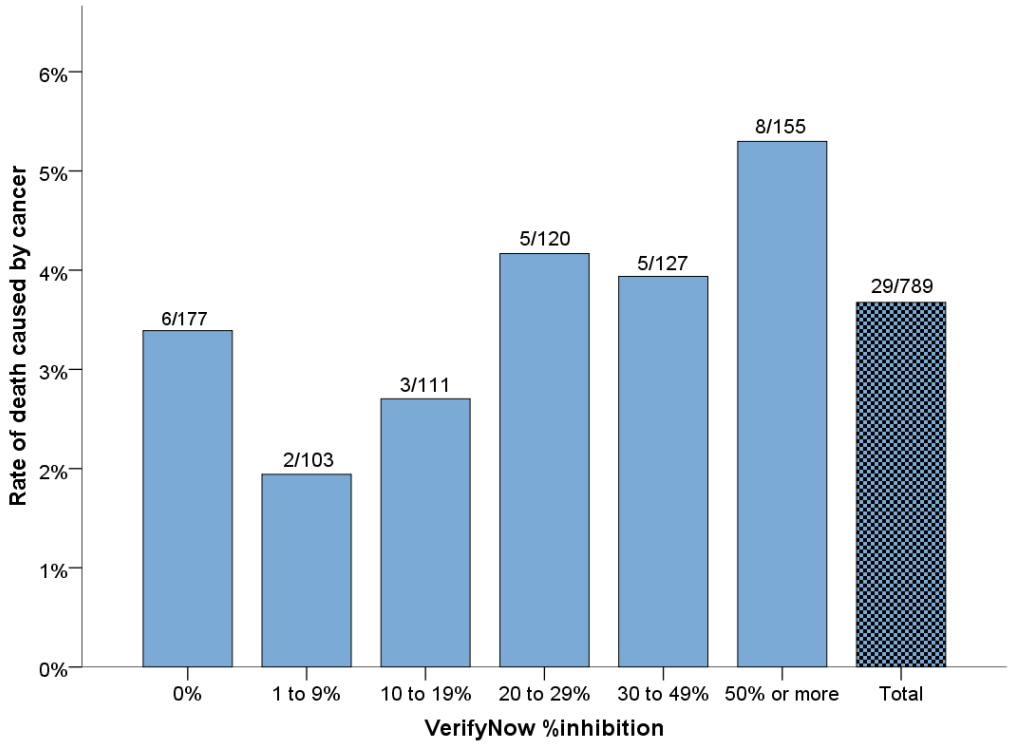



**Figure 7-18 Bar chart showing the rate of death caused by cancer over long-term follow up in patients stratified by VerifyNow %inhibition; number of deaths/number in group shown above each bar**

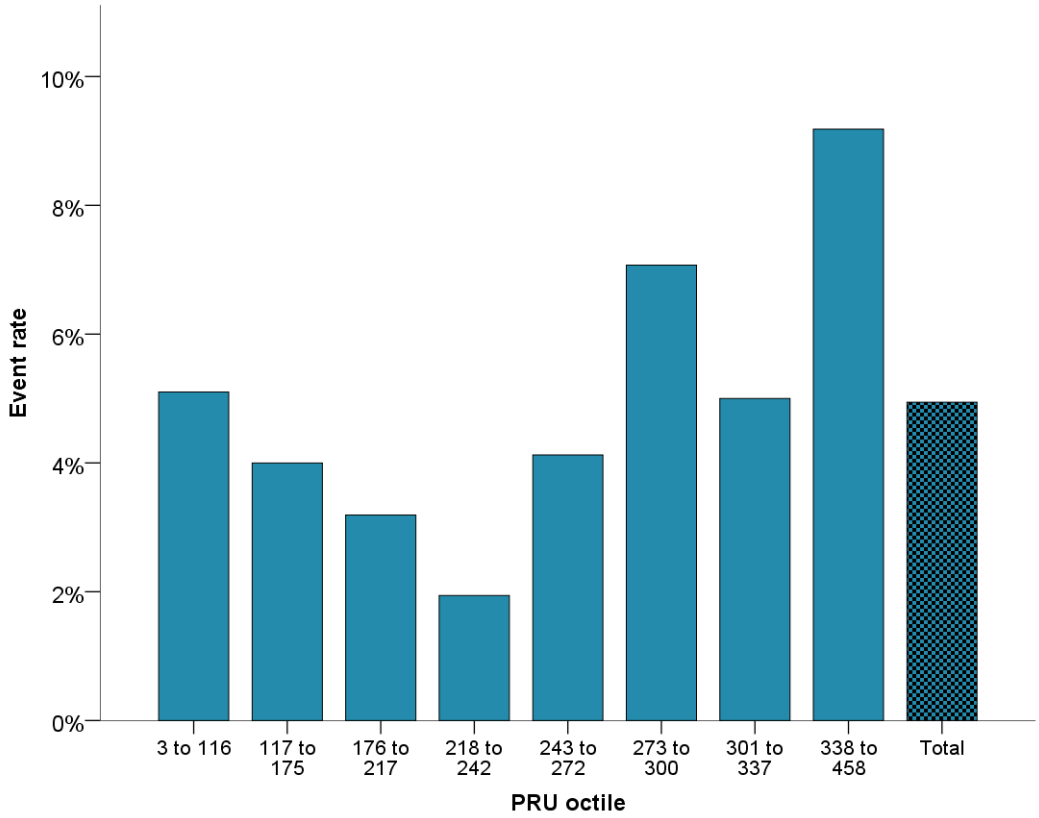



**Figure 7-19 Bar chart showing the rate of the combined endpoint of on-treatment recurrent MI, stent thrombosis and cardiac death over long term follow-up**

## 7.4. Platelet reactivity and stroke

There were 22 cases of stroke or TIA recorded over long-term follow up; only 5 cases occurred within the first year of PCI however. Other than 1 patient with bilateral subdural haematomas, all cases were due to cerebral infarction, although 2 of these underwent haemorrhagic transformation. The stroke rate was higher in patients in the highest 2 quartiles of Base reactivity (>350): 4.6% vs. 1.3%, $p < 0.01$.

## 7.5. Bleeding, anaemia and platelet reactivity

### 7.5.1. On-treatment bleeding and PRU

There were 36 cases of BARC 2 to 5 bleeding while on treatment with the index P2Y12 inhibitor over long term follow up. These bleeds occurred mainly from either the femoral puncture site or from the GI tract. The median PRU (IQR) in patients experiencing a BARC 2 to 5 bleed was non-significantly lower than in those without bleeding: 212 (179) vs. 243 (122), $p = 0.097$; see Figure 7-19. The median *%inhibition* (IQR) for the same groups was 32 (62) vs. 19 (38), $p = 0.02$; see figure 7-20. The bleeding rate was particularly high in patients with the lowest PRU, shown in Figure 7-21.

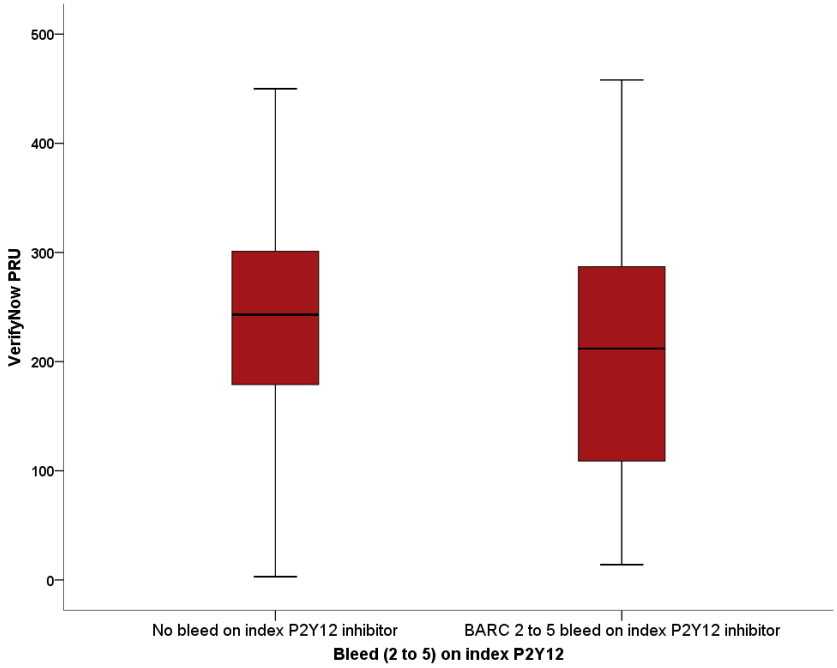



**Figure 7-20 Box and whisker plots showing the distribution of *'VerifyNow PRU'* in patients with and without BARC 2 to 5 bleeding; only events while patient on index P2Y12 inhibitor included**

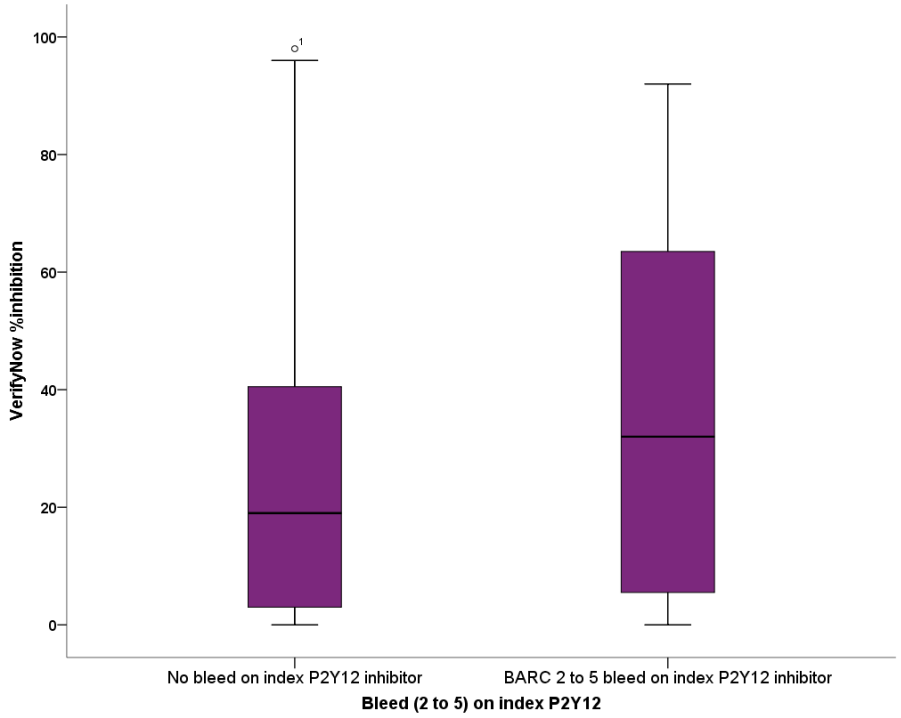



**Figure 7-21 Box and whisker plots showing the distribution of *'VerifyNow %inhibition'* in patients with and without BARC 2 to 5 bleeding; only events while patient on index P2Y12 inhibitor included**

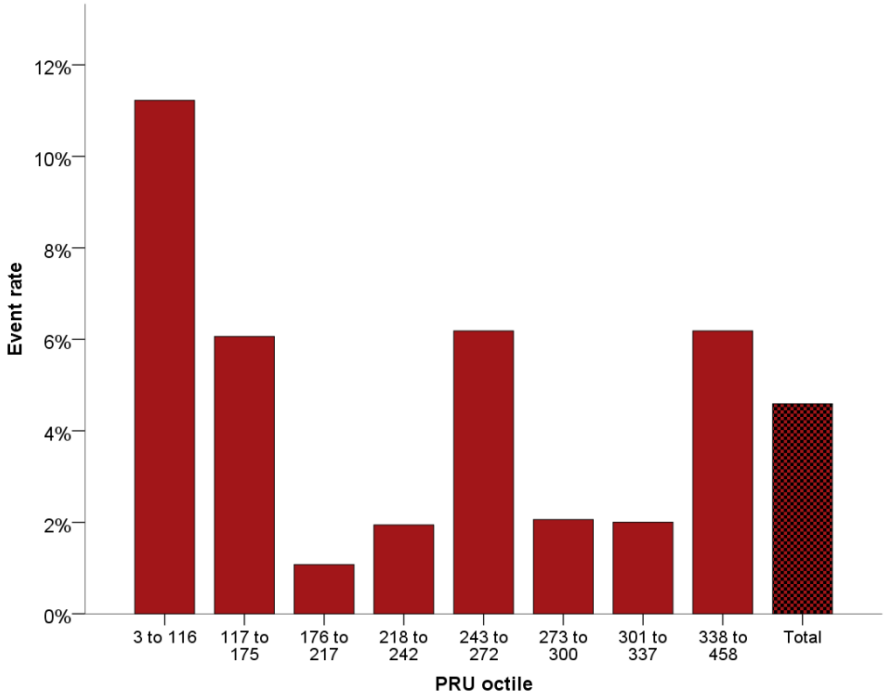



**Figure 7-22 Bar chart showing proportion of patients experiencing BARC 2 to 5 bleeding while on treatment with the index P2Y12 inhibitor according to PRU**

When patients were stratified into groups above and below the ARMYDA-PROVE (Mangiacapra et al, 2012) 'bleeding threshold' of PRU 179 significantly more BARC 2 to 5 bleeding occurred in hyper-responding patients (below 179) than in other patients, illustrated in the KM survival curve, Figure 7-21 [N.B. patients were censored at the first BARC 2 to 5 bleed, cessation of the index P2Y12 inhibitor, last follow-up or death]. There was a high early bleeding rate in hyper-responders, mostly due to femoral haematoma, but there also appears to be a higher rate of bleeding over long term follow up.

Baseline characteristics of patients in these 2 PRU groups are compared in Table 7-4. There was no difference in age, sex, EuroSCORE or SYNTAX score between the 2 groups. However, hyper-responding patients:

- weighed nearly 4 kg less on average than the other patients
- had only half the incidence of diabetes
- were more likely to have poor renal function
- were less likely to be on treatment with proton pump inhibitors
- had higher pre-procedural Hb concentration
- had higher pre-procedural platelet counts
- were more likely to receive a procedural IIbIIIa inhibitor

The characteristics individually associated with on-treatment BARC 2 to 5 bleeding, excluding patients receiving PPCI, are listed in Table 7-5. After adjustment,

the factors independently associated with bleeding were a history of heart failure, PRU below 179 and a history of hypertension. The use of ACE inhibitors and increasing haemoglobin concentration were both independently associated with lower bleeding rates. Age was not independently associated with bleeding after adjustment, either as a continuous variable per year or as a categorical variable as age > 80 years (Table 7-6).

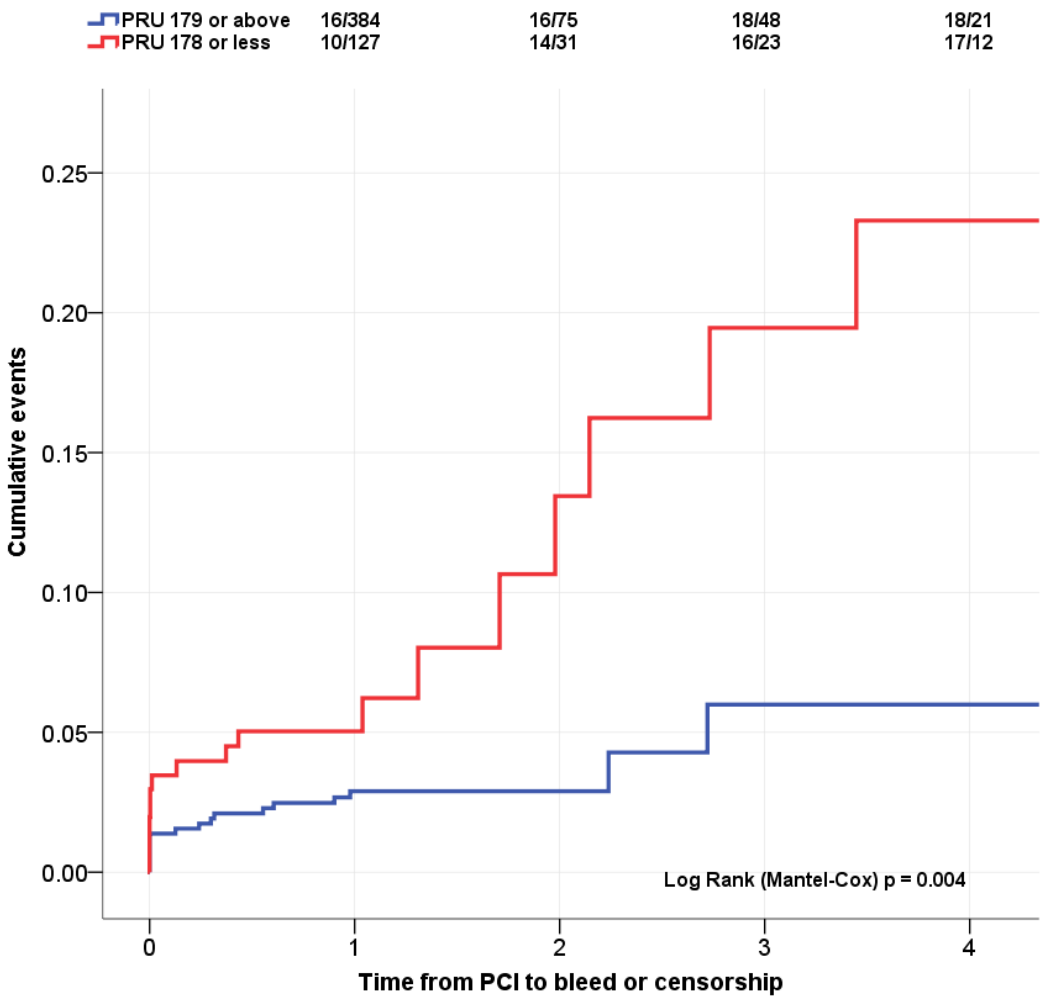



**Figure 7-23 Cumulative incidence of BARC 2 to 5 bleeding while on index P2Y12 inhibitor, stratified into groups above (n = 580) and below (n = 203) PRU 179; PPCI patients excluded**

**Table 7-4 Table comparing baseline characteristics in P2Y12 inhibitor 'hyper-responding' patients (PRU < 179) with patients with PRU ≥ 179**

| Baseline characteristic | PRU <179<br>N = 204 | PRU ≥179<br>N = 585 | P value |
|---|---|---|---|
| *Demographics and clinical syndrome* | | | |
| Mean (SD) Age, years | 61.4 (12.4) | 62.6 (11.9) | 0.20 |
| Age ≥ 80 years | 12 (5.9%) | 45 (7.7%) | 0.39 |
| Female | 58 (28.4%) | 139 (23.8%) | 0.18 |
| Median (IQR) weight, kg | 78.0 (20.4) | 81.6 (19.3) | < 0.01 |
| Median (IQR) Body Mass Index | 26.8 (6) | 28.3 (6) | < 0.01 |
| Any history of smoking | 141 (69.1%) | 405 (69.7%) | 0.875 |
| Presented with unstable angina | 31 (15.2%) | 86 (14.7%) | 0.864 |
| Presented with NSTEMI | 138 (67.6%) | 420 (71.8%) | 0.262 |
| Presented with STEMI | 35 (17.2%) | 79 (13.5%) | 0.20 |
| Median (IQR) EuroSCORE | 2.8 (4.3) | 2.8 (4.2) | 0.43 |
| Median (IQR) SYNTAX score | 9.0 (10.8) | 8.0 (11.0) | 0.55 |
| First phase of recruitment | 175 (85.8%) | 472 (80.7%) | 0.10 |
| Previous medical History | | | |
| Previous revascularisation, any | 33 (16.2%) | 105 (17.9%) | 0.56 |
| Previous clinical IHD, any | 104 (51%) | 291 (49.7%) | 0.76 |
| Diabetes | 21 (10.3%) | 113 (19.4%) | < 0.01 |
| Hypertension | 92 (45.1%) | 314 (53.7%) | 0.04 |
| Stroke | 7 (3.4%) | 26 (4.4%) | 0.53 |
| Heart failure | 6 (2.9%) | 8 (1.4%) | 0.14 |
| *Blood results (pre-procedural)* | | | |
| Median (IQR) serum Cr concentration, µmol/L | 96 (24) | 94 (25) | 0.95 |
| Median (IQR) eGFR, ml/min/1.73m$^2$ | 72 | 76 | 0.15 |
| eGFR < 60 ml/min/1.73m$^2$ | 69 (34.3%) | 179 (31.5%) | 0.46 |
| eGFR <30 ml/min/1.73 m$^2$ | 14 (7.0%) | 17 (3.0%) | 0.01 |
| Cr >200 µmol/L | 10 (4.9%) | 13 (2.2%) | 0.05 |

| | | | |
|---|---|---|---|
| Median (IQR) Hb concentration, g/dL | 14.3 (2.6) | 14.0 (2.0) | 0.05 |
| Hb <12 g/dL | 29 (15.3%) | 61 (11.4%) | 0.17 |
| Median (IQR) platelet count, $x10^9$/L | 252 (80) | 244 (82) | 0.05 |
| Median (IQR) white cell count, $x10^9$/L | 8.0 (2.8) | 8.0 (3.0) | 0.97 |
| Median (IQR) PRU *(VerifyNow)* | 118 (69) | 273 (87) | |
| *Procedural medication* | | | |
| Aspirin | 196 (96.6%) | 564 (96.7%) | 0.90 |
| Clopidogrel | 200 (98.0%) | 585 (100%) | < 0.01 |
| Prasugrel | 4 | 0 | |
| Heparin | 190 (93.1%) | 541 (92.6%) | 0.81 |
| IIbIIIa inhibitor | 68 (33.3%) | 152 (26.0%) | 0.04 |
| Bivalirudin | 15 (7.4%) | 47 (8.1%) | 0.76 |
| ACE inhibitor | 136 (69.7%) | 404 (73.2%) | 0.36 |
| Beta blocker | 157 (80.5%) | 456 (81.7%) | 0.71 |
| HMG-CoA reductase inhibitor | 183 (89.7%) | 524 (89.6%) | 0.96 |
| Proton pump inhibitor | 50 (24.5%) | 207 (35.4%) | < 0.01 |
| Median (IQR) time on index P2Y12 inhibitor, days | 366 (130) | 366 (73) | 0.91 |
| *Procedural characteristics* | | | |
| Femoral access | 179 (87.7%) | 491 (83.9%) | 0.19 |
| DES used | 119 (58.3%) | 331 (56.6%) | 0.66 |
| More than 1 vessel treated | 48 (23.5%) | 127 (21.7%) | 0.59 |
| Calcified lesion | 43 (21.1%) | 109 (18.6%) | 0.45 |
| Thrombotic lesion | 36 (17.6%) | 96 (16.4%) | 0.68 |
| TIMI flow < III in any vessel at start | 5 (2.5%) | 22 (3.8%) | 0.38 |
| Procedural complication, any | 29 (14.2%) | 89 (15.2%) | 0.73 |
| Procedural complication, angiographic | 24 (11.8%) | 83 (14.2%) | 0.38 |
| Procedural complication, clinical (non-haemorrhagic) | 3 (1.5%) | 3 (0.5%) | 0.175 |

**Table 7-5 Table showing univariable characteristics associated with BARC 2 to 5 bleeding before adjustment; events on index P2Y12 inhibitor only; PPCI patients excluded**

| | Hazard Ratio (as univariate) | 95% C.I. | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Haemoglobin, per g/dL | 0.65 | 0.55 | 0.77 | < 0.01 |
| ACE inhibitor | 0.42 | 0.22 | 0.79 | < 0.01 |
| History of heart failure | 9.12 | 3.22 | 25.83 | < 0.01 |
| More than 1 vessel treated | 2.11 | 1.11 | 4.00 | 0.02 |
| Age, per year | 1.08 | 1.05 | 1.11 | < 0.01 |
| Female sex | 3.16 | 1.70 | 5.87 | < 0.01 |
| Weight, per kg | 0.98 | 0.95 | 1.00 | 0.02 |
| Previous IHD | 2.71 | 1.36 | 5.43 | < 0.01 |
| Hypertension | 2.23 | 1.14 | 4.39 | 0.02 |
| Renal dysfunction, eGFR < 30 | 5.36 | 2.24 | 12.85 | < 0.01 |
| Smoking | 0.50 | 0.27 | 0.92 | 0.03 |
| PRU < 179 | 2.75 | 1.42 | 5.34 | < 0.01 |
| PCI to LMS | 6.59 | 2.76 | 15.71 | < 0.01 |
| | | | | |
| EuroSCORE, per point | 1.06 | 1.04 | 1.09 | < 0.01 |
| SYNTAX score, per point | 1.05 | 1.02 | 1.08 | < 0.01 |

**Table 7-6 Baseline characteristics independently associated with BARC 2 to 5 bleeding while on index P2Y12 inhibitor**

| Characteristic | Hazard Ratio | 95% C.I. | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Pre-procedural haemoglobin, per g/dL | 0.769 | 0.632 | 0.934 | < 0.01 |
| ACE inhibitor use | 0.444 | 0.218 | 0.902 | 0.03 |
| History of heart failure | 4.638 | 1.348 | 15.955 | 0.02 |
| History of hypertension | 2.24 | 1.020 | 4.922 | 0.05 |
| PRU < 179 | 2.775 | 1.365 | 5.641 | < 0.01 |

## 7.6. Anaemia and adverse events

Platelet reactivity and Hb level had a strong inverse relationship in patients in the OPERA study, as discussed in section 7.1.2. Anaemic patients were more likely to experience BARC 2 to 5 bleeding, as discussed in Chapter 6. The bleeding rates for the cohort of OPERA patients who had platelet function testing (as studied in this chapter so far) relative to baseline Hb level are shown in Figure 7-24.

Anaemia was also associated with a higher rate of non-haemorrhagic cardiovascular events, as discussed in Chapter 5. Figures 7-25 to -27 show the rates of recurrent MI, all-cause death and combined cardiac death and MI over long-term follow-up in patients stratified by Hb octile. The baseline Hb level was a strong risk factor for death in this study: over a median follow up of 3.7 years, 1 third of patients with a Hb level of < 12 g/dL at recruitment had died.

The 3-way relationship between platelet reactivity, anaemia and adverse events is illustrated in Figure 7-28, showing by far the highest risk patients over long-term follow up are those who have the combination of anaemia and high platelet reactivity. The 91 patients in this group had a 70% event rate. In contrast, anaemic patients with low platelet reactivity had a relatively good prognosis, as did those with high platelet reactivity who were not anaemic.

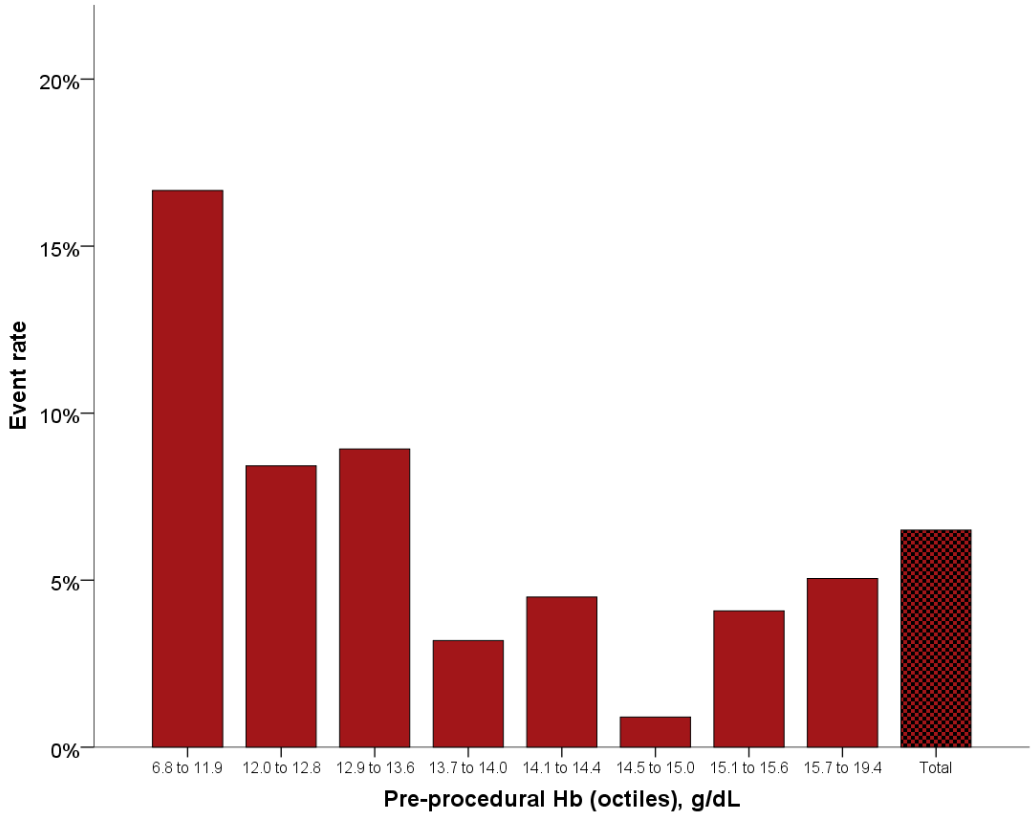



**Figure 7-24 Rates of BARC 2 to 5 bleeding during long-term follow-up stratified by pre-procedural Hb level**

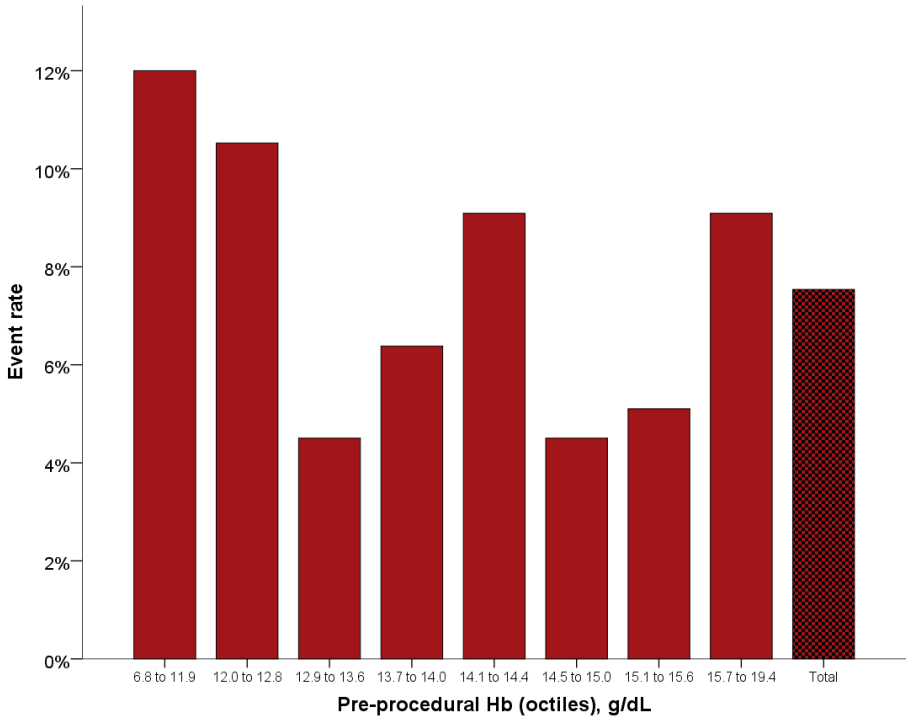



**Figure 7-25 Rates of recurrent troponin positive acute coronary syndrome during long-term follow-up stratified by pre-procedural Hb level**

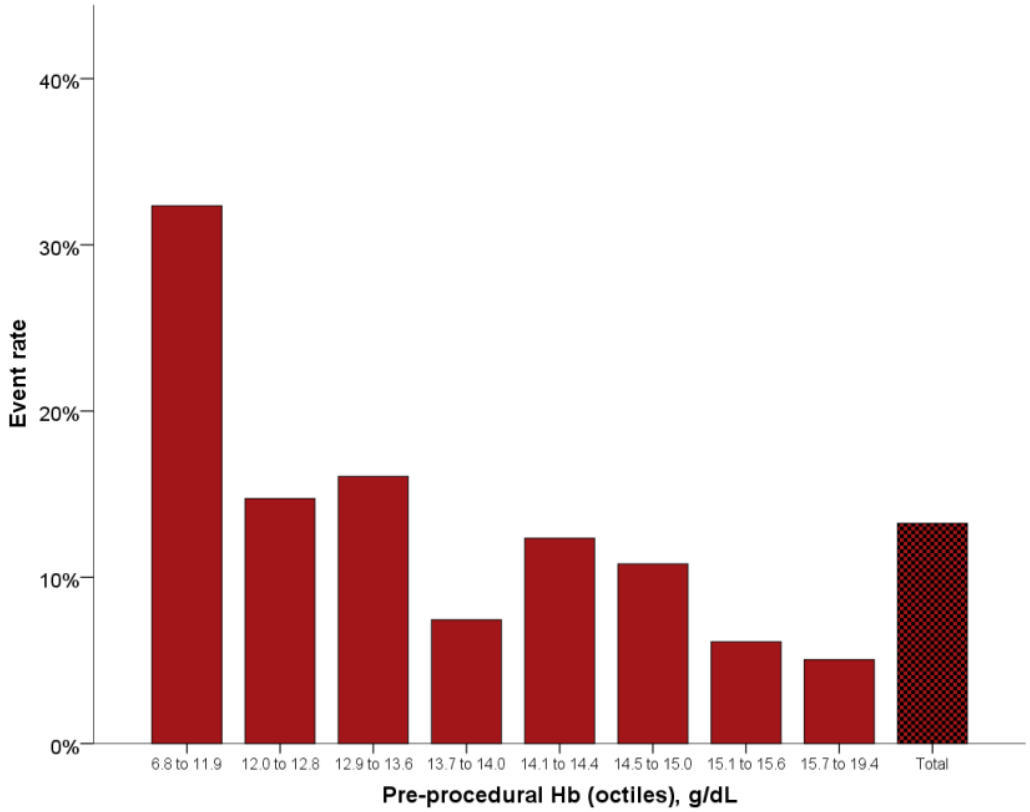



**Figure 7-26 Rates of all-cause death over long-term follow-up stratified by pre-procedural Hb level**

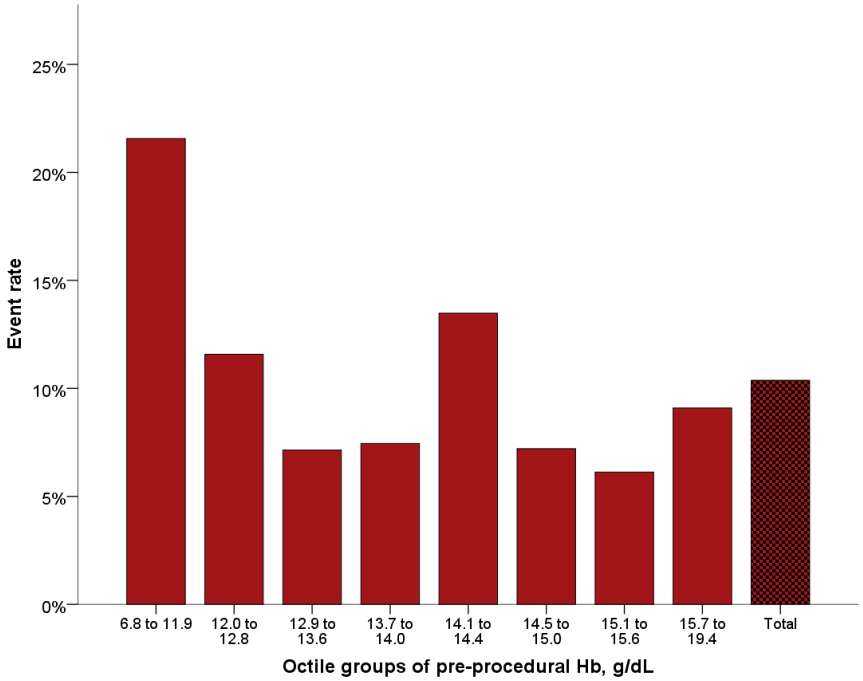



**Figure 7-27 Rates of the combined endpoint of cardiac death and MI during long-term follow-up, stratified by pre-procedural Hb level**

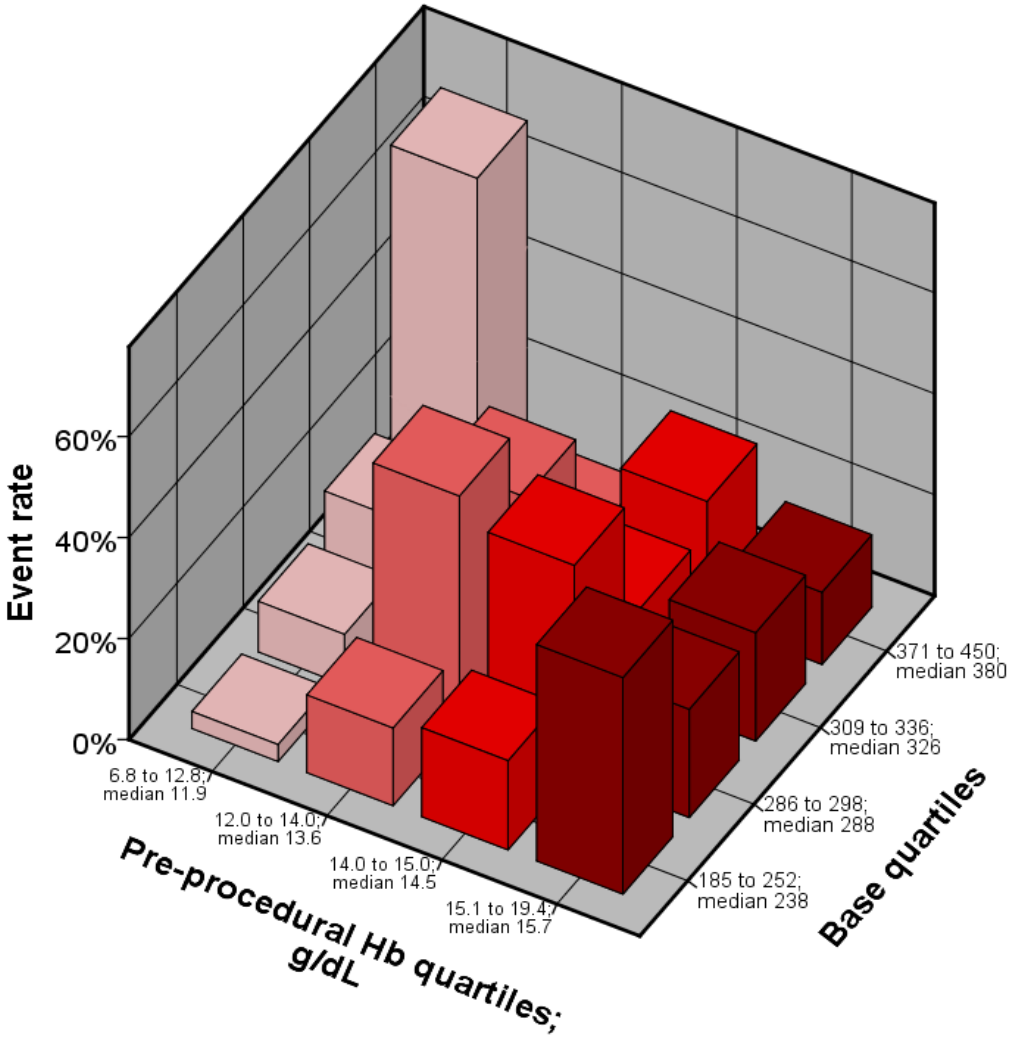



**Figure 7-28 3-dimensional bar chart showing the combined rate of MI and cardiac death over long term follow up; patients are stratified into 16 groups based on quartiles of pre-procedural Hb and quartiles of VerifyNow Base platelet reactivity**

## 7.7. Combined ischaemic and haemorrhagic adverse endpoint and PRU

The rate of the combined adverse endpoint of on-treatment BARC 2 to 5 bleeding, cardiac death, MI and stent thrombosis over long-term follow up according to PRU is shown in Figure 7-29. The lowest combined event rate occurs in patients with PRU 176 to 242; patients in the lowest and highest PRU octiles have equally high event rates.

Figure 7-30 shows this combined event rate over the first year from PCI for the cohort when patients are divided into the 3 groups determined by the 'ARMYDA-PROVE' investigators: hyper-responders (PRU < 179), optimal range PRU and hypo-responders (PRU > 238).

The characteristics associated with the combined adverse endpoint are shown in Table 7-7. After adjustment, the strongest independent predictors of the combined adverse event were non-optimal PRU, SYNTAX score and decreasing Hb. In a model with these 3 parameters, adjusted HRs for optimal PRU, SYNTAX score per point and Hb per g/dL were 0.35 (0.13 to 0.96, p = 0.04), 1.03 (1.01 to 1.06, p = 0.02) and 0.80 (0.68 to 0.97, p < 0.01).

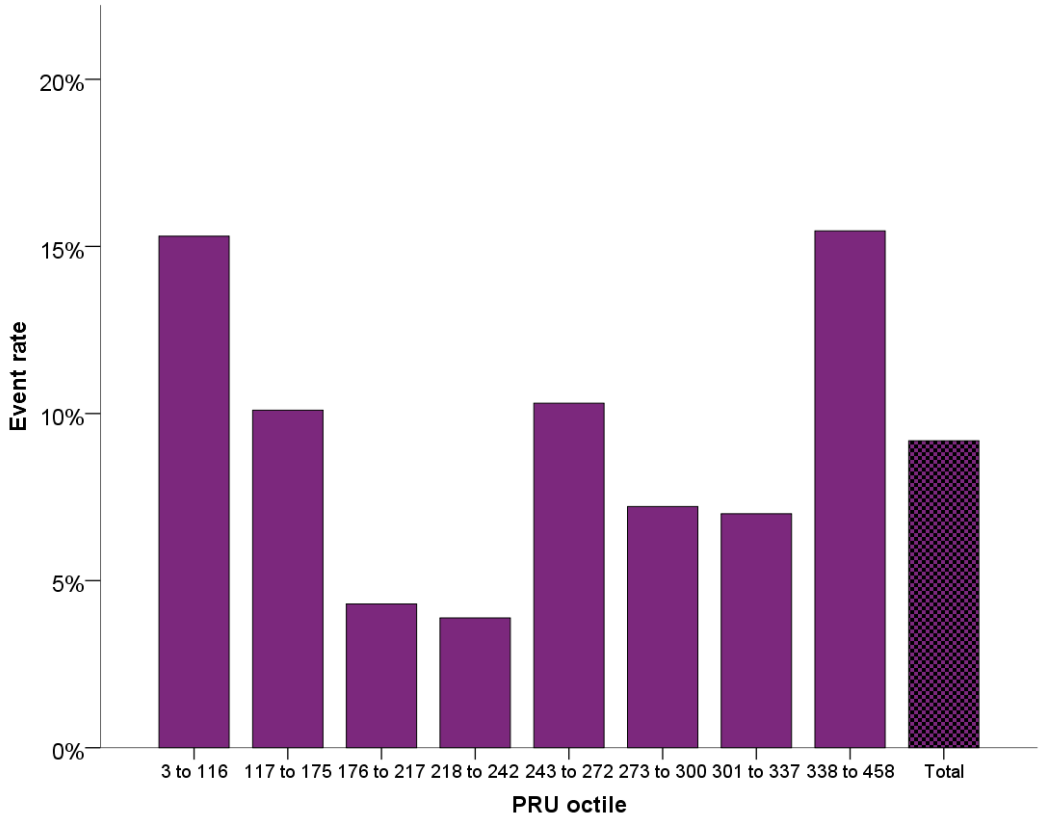



**Figure 7-29 Rate of the combined adverse on-treatment event rate of BARC 2 to 5 bleeding, cardiac death, MI and stent thrombosis over long term follow up stratified by PRU octile**

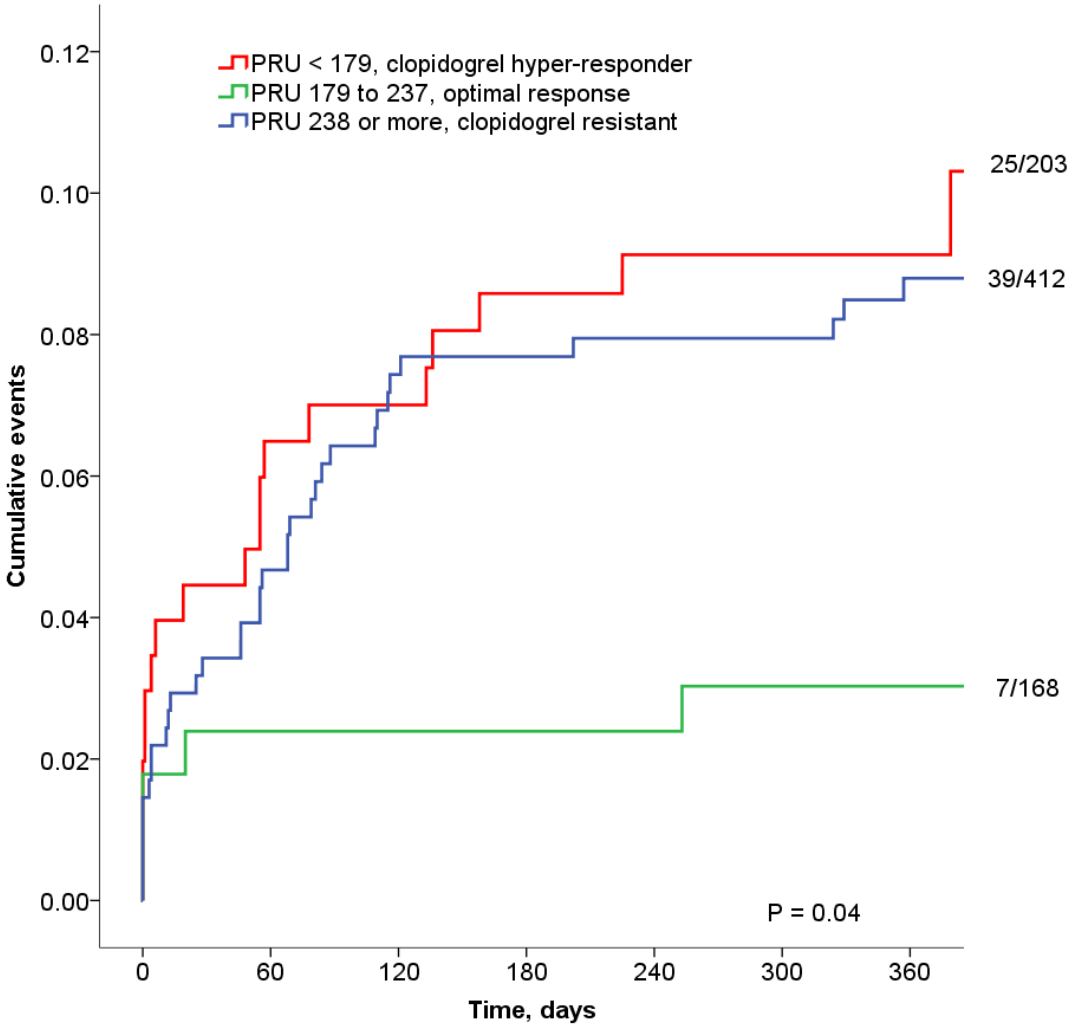



**Figure 7-30 Kaplan-Meier estimates of combined adverse events rates (cardiac death, MI, stent thrombosis or BARC 2 to 5 bleeding) for patients stratified into 3 PRU groups as defined by the ARMYDA-PROVE investigators; censored on cessation of index P2Y12 inhibitor**

## 7.1. Long-term off-treatment ischaemic adverse events and baseline platelet reactivity

Patients with higher Baseline platelet reactivity appeared to have a worse long-term prognosis after coming off DAPT, with higher rates of MI, cardiac death and stroke. Figure 7-30 shows the estimated rates of the combined adverse endpoint of off-treatment stroke, MI and cardiac death in patients stratified into Base quartiles. In a model with VerifyNow Base, SYNTAX score and EuroSCORE, Base was an independent predictor of this combined endpoint: aHRs (95% C.I.s) 1.53 (1.21 to 1.92) per 50 Base points, $p < 0.01$; 1.05 (1.02 to 1.08) per EuroSCORE point, $p < 0.01$; and 1.04 (1.01 to 1.07) per SYNTAX score point, $p = 0.02$.

**Table 7-7 Table showing characteristics associated with 1 year on-treatment combined adverse endpoint**

| Characteristic | Hazard Ratio (as univariate) | 95% C.I. | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| 2nd recruitment phase | 1.83 | 1.06 | 3.17 | 0.03 |
| Presented with NSTEMI | 2.12 | 1.08 | 4.16 | 0.03 |
| Presented with STEMI (convalescent PCI) | 0.30 | 0.09 | 0.95 | 0.04 |
| Haemoglobin, per g/dL | 0.71 | 0.61 | 0.82 | < 0.01 |
| ACE inhibitor | 0.57 | 0.34 | 0.95 | 0.03 |
| History of heart failure | 7.79 | 3.35 | 18.10 | < 0.01 |
| Age, per year | 1.05 | 1.02 | 1.07 | < 0.01 |
| Female sex | 1.70 | 1.01 | 2.87 | 0.05 |
| BMI, per point | 0.93 | 0.88 | 0.99 | 0.02 |
| Previous revascularisation | 2.27 | 1.33 | 3.86 | < 0.01 |
| Renal dysfunction, eGFR < 60 | 2.92 | 1.75 | 4.86 | < 0.01 |
| Optimal PRU | 0.34 | 0.13 | 0.84 | 0.02 |
| PCI to LMS | 5.06 | 2.30 | 11.12 | < 0.01 |
| PCI to calcified lesion | 2.58 | 1.54 | 4.31 | < 0.01 |
| EuroSCORE, per point | 1.05 | 1.04 | 1.07 | < 0.01 |
| SYNTAX score, per point | 1.05 | 1.03 | 1.08 | < 0.01 |

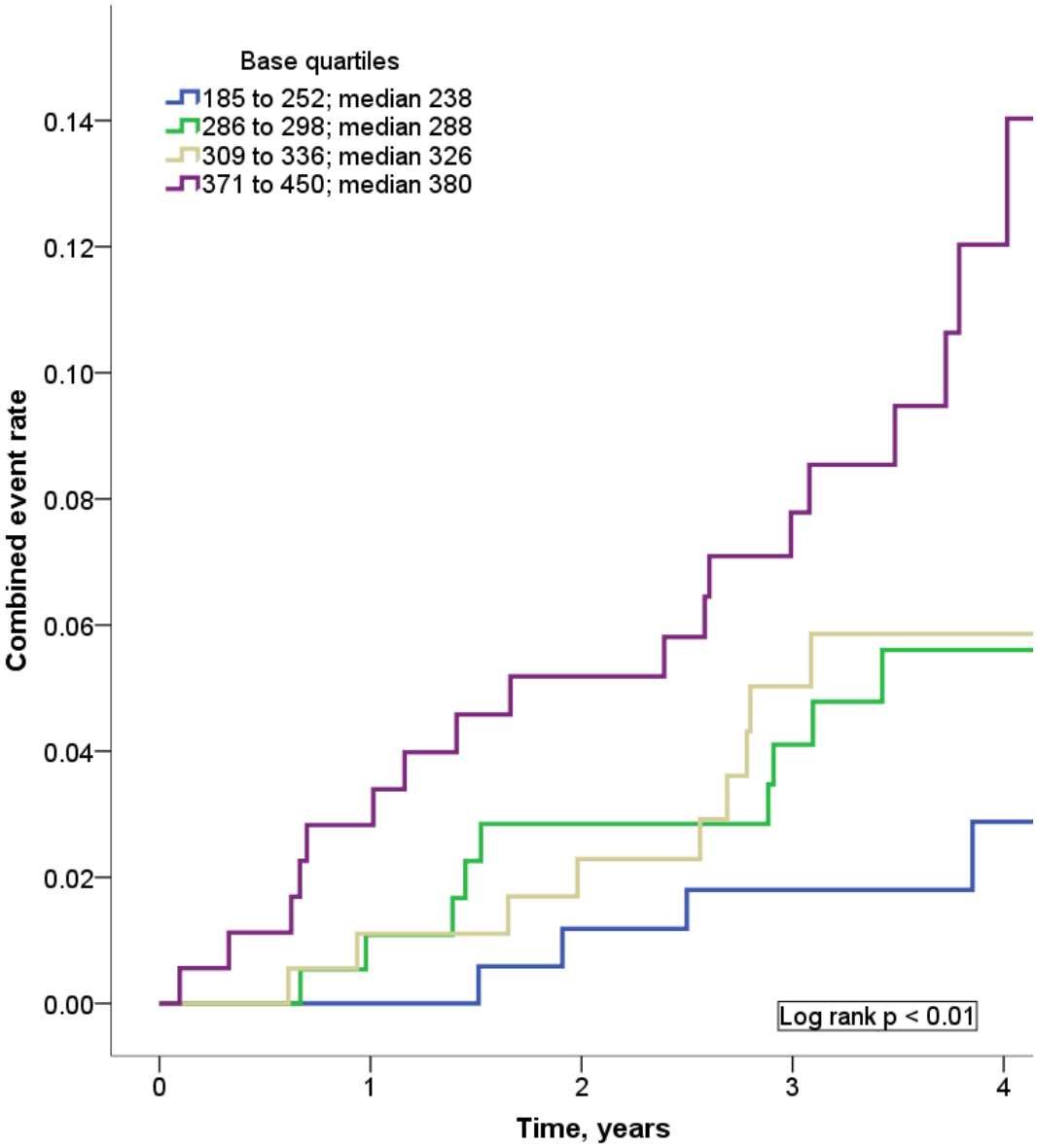



**Figure 7-31 KM curves showing time from PCI to combined MI, stroke and cardiac death after cessation of treatment with index P2Y12 inhibitor, stratified into quartiles of VerifyNow Baseline platelet reactivity**

## 7.2. Chapter 7 summary points and discussion

- All 3 VerifyNow measures of platelet reactivity were highly variable. The Baseline value (reactivity to thrombin) was normally distributed but the distribution of PRU (reactivity to ADP) was moderately skewed. The distribution of %inhibition values was extremely skewed, as about a fifth of patients had no measureable P2Y12-mediated inhibition.
- The PRU level was influenced by multiple baseline factors. The VerifyNow Base value (platelet reactivity to a thrombin-like agonist) and the pre-procedural Hb level were the 2 factors with the greatest apparent effect on PRU.
- Proton pump inhibitors had no effect on %inhibition values in this study.
- A PRU level above around 200 was associated with higher rates of recurrent MI and of cardiac death, although these associations were of only borderline statistical significance.
- There was no dichotomous association between all-cause death and PRU above the 'ischaemic cut-off'; however, death rate and PRU formed a U-shaped curve, with the lowest death rates at mid-level PRU.
- There were more deaths due to cancer in patients with the greatest levels of platelet inhibition.
- Very low PRU (below about 100) was associated with increased rates of BARC 2 to 5 bleeding and anaemia.
- The previously published ARMYDA-PROVE optimal PRU range of 179 to 238 appeared to fit very well with optimal combined clinical outcomes at 1 year in the OPERA study.
- Base reactivity was an important independent predictor of long-term off-treatment ischaemic adverse events including stroke, MI and cardiac death.
- Anaemia was predictive of all types of adverse event including bleeding, death and MI, but only in patients with high Baseline reactivity values. Patients with both Hb in the lowest quartile and Base in the highest quartile had a 60% adverse event rate over 3.5 years.

### 7.2.1. Comparison of levels of platelet inhibition in OPERA with the published literature

The mean PRU level in ARMYDA-PROVE study (elective PCI) by Mangiacapra et al, 2012, was 206 +/- 72. Mean PRU in OPERA was considerably higher at 235 +/- 91. This is a large difference and possibly relates to the relative timing of the clopidogrel loading dose in the 2 studies. In OPERA, most patients had been on 75 mg per day at the time of PCI with a remote loading dose given on the day of admission

with ACS; in contrast, in ARMYDA-PROVE, 88% of participants received a 600mg loading dose >6 hours before PCI, close to the time of the platelet function test which was performed in the catheter laboratory at the time of PCI, as in OPERA. It is known that higher doses of clopidogrel produce greater platelet inhibition as measured by VerifyNow, shown in several large studies (Gladding et al, 2008; Price et al, 2011). In the GRAVITAS study (Price et al) the median PRU in patients on 75 mg of clopidogrel for 30 days was even higher than OPERA at 250 (IQR 206 to 298). In another important study of platelet reactivity and PCI, ADAPT-DES (Stone et al, 2013), the mean PRU was 188 which is lower than all the other studies. In ADAPT-DES, clopidogrel could be given either as 600mg loading >6 hours, 300 mg loading >12 hours or 75 mg for at least 5 days before VerifyNow testing – the PRU by subgroup is not given, nor is the proportion of patients in each subgroup. These large variations in average PRU between different studies shows that, if optimal PRU cut-offs are to be established, it is important that the timing of the VerifyNow test in relation to the antiplatelet loading dose is specified.

### 7.2.2. PRU and clinical outcomes

In common with other similar studies the PRU level in OPERA appeared to be related to adverse clinical events. Patients with a PRU above 238 had 2 to 3 times the rate of MI at 12 months than those with PRU <238: 6.3% vs. 2.5%; p = 0.01 (log rank test). In the same groups there was little difference in the rates of cardiac death (2.7% vs. 1.6%, p = 0.32); although there was a trend for greater all-cause mortality (4.3% vs. 2.1%, p = 0.09). These results are not dissimilar to those from the large ADAPT-DES study (Stone et al, 2013), in which the rates of MI and all-cause death at 12 months in patients with PRU above and below the ischaemic PRU threshold were 3.9% and 2.7% (p = 0.01) and 2.4% vs. 1.5% (p = 0.3) (the ischaemic threshold was PRU 208 in that study; however, identical results were seen using PRU 230).

It is unclear why a reduction in MI rate in clopidogrel responders compared to non-responders does not translate into a more definite reduction in death rate. However, in most platelet reactivity studies the death rate and recurrent MI rate do not follow the same pattern of relationship to PRU. In OPERA while there is a clear ‘threshold effect’ for recurrent MI with a lower rate below PRU 238 and a higher rate above this (Figure 7-14), this is not so obvious for death (Figures 7-16 and 7-17). Death and PRU seem to have a more u-shaped relationship, with a higher rate at both very low and very high PRU levels and a low rate in the mid PRU range.

In ADAPT-DES the finding of excess deaths in patients with very low PRU was assumed to be related to bleeding, as bleeding rates were seen to be higher in those with

low PRU. However, in OPERA deaths were studied in greater detail than is usual in this type of study and deaths were found to be very rarely due to bleeding. Although bleeding was indeed more common in those with low PRU (see Figures 7-22 and 7-23), as in ADAPT-DES, the commonest cause of death in OPERA patients with very low PRU levels was in fact cancer, with only 1 death involving haemorrhage. It may be that very low PRU in clopidogrel-treated patients is simply a marker of general frailty or co-morbidity. Whatever the explanation, there is a strong signal that the safest PRU to have after PCI for ACS on DAPT with aspirin and clopidogrel is in the mid range, around PRU 200, similar to the ARMYDA-PROVE optimal range; see Figures 7-19 and 7-30.

### 7.2.3. The VerifyNow Base value

In OPERA the VerifyNow Base value, like PRU, was highly variable. The Base value indicates platelet reactivity mediated via activation of platelet PAR-1 thrombin receptors. The VerifyNow test manufacturers noted the high variation in general baseline platelet reactivity between individuals and incorporated the thrombin channel into their device to act as an individual control for each patient, producing the VerifyNow '%inhibition' value. The Base value has not generally been a focus of investigation in clinical research and rarely even mentioned in platelet function testing studies. However, it is useful for the study of the platelet reactivity variability in ACS patients because it is unaffected by either of the oral antiplatelet agents used to treat these patients (aspirin and P2Y12 receptor inhibitors).

In OPERA, Base reactivity was associated with the level of platelet reactivity in response to ADP (the PRU) in clopidogrel-treated patients. High Base reactivity conferred a much higher chance of 'clopidogrel resistance'. Clopidogrel resistance as defined by PRU ≥238 was seen in 23.7% and 76.0% of those in the lowest and highest Base quartiles. Furthermore, the Base value in OPERA carried substantial long-term prognostic information after cessation of DAPT (Fig 7-31), being an independent predictor of MACE. For some reason neither of these important observations have been studied to any great extent in the recent literature. It is largely unknown why platelet reactivity to thrombin varies so widely between individuals; and whether it is a relatively fixed quantity for each person or whether it can change and, if so, how often and in response to what stimuli. There is some limited evidence to suggest it can be changed, explored further in Chapter 9. In OPERA, the main baseline characteristic to be associated with Base reactivity was the haemoglobin level.

### 7.2.4. Speculation on the cause of the link between anaemia, platelet reactivity and adverse events

Anaemia is present in approximately 20% of patients presenting with ACS and has long been recognised as an independent predictor of adverse events, including all-cause mortality, as described in the meta-analysis by Lawler et al, 2013. However, the exact reasons for the adverse cardiovascular prognosis conferred by anaemia remain elusive. In OPERA there were strong relationships between platelet reactivity and haemoglobin level (Figures 7-6 and 7-7); and between platelet reactivity, Hb and adverse clinical outcomes (Figure 7-28). Anaemic patients with highly reactive platelets were the highest-risk group in OPERA, although not all anaemic patients had high platelet reactivity and in fact anaemic patients with low platelet reactivity did as well as those with normal haemoglobin levels. I would like to propose that changes in platelet reactivity can occur as a direct response to changes in Hb level; and that this is a potential pathophysiological mechanism for the increased cardiovascular event rates seen in anaemia.

It may seem odd in evolutionary terms that humans might have developed a mechanism to increase platelet reactivity in response to anaemia when it seems clear that this could lead to more frequent arterial thrombotic events. However, the usual cause of anaemia in wild animals is traumatic bleeding. A mechanism for increasing platelet reactivity in response to anaemia could have evolved in our common ancestors in order to stop bleeding, conserve blood and more rapidly regain normal oxygen carrying capacity. This mechanism would certainly confer a survival advantage in wild animals. Although atherosclerosis is well documented in primates (Stout and Lemmon, 1969) and other animals, in the wild animals die of predation, starvation and disease before they die of myocardial infarction. Biological systems for homeostatic regulation of bleeding, thrombosis and platelet reactivity which might predispose to arterial thrombosis therefore persist into old age, as there are no evolutionary mechanisms to modify them. A sudden increase in platelet reactivity due to a drop in Hb level might get a young wild animal up and running sooner than its competitors; but in an old human with degenerative atherosclerotic disease it might be enough to trigger an MI. Atherosclerosis, MI and anaemia all become more common with age, amplifying the problem as the years go by.

By what mechanism might haemoglobin concentration affect platelet reactivity? One obvious possible intermediary is erythropoietin, a hormone released in response to anaemia to mediate erythrocyte production. The administration of erythropoietin has been known for some time to cause an increase in whole blood platelet aggregation (Taylor et al, 1991). Furthermore, the treatment of anaemia by the administration of

erythropoietin has never been shown to lower cardiovascular risk and in fact seems to have the reverse effect. These observations have been made mainly from studies of patients with renal failure with anaemia, in whom recombinant erythropoietin injections are a common treatment (Horl, 2013). There is some data to suggest that erythropoietin can directly and markedly increase platelet reactivity in humans (Stohlawetz et al, 2000). An interaction between anaemia, platelet reactivity, erythropoietin and adverse thrombotic events is plausible and certainly requires further study.

# Chapter 8 Myocardial injury from peri-procedural biomarker levels and its association with prognosis

Chapter 8 explores the OPERA study biomarker data, with a focus on identification of PCI-induced myocardial injury and its relationship, if any, with prognosis. Patients undergoing emergent PCI for STEMI (PPCI) were excluded from most of these analyses, as there is no way to distinguish procedure-induced myocardial injury from that caused by the acute MI. Biomarkers were measured using the Cardiac Plus Array from Randox, except for Troponin I, measured with the Advia Centaur highly sensitive assay from Siemens. The Randox array was a research tool at the time of this study whereas the Siemens troponin assay was in current clinic use. Technical specifications for these assays are available in the Appendices.

The biomarkers measured were:

- CK-MB and highly sensitive Troponin I (TnI), recommended as biomarkers of choice for the diagnosis of MI in current clinical guidelines
- Myoglobin; no longer recommended for use in diagnosis of MI as not cardio-specific, the same isoform of the enzyme being released readily from both skeletal and cardiac muscle
- Glycogen phosphorylase BB (GPBB) and Human Fatty Acid Binding Protein (H-FABP), 2 novel cardiac biomarkers
- Carbonic Anhydrase III; not a cardiac biomarker and released only from skeletal muscle; intended to be of use to establish the proportion of myoglobin which is of cardiac origin

## 8.1. Biomarker data completeness

There was a significant amount of missing biomarker data, particularly at the 12 hour time point when blood samples were sometimes not obtained. Table 8-1 shows the overall data completeness for each biomarker at each time point.

**Table 8-1 Data completeness for serum biomarkers at each time point for 872 patients; patients undergoing PPCI excluded**

| Assay | | 0 hours | 4 hours | 12 hours | All 3 time points |
|---|---|---|---|---|---|
| Randox Array: | Myoglobin | 96.1% | 88.2% | 80.0% | 77.4% |
| | CK-BM | | | | |
| | HFABP | | | | |
| | GPBB | | | | |
| | CAIII | | | | |
| Advia Centaur TnI | | 90.5% | 82.1% | 73.3% | 66.5% |

## 8.2. Biomarker Upper Limit of Normal values

The upper limit of the normal range (ULN) for each biomarker was available from the assay manufacturers' product information sheets as the 95$^{th}$ or 99$^{th}$ percentile population of healthy individuals. To verify these normal range values for the OPERA study population, Time 0 biomarker values were displayed graphically in ascending order to identify an *inflection point* (the first major deviation of the graph). Inflection points could be seen for myoglobin, CK-MB, H-FABP, CAIII and GPBB; see Figures 8-1 to 8-5. These visually estimated values, all slightly lower than the published 99$^{th}$ percentile cut-off values, were used as the ULN in the following analyses. For TnI an inflection point could not be discerned and the published value of the 99$^{th}$ percentile of a normal population was used for the analysis.

### 8.2.1. Myoglobin normal range

Figure 8-1 shows the distribution of *time 0* myoglobin levels sorted into ascending order, from which a conservative cut-off for a normal range of < 45 ng/mL can be estimated for our population. This level is similar to the 99$^{th}$ percentile of a healthy population published by the company of 74 ng/mL.

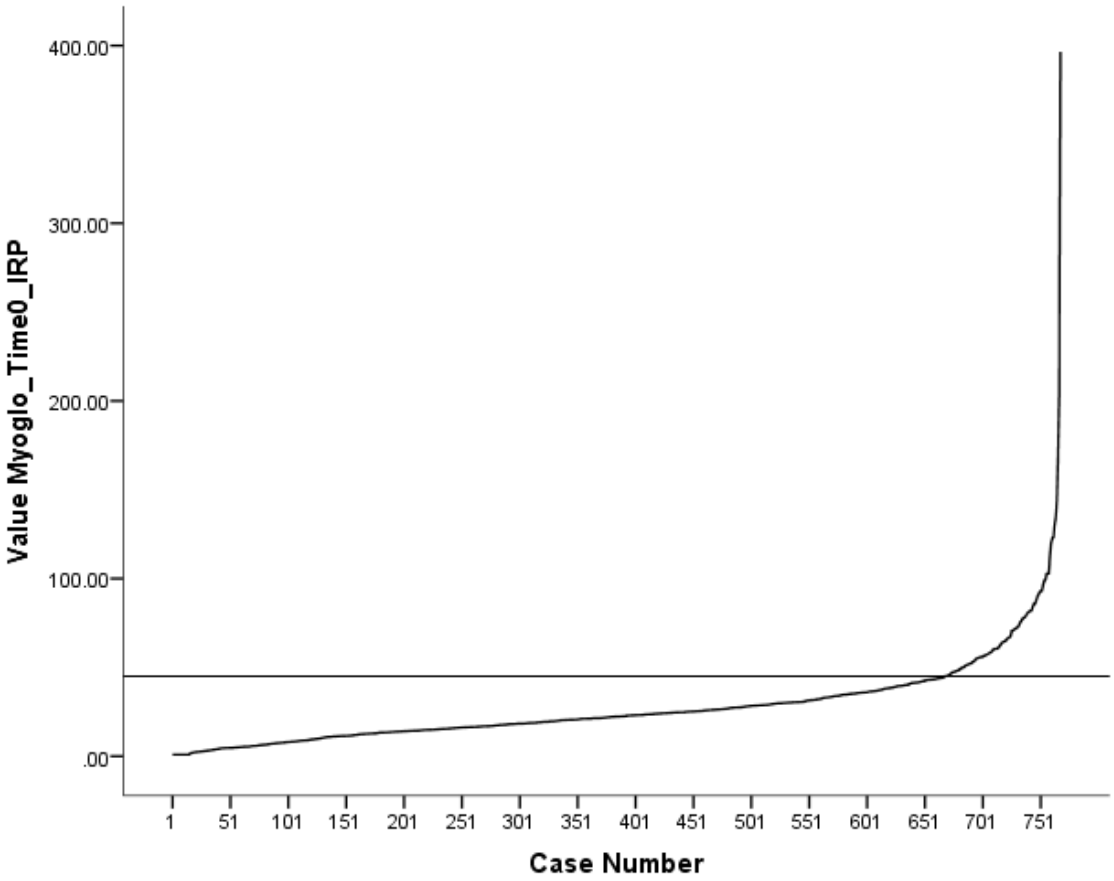



**Figure 8-1 Myoglobin (Randox array) levels at Time 0 for non-PPCI patients sorted into ascending order, showing first major deviation from normal range at approximately 45ng/mL, illustrated with horizontal line**

### 8.2.2. CK-MB normal range

Figure 8-2 suggests an approximate normal range for CK-MB of < 2.4 ng/mL for OPERA patients, close to the manufacturer's published value for the 99$^{th}$ percentile of a healthy population of 2.69 ng/mL.

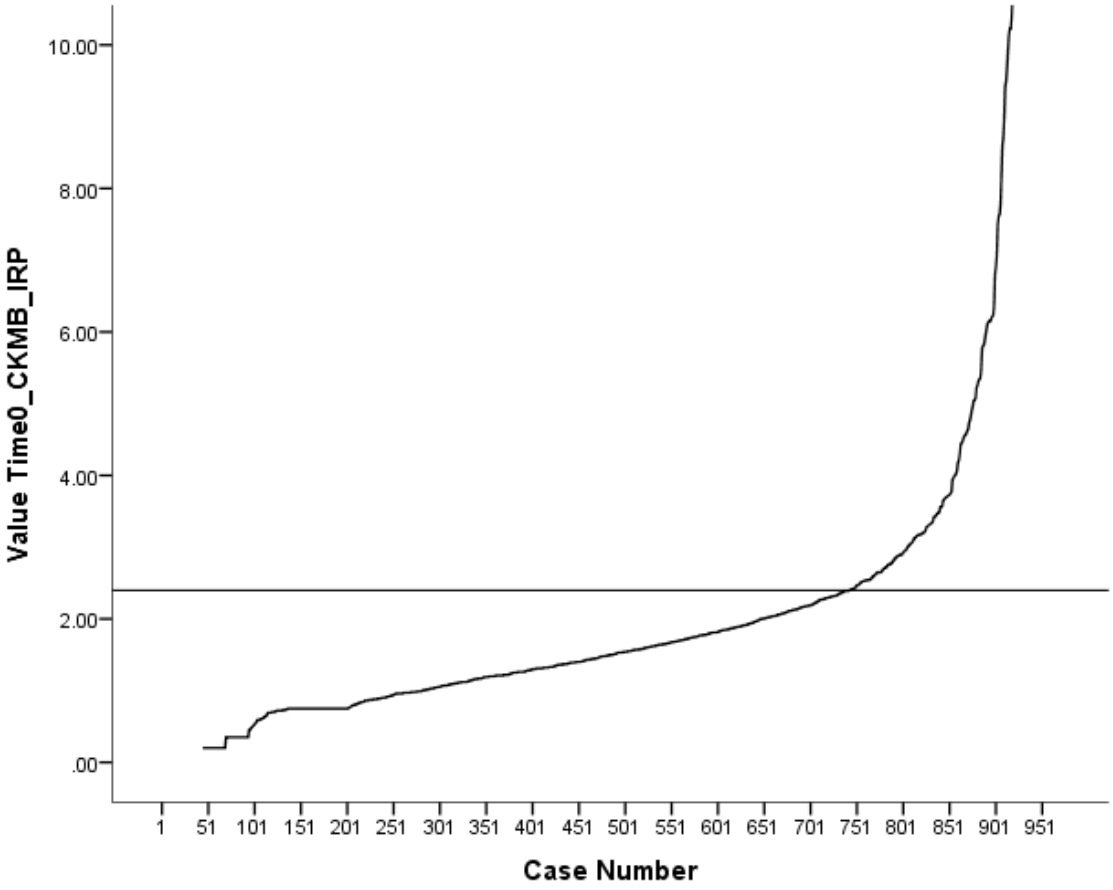



**Figure 8-2 CK-MB (Randox array) Time 0 levels for non-PPCI patients sorted into ascending order; first major deviation from normal range at approximately 2.4 ng/mL shown with horizontal line**

### 8.2.3. H-FABP normal range

The published 99th percentile of the Randox Cardiac Plus Array H-FABP in a healthy population is 3.0 ng/ml. I have used a cut-off of 2.0 ng/ml for OPERA patients, derived as shown in Figure 8-3.

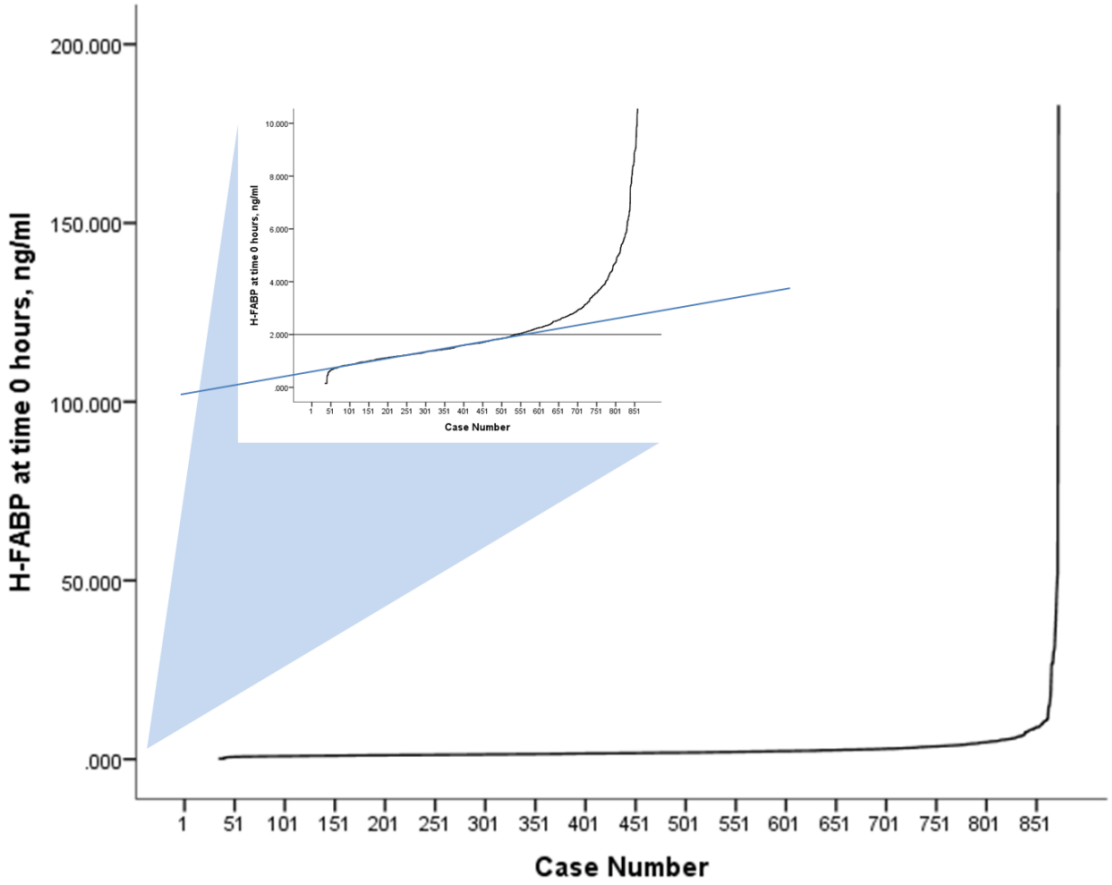



**Figure 8-3 H-FABP (Randox array) levels at Time 0 for non-PPCI patients sorted into ascending order. Inset shows same curve magnified with an oblique line to illustrate probable normal range. The first major deviation from normal range, at approximately 2.0 ng/mL, is marked by horizontal line**

### 8.2.4. Glycogen phosphorylase BB (GPBB)

The published 99$^{th}$ percentile for GPBB is 7.88 ng/ml. I have used a conservative cut-off 4.0 ng/ml for the normal range, see Figure 8-4.

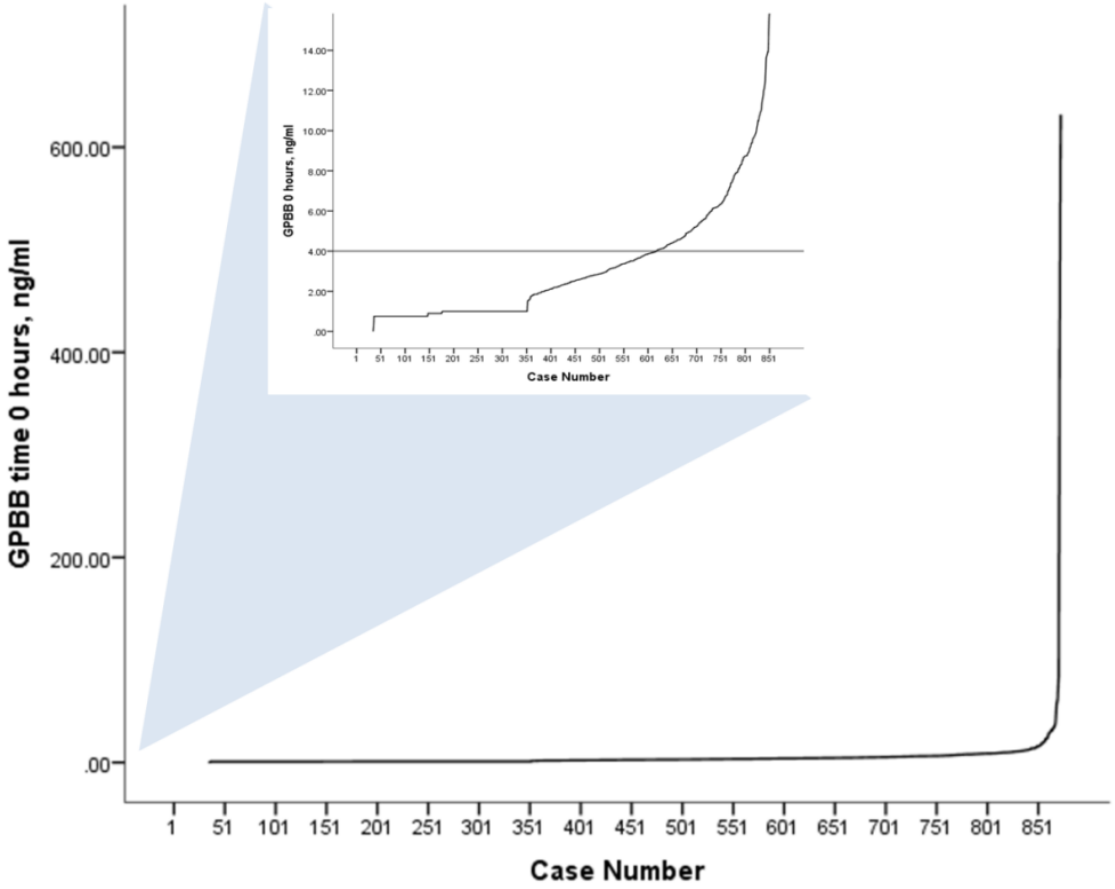



**Figure 8-4 Time 0 GPBB (Randox array) levels in non-PPCI patients sorted into ascending order, with inset showing the same curve magnified; first major deviation from normal at approximately 4.0 ng/mL shown by horizontal line**

### 8.2.5. Carbonic anhydrase III

Figure 8-5 shows the approximate normal range of CA III level for our population was < 30 ng/mL. The published 99$^{th}$ percentile for a normal population is 86 ng/mL.

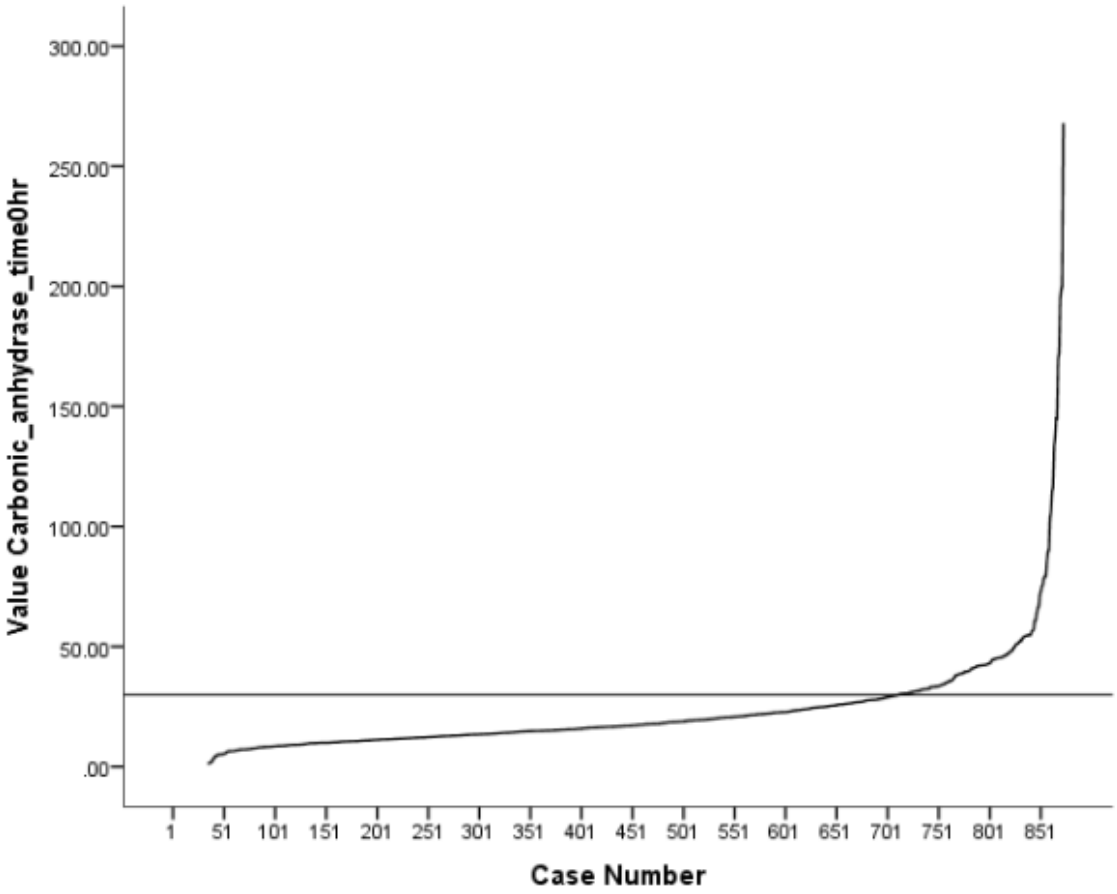



**Figure 8-5 Carbonic anhydrase III (Randox array) levels at Time 0 in non-PPCI patients sorted into ascending order; first major deviation from normal range at approximately 30 ng/mL shown by horizontal line**

### 8.2.1. Cardiac Troponin I (cTnI) normal range

In contrast to the other biomarkers, a clear 'normal range' cut-off value for TnI could not be identified using the inflection point method, as shown in Figure 8-6. For the high-sensitivity troponin assay used in this study (Advia Centaur TnI Ultra, Siemens), the published 99th percentile in healthy patients, 0.04 ng/mL, was taken as the ULN in these analyses.

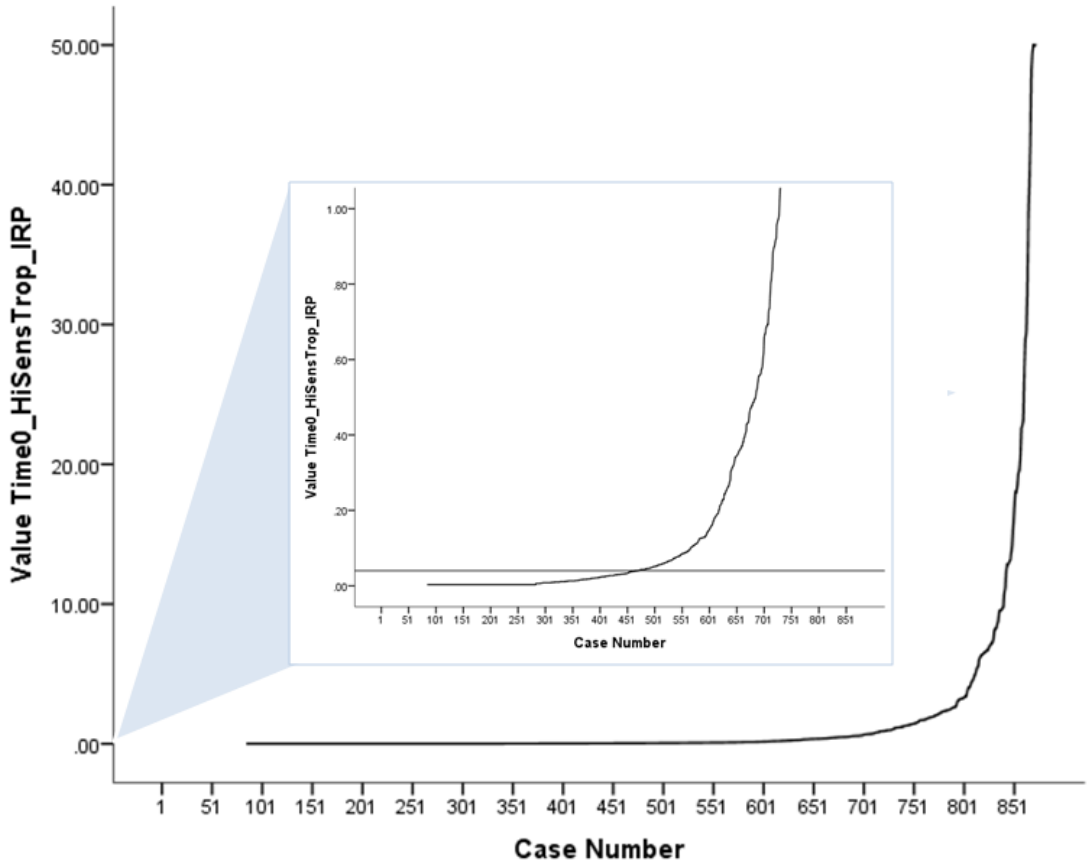



**Figure 8-6 Highly sensitive troponin I (Advia Centaur) at Time 0 for non-PPCI patients sorted into ascending order, with inset showing the same curve magnified; horizontal line at 0.04 ng/mL, the 99$^{th}$ percentile from a normal population.**

## 8.3. Biomarker levels pre-PCI

The proportion of patients with biomarker levels < ULN (OPERA study values) at the start of the procedure varied widely between biomarkers, from 48% for TnI to 87% for myoglobin (Table 8-3). There were associations between biomarker elevation > ULN at Time 0 and patient baseline characteristics, shown in Table 8-2.

One factor was associated with elevation of all 6 biomarkers: enrolment into the study during the 2nd recruitment phase (2010 to 2012). There was a significantly shorter time from admission to hospital with ACS to PCI in patients in the 2nd recruitment stage compared with those in the 1st: 5 days vs. 7 days, $P < 0.01$.

The only other characteristic associated with elevation of TnI at Time 0 was an ACS presentation with MI (as opposed to TnI negative ACS/UA). In contrast, elevation of CK-MB, myoglobin and H-FABP was associated with multiple baseline characteristics. Note that elevation of the skeletal muscle biomarker, CAIII, was associated with much the same characteristics. There was no sign that GPBB level was affected by baseline characteristics.

**Table 8-2 Table showing all clinical features significantly associated with elevation of any biomarker above the ULN at Time 0 (just prior to PCI); P < 0.05; PPCI patients excluded**

| | TnI† | CK-MB* | Myoglobin* | H-FABP* | GPBB* | CAIII* |
|---|---|---|---|---|---|---|
| Recruited 2nd stage | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| > 80 years old | × | ✓ | ✓ | ✓ | × | ✓ |
| Presented with MI | ✓ | ✓ | × | ✓ | × | × |
| Highest EuroSCORE quartile | × | ✓ | ✓ | ✓ | × | ✓ |
| Highest SYNTAX quartile | × | ✓ | ✓ | ✓ | × | ✓ |
| eGFR < 60 ml/min/1.73 $m^2$ | × | ✓ | ✓ | ✓ | × | ✓ |
| Diabetes | × | × | ✓ | ✓ | × | ✓ |
| Hypertension | × | × | ✓ | ✓ | × | ✓ |
| Hb < 12 g/dL | × | × | ✓ | ✓ | × | ✓ |
| Peripheral vascular disease | × | × | × | ✓ | × | ✓ |

✓ There were significantly more patients with biomarker levels above the ULN when characteristic present

× There was no significant difference in the proportion of patients with biomarker levels above the ULN when characteristic present

* *Randox Cardiac Plus Array* assay † Siemens' *ADVIA Centaur TnI-Ultra* assay

## 8.4. Biomarkers levels post-PCI

For patients with normal range biomarker levels at Time 0 hours there was a small increase in average levels at 4 hours for all biomarkers, and a further small increase from 4 to 12 hours (except for the non-cardiac biomarker CAIII), displayed in Table 8-3.

On an individual patient basis, biomarker levels above the upper limit of the normal range (ULN) following PCI, either at 4 hours, 12 hours or both, was very common. TnI was the biomarker most frequently elevated, with levels above the ULN seen in 71.7% at 12 hours; and myoglobin the least frequently elevated, seen in 20.6% at 12 hours (Table 8-4). H-FABP, myoglobin and GPBB had a heterogeneous pattern of elevation over the 4 and 12 hour time points. In contrast, patients with elevation of CK-MB and troponin I had a much more ordered pattern of release, with a small early rise and a greater late rise. These patterns of biomarker elevation are shown in Figures 8-7 to 8-11.

**Table 8-3 Median (IQR) biomarker levels in non-PPCI patients; only those with biomarker levels < ULN at Time 0 AND with results available at all 3 time points are included**

| | ULN, ng/mL | Total number patients with biomarker levels < ULN at Time 0 (% of total non-PPCI cohort) | Number of patients with biomarker levels < ULN, with complete results (% of previous column) | Median (IQR) biomarker level, ng/mL | | |
|---|---|---|---|---|---|---|
| | | | | Time point: 0 hours | 4 hours | 12 hours |
| **CK-MB*** | 2.4 | 675 (80.5%) | 548 (81.2%) | 1.26 (0.89) | 1.41 (1.02) | 2.04 (2.16) |
| **Myoglobin*** | 45.0 | 729 (87.0%) | 583 (80.0%) | 20.32 (15.8) | 23.53 (22.58) | 27.21 (23.75) |
| **H-FABP*** | 2.0 | 500 (60.0%) | 412 (82.4%) | 1.29 (0.61) | 1.63 (1.16) | 1.89 (1.54) |
| **GPBB*** | 4.0 | 583 (70.0%) | 472 (81.0%) | 1.0 (1.64) | 2.06 (2.6) | 2.23 (2.65) |
| **CA III*** | 30.0 | 676 (80.7%) | 540 (79.9%) | 15.23 (9.31) | 18.07 (14.8) | 18.91 (14.78) |
| **TnI†** | 0.04 | 381 (48.3%) | 275 (72.2%) | 0.003 (0.014) | 0.019 (0.05) | 0.097 (0.342) |

*Randox *Cardiac Plus Array* assay

†Siemens' *ADVIA Centaur TnI-Ultra* highly sensitive troponin I assay

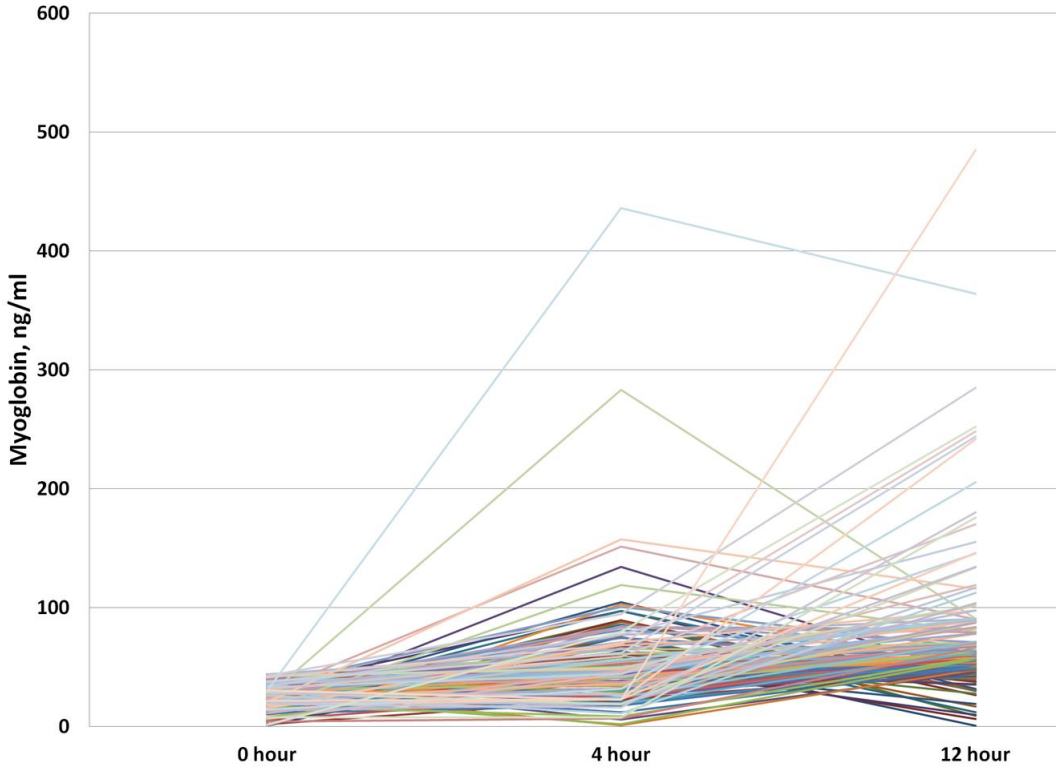



**Figure 8-7 Changes in myoglobin level after PCI in 150 patients who were myoglobin negative at Time 0 but positive at either 4 or 12 hours**

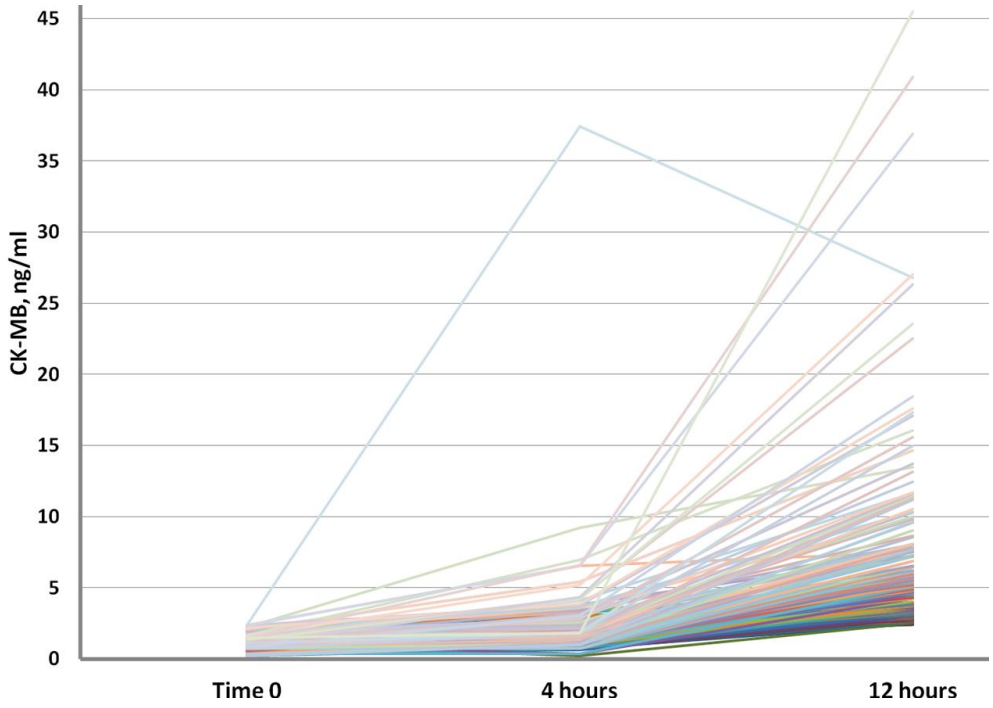



**Figure 8-8 Changes in CK-MB levels after PCI in 213 patients who were CK-MB negative at Time 0 but positive at either 4 or 12 hours**

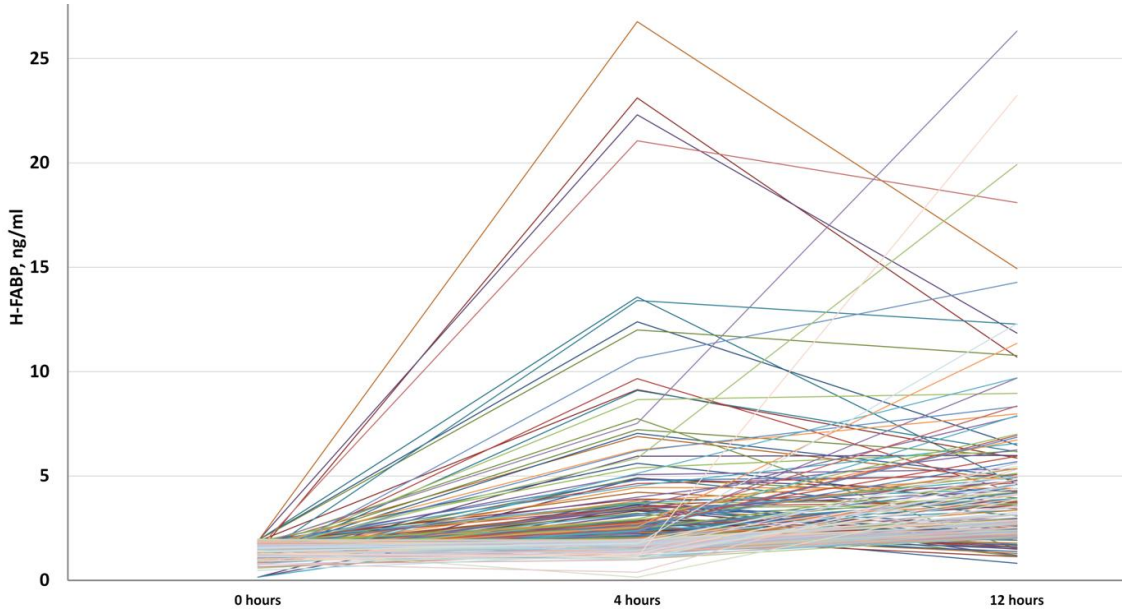



**Figure 8-9 Changes in H-FABP level after PCI in 216 patients who were H-FABP negative at Time 0 but positive at either 4 or 12 hours**

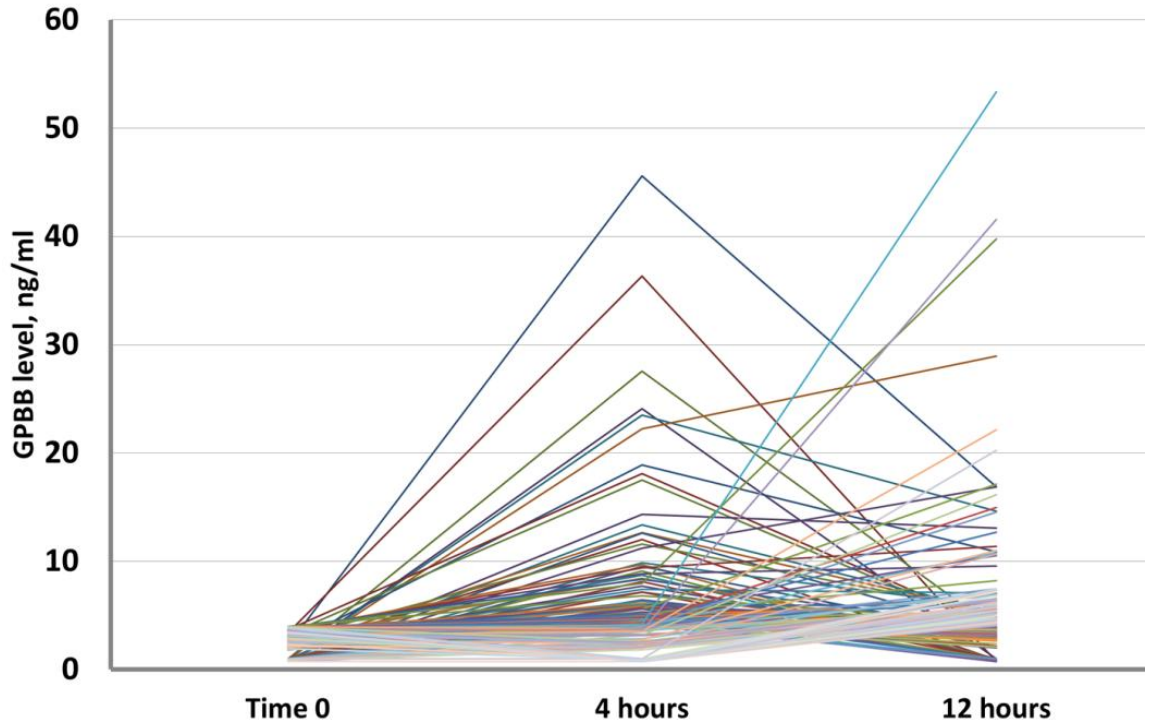



**Figure 8-10 Changes in GPBB level after PCI in patients who were GPBB negative at Time 0 but positive at either 4 or 12 hours (1 extreme outlier, PAT ID 578, excluded for clarity)**

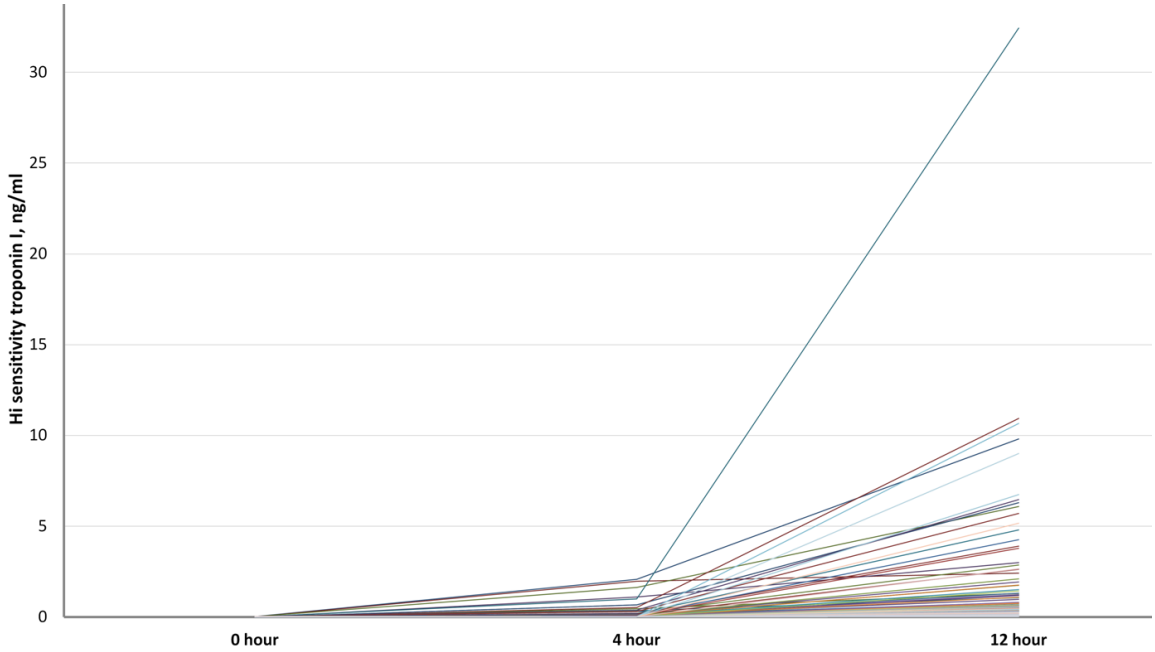



**Figure 8-11 Changes in high sensitivity TnI level after PCI in 193 patients who were TnI negative at Time 0 but positive at either 4 or 12 hours**

**Table 8-4 Number (%) of patients (biomarker negative at Time 0, results at all 3 time points) with biomarker levels at various thresholds above the upper limit of the normal range (ULN) at 4 and 12 hours post PCI**

| | ULN (OPERA study values), ng/mL | Above ULN | | ≥ 5× ULN | | ≥ 10× ULN | | ≥ 50× ULN | |
|---|---|---|---|---|---|---|---|---|---|
| | | 4hr | 12hr | 4hr | 12hr | 4hr | 12hr | 4hr | 12hr |
| CK-MB, ng/mL * | 2.4 | 3 (0.5) | 218 (38.6) | 3 (0.5) | 23 (4.1) | 3 (0.5) | 7 (1.2) | 0 | 0 |
| Myoglobin, ng/mL* | 45.0 | 104 (15.6) | 124 (20.6) | 3 (0.5) | 8 (1.3) | 0 | 1 (0.2) | 0 | 0 |
| H-FABP, ng/mL* | 2.0 | 150 (32.7) | 200 (47.2) | 12 (2.6) | 12 (2.8) | 4 (0.9) | 2 (0.5) | 0 | 0 |
| GPBB, ng/mL* | 4.0 | 112 (21.2) | 101 (20.7) | 8 (1.5) | 6 (1.2) | 2 (0.4) | 2 (0.4) | 1 (0.2) | 0 |
| CA III, ng/mL* | 30.0 | 117 (19.0) | 128 (23) | 5 (0.8) | 6 (1.1) | 0 | 0 | 0 | 0 |
| Hi Sens TnI, ng/mL† | 0.04 | 112 (33.4) | 213 (71.7) | 18 (5.4) | 103 (34.7) | 10 (3.0) | 70 (23.6) | 1 (0.3) | 23 (7.7) |

* *Randox Cardiac Plus Array* assay † *ADVIA Centaur TnI-Ultra* assay

## 8.5. Comparison between serum and plasma biomarker levels

The Time 0 blood sample taken for biomarker measurement was stored both as serum (derived from centrifugation of coagulated blood) and plasma (from centrifugation of anticoagulated blood, thereby containing clotting factors and fibrinogen). Blood was stored as serum only at the 4 and 12 hour time points. Biomarker levels at Time 0 were measured both in the serum and in the plasma of about half the patient cohort. Patients in this subgroup all enrolled during the first recruitment phase of the study but were otherwise similar to the overall cohort.

Correlation between the plasma and serum results was strong for TnI and CK-MB, slightly weaker for H-FABP and only modest for myoglobin and GPBB (Table 8-4).

Scatter plots of serum and plasma levels for H-FABP and TnI can be seen in Figure 8-12 and 8-13, showing reasonably linear associations (more so for TnI) but with scattered outliers. Particularly when TnI was in the lower range many patients can be seen to test positive on the serum sample but negative on the plasma sample, and vice versa.

**Table 8-5 Median (IQR) biomarker levels at Time 0 in subgroup of non-PPCI patients who had both plasma and serum samples tested; with Pearson's Correlation Coefficient**

| | Number of patients | Median level in serum, ng/ml | Median level in plasma, ng/ml | Correlation | P value |
|---|---|---|---|---|---|
| CK-MB, ng/mL * | 423 | 1.32 (1.33) | 1.42 (1.28) | 0.96 | < 0.01 |
| Myoglobin, ng/mL* | 423 | 20.34 (21.71) | 20.7 (17.0) | 0.79 | < 0.01 |
| H-FABP, ng/mL* | 423 | 1.63 (1.48) | 1.49 (1.41) | 0.92 | < 0.01 |
| GPBB, ng/mL* | 423 | 1.85 (2.82) | 3.84 (4.52) | 0.61 | < 0.01 |
| CA III, ng/mL* | 423 | 15.8 (13.27) | 12.64 (10.76) | 0.74 | < 0.01 |
| Hi Sens TnI, ng/mL† | 340 | 0.037 (0.345) | 0.046 (0.43) | 0.98 | < 0.01 |

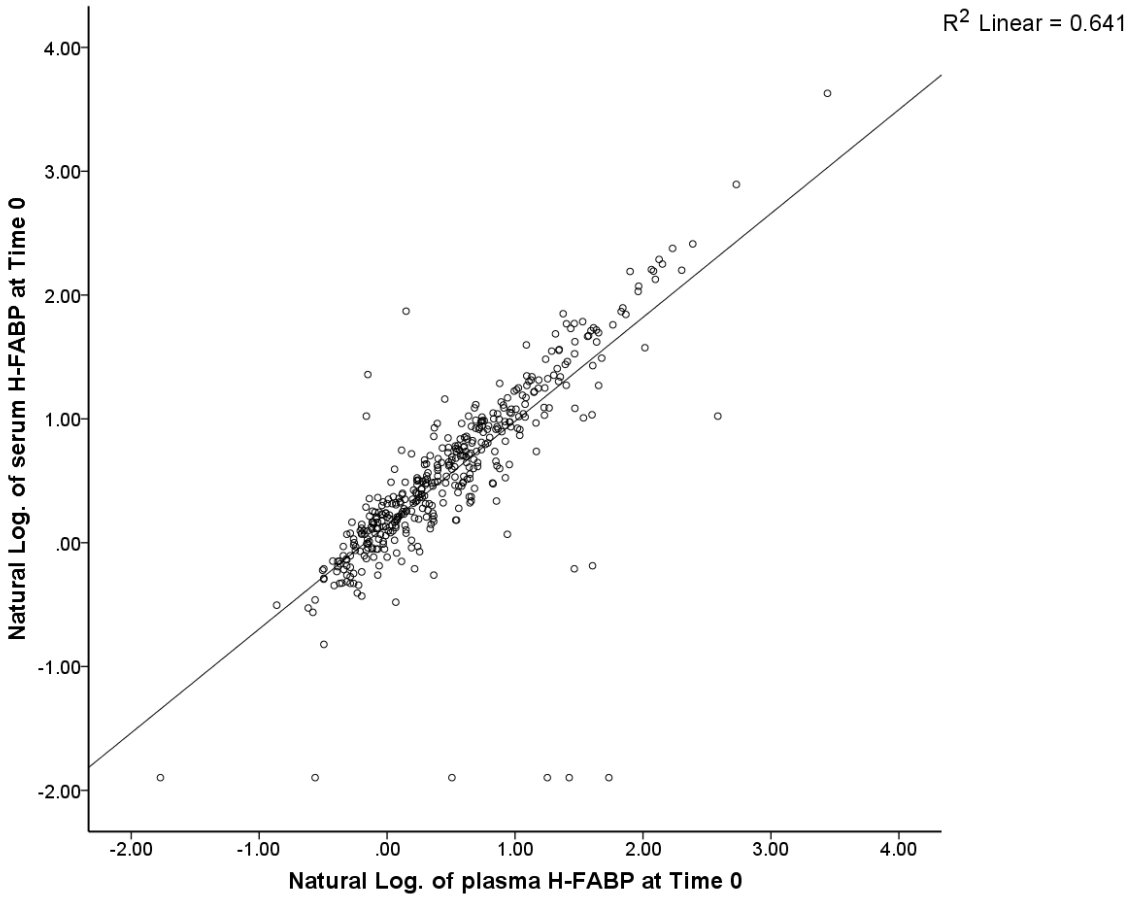



**Figure 8-12 Scatter plot of Time 0 H-FABP results obtained from plasma vs. serum, non PPCI patients; logarithmic transformation performed for clarity; automatic straight line of best fit added**

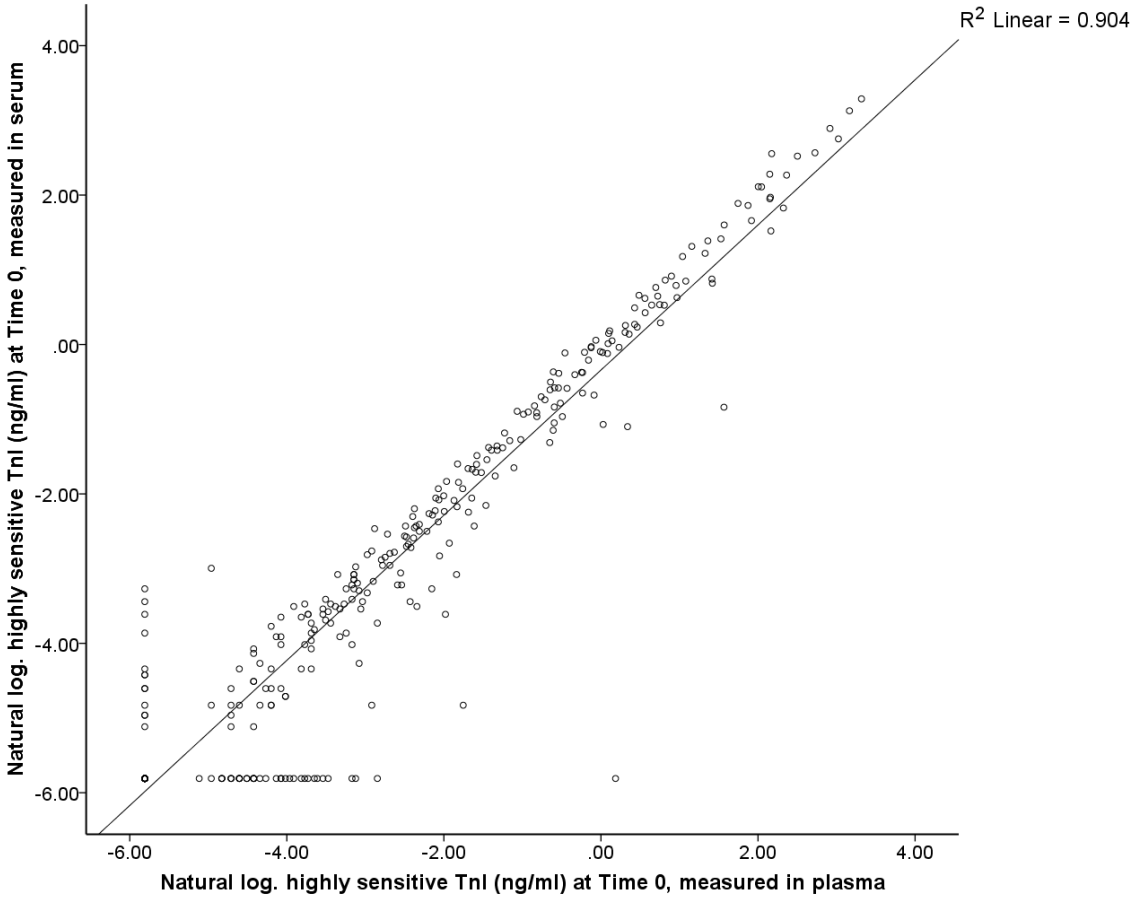



**Figure 8-13 Scatter plot of Time 0 highly sensitive TnI results obtained from plasma vs. serum, non PPCI patients; logarithmic transformation performed for clarity. Automatic straight line of best fit added**

## 8.6. Biomarker elevation post-PCI and baseline characteristics

Elevation of biomarker levels following PCI was associated with a variety of baseline and procedural characteristics, although these elevations were often inconsistent between the different biomarkers. Table 8-7 lists the characteristics significantly associated with any biomarker elevation above the ULN post PCI ($P < 0.05$). The more biomarkers that were elevated with each characteristic, the higher its position in the table.

There were 10 factors which were associated with a rise in all 3 of the traditional biomarkers (CK-MB, troponin I and myoglobin) at either 4 or 12 hours: prolonged fluoroscopy time, multi-vessel PCI, intervention to calcified lesions and Type C lesions, long stent length, high EuroSCORE, anaemia, age over 80 years, severe renal dysfunction and diabetes. A further 6 factors were associated with a rise in 2 out of 3 of the traditional biomarkers. For all the characteristics associated with a TnI rise at 12

hours, and for most of those associated with a rise in 12 hour myoglobin, a significant rise was detectable by 4 hours. In contrast, the 4 hour time point was too early to predict a 12 hour rise with CK-MB.

Regarding the novel biomarkers, HFABP was more likely to be elevated above normal at either 4 or 12 hours in patients with all the above 10 main characteristics associated with traditional biomarker elevation. In marked contrast, GPBB was elevated above normal in patients with only 1 of those characteristics (age > 80 years). CA III (the non-cardiac skeletal muscle marker) was elevated above normal in patients with prolonged fluoroscopy time, high EuroSCORE and diabetes.

## 8.7. Cardiac biomarkers and platelet reactivity

Median biomarker levels for patients grouped into the 3 ARMYDA-PROVE ranges for optimal, hypo- and hyper-response to clopidogrel are shown in Table 8-7. Platelet function did not have any association with biomarker levels in the OPERA study.

**Table 8-6 Median (IQR) biomarker levels in biomarker-negative patients (for all 4 biomarkers) at Time 0 hours, stratified by PRU level according to ARMYDA-PROVE thresholds for clopidogrel response; PPCI patients excluded; patients with any missing values excluded**

| **Biomarker** (ng/mL) | **PRU < 179 N = 42** | **PRU 179 to 238 N = 46** | **PRU > 238 N = 89** | **P value across 3 groups** |
|---|---|---|---|---|
| Myoglobin, 4 hour | 18.47 (14.31) | 20.14 (22.11) | 21.61 (18.65) | 0.24 |
| Myoglobin, 12 hour | 22.68 (21.06) | 23.83 (24.87) | 20.85 (23.83) | 0.59 |
| CK-MB, 12 hour | 1.44 (2.16) | 1.41 (1.72) | 1.62 (1.56) | 0.87 |
| TnI, 4 hour | 0.01 (0.04) | 0.02 (0.05) | 0.15 (0.03) | 0.15 |
| TnI, 12 hour | 0.06 (0.25) | 0.12 (0.26) | 0.08 (0.24) | 0.49 |
| H-FABP, 4 hour | 1.33 (0.91) | 1.67 (1.1) | 1.56 (0.97) | 0.47 |
| H-FABP, 12 hour | 1.79 (1.39) | 1.77 (1.17) | 1.6 (0.92) | 0.64 |

**Table 8-7 Characteristics associated with cardiac biomarker elevation above ULN post PCI at either 4 or 12 hours (P < 0.05), in non-PPCI patients with levels < ULN at baseline**

| | TnI† | CK-MB* | Myoglobin* | H-FABP* | GPBB* |
|---|---|---|---|---|---|
| Fluoroscopy time ≥ 10 minutes | ✓ | ✓ | ✓ | ✓ | × |
| PCI to more than 1 vessel | ✓ | ✓ | ✓ | ✓ | × |
| Total stent length ≥ 30 mm | ✓ | ✓ | ✓ | ✓ | × |
| EuroSCORE 4$^{th}$ quartile (≥ 6.4) | ✓ | ✓ | ✓ | ✓ | × |
| Anaemia; Hb ≤ 12.0 g/dL | ✓ | ✓ | ✓ | ✓ | × |
| Calcified lesion treated | ✓ | ✓ | ✓ | ✓ | × |
| Patient aged ≥ 80 years | ✓ | ✓ | ✓ | ✓ | ✓ |
| Diabetes | ✓ | ✓ | ✓ | ✓ | × |
| Type C lesion treated | ✓ | ✓ | ✓ | ✓ | × |
| Renal dysfunction; eGFR ≤ 30 | ✓ | ✓ | ✓ | ✓ | × |
| Patient presented with MI | ✓ | ✓ | × | × | × |
| More than 1 stent implanted | ✓ | × | ✓ | ✓ | × |
| PCI to left main stem | × | ✓ | ✓ | ✓ | ✓ |
| Bifurcation lesion treated | × | ✓ | ✓ | ✓ | × |
| IIbIIIa inhibitor given | × | ✓ | ✓ | × | × |
| History of stroke | ✓ | × | ✓ | × | × |
| Previous revascularisation | × | ✓ | × | ✓ | × |
| Hypertension | × | × | ✓ | × | ✓ |
| SYNTAX 4$^{th}$ quartile (≥ 15.5) | ✓ | × | × | ✓ | × |
| Peripheral vascular disease | × | × | ✓ | ✓ | × |
| Treated with B blocker | ✓ | × | × | × | × |
| PCVI to thrombotic lesion | ✓ | × | × | × | × |
| Saphenous vein graft treated | × | × | ✓ | × | × |
| Severe aortic stenosis | × | × | ✓ | × | × |
| Localised dissection | × | ✓ | × | × | × |
| Poor flow, any vessel | × | × | × | ✓ | × |
| Restenotic lesion treated | × | × | × | × | ✓ |
| Stent diameter 2.5 mm or less | × | × | × | ✓ | × |

✓ There were significantly more patients with biomarker levels above the ULN when characteristic present

× There was no significant difference in the proportion of patients with biomarker levels above the ULN when characteristic present

* *Randox Cardiac Plus Array* assay † Siemens' *ADVIA Centaur TnI-Ultra* assay

## 8.8. Cardiac biomarkers and clinical outcome

### 8.8.1. Troponin elevation and adverse events

In patients who were TnI negative at Time 0 hours, elevation of TnI above the ULN at 4 hours was associated with approximately twice the rate of the combined endpoint of recurrent ACS, death or further unplanned revascularisation at 1 year, see Figure 8-14. There was no significant difference in individual or composite adverse event rate in patients who did and did not have elevation of TnI above the ULN at 12 hours, but note that this was a smaller group of 296 patients due to missing 12 hour biomarker results.

The characteristics individually associated with this composite adverse endpoint are listed in Table 8-7. After adjustment, elevation of TnI lost statistical significance; the most important factors were the occurrence of a major procedural complication and a high SYNTAX score. In a model including major complications, high SYNTAX, high EuroSCORE and 4 hour TnI >ULN, respective aHR (95% CI) were 5.24 (1.52 to 17.98), $p < 0.01$; 5.93 (1.77 to 19.95), $p < 0.01$; 2.13 (0.83 to 5.47), $p = 0.12$; and 1.99 (0.90 to 4.40), $p = 0.09$.

### 8.8.2. H-FABP and adverse events

In patients who were H-FABP negative at Time 0 hours, there was no association with elevation at 4 hours and combined adverse events at 1 year.

Time 0 hours (immediately pre-PCI) H-FABP levels were associated with adverse events, but only for patients who were clopidogrel-resistant. Patients who were both clopidogrel resistant and who had elevation of H-FABP at Time 0 hours were 2 to 3 times more likely to experience an adverse event than other patients (Figure 8-15).

### 8.8.3. Procedural MI

Only 1 patient could be said to have had a procedural MI according to the Universal Definition criteria: PAT ID 355 had prolonged chest pain following the procedure. He had another coronary angiogram to investigate this pain the following day. No angiographic abnormalities were seen and his ECGs shown no dynamic changes but TnI and CK-MB, both < ULN at Time 0 hours, were elevated at 12 hours (1.79 and 11.3 ng/ml respectively). This patient died 5 years later of a recurrent MI and gastric cancer.

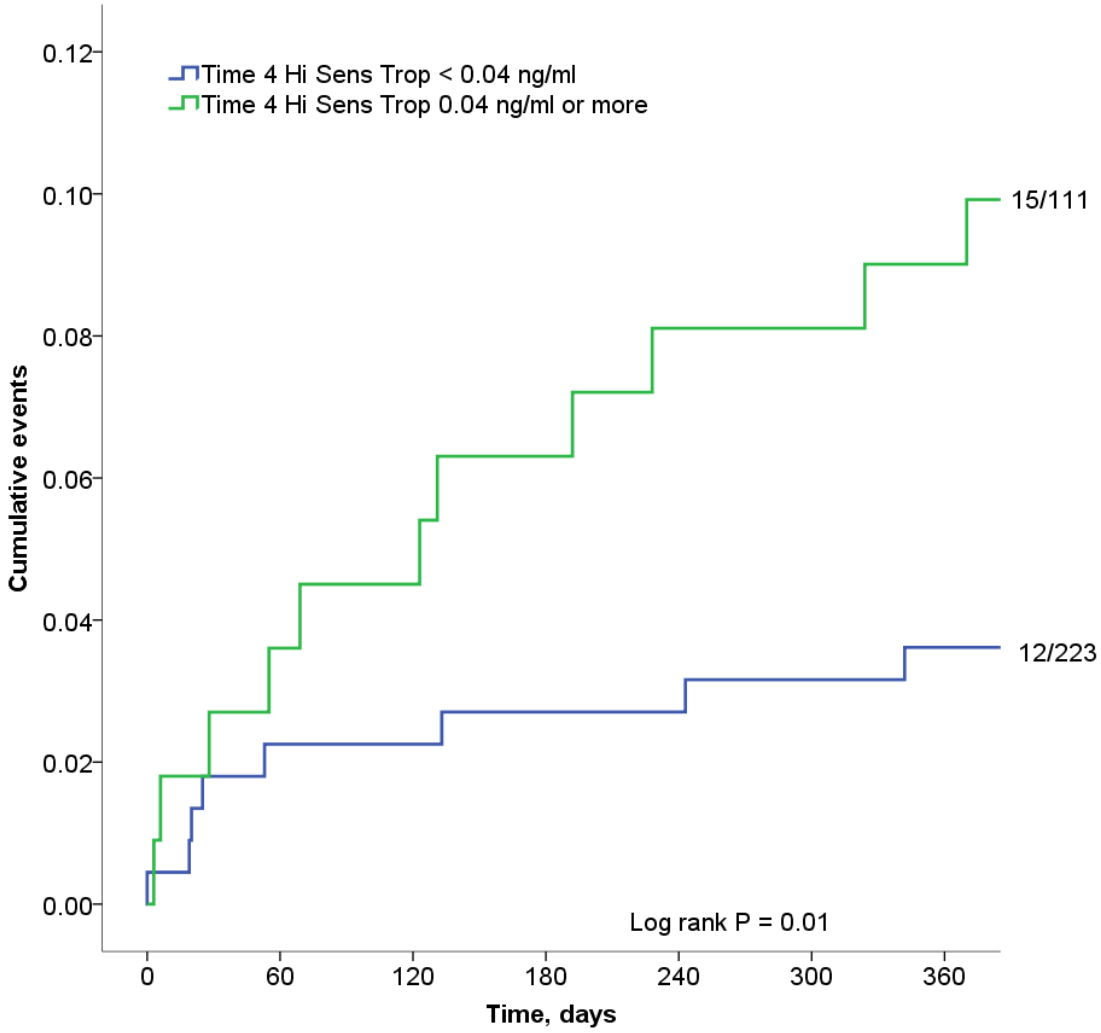



**Figure 8-14 KM unadjusted estimates of combined recurrent MI, death or further revascularisation at 1 year in patients who were TnI negative at Time 0, stratified by elevation and no elevation of TnI above the ULN at 4 hours; PPCI patients excluded**

**Table 8-8 Univariable characteristics associated with 1 year composite outcome of MI, death or further revascularisation in 381 patients who were TnI negative at Time 0 hours (P ≤ 0.01)**

| | HR (unadjusted) | 95% CI | | P Value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Major procedural complication* | 5.08 | 1.55 | 16.65 | < 0.01 |
| SYNTAX ≥ 8 (highest 2 quartiles) | 5.03 | 1.94 | 13.06 | < 0.01 |
| SVG intervention | 3.64 | 1.41 | 9.43 | < 0.01 |
| EuroSCORE ≥ 3.2 (highest 2 quartiles) | 3.27 | 1.47 | 7.24 | < 0.01 |
| > 1 stent implanted | 3.12 | 1.85 | 7.48 | < 0.01 |
| Previous revascularization | 2.93 | 1.47 | 5.84 | < 0.01 |
| > 1 vessel intervention | 2.67 | 1.34 | 5.33 | < 0.01 |
| TnI > ULN at 4 hours | 2.60 | 1.22 | 5.56 | 0.01 |
| Smallest stent diameter ≤ 2.5 mm | 2.48 | 1.24 | 4.94 | 0.01 |
| Total fluoroscopy time > 10 minutes | 2.45 | 1.20 | 4.98 | 0.01 |

*8 patients in this subgroup of 381 had a major procedural complication. These were: 1 case of coronary dissection with poor flow; 3 cases poor flow without obvious dissection; 1 case of occlusion of a large side branch; 2 cases of VF; and 1 case of self-limiting coronary perforation.

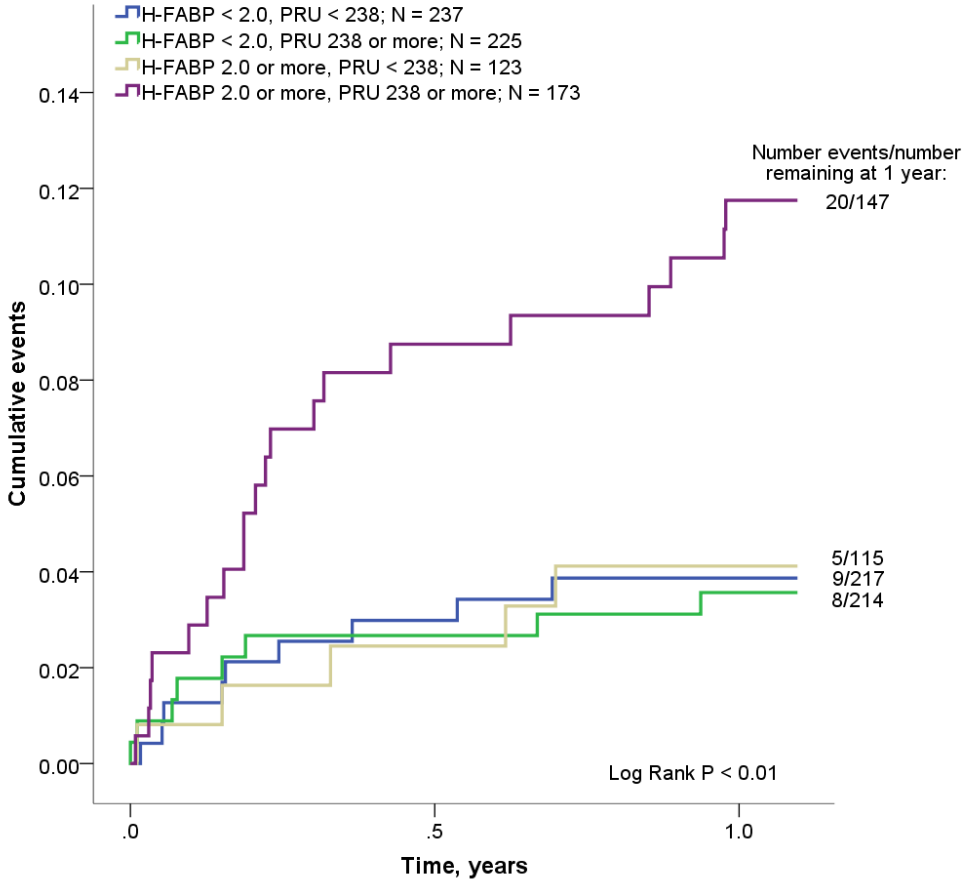



**Figure 8-15 KM estimated combined adverse event rate (all cause death, definite or probable stent thrombosis and recurrent myocardial infarction) at 1 year in patients stratified into 4 groups based on Time 0 H-FABP less than or greater than the ULN, and clopidogrel response or non-response**

## 8.9. Chapter 8 Summary Points and Discussion

- Elevation of cardiac biomarkers following PCI in ACS patients was common, but procedural MI according to the latest Universal Definition very rare (1 patient).
- Biomarker elevation was more common in patients with a variety of baseline characteristics which would tend to prolong the procedure or require multiple balloon inflations such as calcified lesions, long lesions and more than 1 stent.
- High sensitivity cTnI was not ideal for detection of procedural myocardial injury in this ACS population: the high rate of elevation of baseline levels and the low rate of clearance from the body did not allow a clear peri-procedural injury signal. H-FABP levels however were more commonly back to normal pre-procedure and gave a more plausible injury signal
- H-FABP was often elevated at 12 hours which could indicate subclinical ongoing myocardial ischaemia in some patients after PCI
- There was little evidence of an increase in myocardial injury in patients with an apparent angiographic procedural complication such as poor flow or dissection.
- Clopidogrel-resistant patients (bedside VerifyNow test, PRU ≥238) did not have any greater myocardial injury than responders; there was no correlation between PRU and post-procedural biomarker levels.
- TnI elevation above the ULN in patients who are negative at the start of the procedure was associated with a worse prognosis in an unadjusted analysis, but after adjustment the difference was no longer significant. The most important determinates of outcome were high SYNTAX score and the occurrence of a major procedural complication.
- Post-procedural H-FABP elevation was not associated with adverse events; however, pre-procedural elevation was strongly associated with adverse events, but only in clopidogrel-resistant patients.

### 8.9.1. Troponin elevation following PCI in previous studies

Troponin elevation following PCI has been reported in many studies. In a meta-analysis of 15 trials and 7578 patients with normal baseline troponin (Testa et al, 2009), 28.7% following PCI had elevation above the URL. Regarding procedural MI, 14.5% had elevation of troponin more than 3 times the URL, the cut-off suggested by the

Universal Definition of MI document at the time (Thygesen et al, 2007; note that this document has since been updated). Testa et al found an association between procedural MI and subsequent adverse events and recommended that troponin testing following PCI became mandatory:

*"At follow up of 18 months any troponin elevation was associated with an increased risk of MACE [OR 1.48 (1.12–1.96), NNH = 20], death [OR 2.19 (1.59–3.00), NNH = 50], MI [OR 3.29 (2.71–6.31), NNH = 33] and re-PCI [OR 1.47 (1.06–2.03), NNH = 25]"*

However, both the causal nature of these findings and the relevance of troponin elevation to patient management have been disputed. Neither the 2014 ESC/EACTS Guidelines on myocardial revascularization nor the 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation even mention testing for procedural biomarker release, let alone make it a mandatory requirement.

Of 275 patients who had baseline troponin within the normal range in OPERA and results available at all 3 time points, 33.4% had elevation at 4 hours and 71.7% at 12 hours. This was a remarkably high proportion and suggests that troponin elevation following PCI is the norm, at least in an ACS population. Note that a highly sensitive assay was used in OPERA which was unavailable at the time of the studies included in the Testa meta-analysis – older, less sensitive assays had higher thresholds for the ULN. Also, it is possible some of the troponin released following PCI in patients who have recently had an MI is due to a 'washout' effect from reperfused myocardium rather than from myocardial necrosis occurring secondary to the procedure; this is purely speculative however.

### 8.9.2. Possible causes of enzyme elevation following PCI

Balloon inflation within a coronary artery causes a period of iatrogenic myocardial ischaemia. This is often severe enough to cause transient ischaemic ST segment changes on the ECG which virtually always resolve within a short time after restoration of circulation. In a study of 108 patients undergoing single vessel elective PCI, Balian et al (2006) observed >1mm of ST shift (using intracoronary ECG) during PCI in 37%. Cardiac enzyme release was much more common during cases with ECG ischaemia during the procedure than without (93% vs. 19%, $p < 0.001$). No obvious angiographic complications occurred but lesion type B2/C (complex), balloon inflation time, stent length and the number of stents per patient were all factors associated with ECG

ischaemia. It seems the more complex the disease, the longer the procedure and a higher chance of myocardial ischaemia and necrosis.

Troponin release in the OPERA study was associated with many different patient baseline characteristics (Table 8-7). Elevations in all 4 of the cardiac biomarkers we studied (troponin I, myoglobin, H-FABP and CK-MB) were seen in patients with the following: fluoroscopy time ≥ 10 minutes, PCI to more than 1 vessel, total stent length ≥ 30 mm, calcified lesions, type C lesions, EuroSCORE 4th quartile (≥ 6.4), patient aged ≥ 80 years, diabetes, anaemia, and renal dysfunction. All these factors would be seen more often in cases in which more complex and difficult angioplasty was required, equating to longer myocardial ischaemia times and cardiac biomarker release. Another possible explanation put forward is embolization of plaque debris and cholesterol released during PCI, with ischaemic damage to the myocardium due to occlusion of tiny vessels not visible angiographically. This is plausible but would be very hard to objectively demonstrate. The above factors, however, would all predispose to a higher chance of micro-embolisation during PCI.

The reason for more myonecrosis in anaemic patients is less clear. Izgi et al (2006) found anaemia and the number of balloon inflations performed to be the only independent predictors of myocardial necrosis following PCI in elective patients. Anaemia was associated with complex disease in OPERA patients: the difference between median SYNTAX score in patients with Hb below and above 12 g/dL was considerable: 15.5 vs. 8.0, $p < 0.01$. Anaemic patients therefore would require more complex and prolonged procedures (median fluoroscopy times were 11.3 vs. 9.0 minutes in those with Hb below and above 12 g/dL; $p < 0.01$). However, anaemia itself could potentially cause more myonecrosis by worsening myocardial hypoxia during balloon inflations.

It has also been postulated that thrombus formation during PCI could be a potential source of procedural myocardial necrosis (Balian et al). In OPERA, platelet reactivity had no association with cardiac biomarker release which would tend to discourage this hypothesis. If intracoronary thrombus formation during the procedure was a significant cause of myocardial necrosis, patients with highly reactive platelets should have a higher risk of thrombosis and therefore more myocardial necrosis, which was not seen. Patients are anticoagulated during PCI and obvious thrombus formation is rarely a problem.

### 8.9.3. Association of procedural biomarker elevation and prognosis

As in most previous similar studies, post procedural troponin elevation in OPERA was associated with a higher rate of adverse events during follow-up (Figure 8-14). However, after adjustment for other baseline characteristics this was no longer statistically significant. Virtually all the troponin elevations in OPERA were too small to indicate the loss of any significant volume of myocardium as a result of the procedure. It is likely that troponin elevation is a marker of advanced atherosclerotic disease and it is this which carries the adverse prognosis. Post-procedural H-FABP elevation had very little association with subsequent risk of clinical events, which reinforces the impression that transient balloon-associated myocardial ischaemia is of little consequence per se.

An interesting finding was that elevated H-FABP levels were found in 40% of patients just prior to PCI (the baseline blood sample) and furthermore this was associated with adverse events during follow-up. A high rate of troponin and CK-MB would be expected in an ACS population several days after admission, as these biomarkers take days to clear from the blood. However, H-FABP is both very rapidly released from ischaemic myocardium (Viswanathan et al, 2012) and very rapidly excreted via the kidneys. In a study of 50 patients with STEMI undergoing reperfusion therapy (Glatz et al, 1994), H-FABP levels peaked at 5.7 hours and plasma levels were normal by 24 hours. In contrast, CK-MB level peaked at 11.7 hours but took 50 to 70 hours to normalise. The average wait from admission to PCI for OPERA patients was 6 days. This high rate of ongoing myocardial ischaemia in these patients indicated by elevated H-FABP levels, days after their admission with chest pain, occurred despite standard treatment with bed rest, anticoagulants and dual antiplatelet therapy. Similar rates were seen in patients presenting with UA, NSTEMI and STEMI, even in clopidogrel-responders. This implies that a long delay to angiography and intervention following ACS is a high risk strategy which should be avoided.

The second interesting finding regarding pre-procedural H-FABP elevation was its association with adverse outcomes following PCI, but only in patients resistant to clopidogrel (Figure 8-15). Patients H-FABP-negative at baseline had a low risk of further events following PCI regardless of whether or not they responded to clopidogrel. In OPERA, persistent elevation of H-FABP following admission (but prior to revascularisation) probably indicated a subset of patients with more critical coronary disease. These patients were more likely to have thrombotic lesions; thrombotic vs. non-thrombotic lesions were associated with baseline elevation of H-FABP, TnI and CK-MB: 34.4% vs. 16.7%, 32.4% vs. 16.3% and 41.9% vs. 19.2% respectively; $p < 0.01$ for all. Furthermore, patients with elevated baseline H-FABP were older and had both

higher EuroSCOREs and SYNTAX scores. However, effective P2Y12 inhibition with clopidogrel reduced their risk of adverse events following PCI to a level identical with lower-risk, H-FABP-negative patients. Pre-procedural H-FABP was an excellent marker of adverse prognosis after PCI in OPERA and may indicate patients who would benefit from a longer duration of antiplatelet therapy.

# Chapter 9 Final overview and Discussion

Acute coronary syndrome (ACS) is not only the commonest cause of death worldwide, but survivors are known to be at considerable risk of recurrent adverse clinical events for years after the index event. In a recent highly detailed national registry study from Sweden of 97254 ACS survivors (Jernberg et al, published on-line 2015), the estimated rate of a composite endpoint of MI, stroke or cardiovascular death at 1 year was 18.3%, with a further 20% experiencing this endpoint during the subsequent 36 months. This is despite modern treatment of ACS including high rates of coronary revascularisation, usually by PCI, accompanied by extensive pharmacological treatment designed to inhibit platelet thrombus activation and to modify the conventional cardiovascular risk factors for coronary disease such as hypertension, hyperlipidaemia and diabetes.

Recurrent adverse events are often be partially iatrogenic, resulting from the treatments delivered during the index event such as stent failure and bleeding while on DAPT. Recurrent ACS may be caused either by ‘de novo’ coronary lesions or by a stent malfunction such as restenosis or stent thrombosis, the latter more frequent in patients with inadequate platelet inhibition. Regarding haemorrhagic adverse events, DAPT must be continued for 12 months in most ACS patients undergoing PCI, but this therapy is known to increase bleeding rate. The risk of bleeding is the factor which limits the intensity of platelet inhibition which can be achieved in ACS patients. Clinically significant bleeding may lead to hospitalisation, the discontinuation of antiplatelet therapy (which in turn may increase the risk of ischaemic events), disability and death. A small proportion of bleeding events are directly fatal, and even non-fatal bleeding appears to be a strong risk factor for subsequent mortality, although the reasons for this are not entirely clear.

There were 2 main aims of this prospective observational study: to document, classify and investigate all adverse events experienced by ACS patients over a minimum period of 1 year post-PCI; and to find any independent associations between baseline patient characteristics and these adverse events, to risk stratify patients. Characterising those at risk of treatment failure can help us to understand why recurrent adverse events occur in some patients and not others, and may lead to changes in management which might improve outcomes. Furthermore, cardiovascular therapeutics is a large and continually developing field and, even if current technology is insufficient to help all patients with ACS, the ability to define the highest risk patients is essential to

develop and target new treatments which could improve the health and prospects of survival of ACS patients in the future.

The adverse outcomes we documented were recurrent ACS and its various clinical syndromes, stent thrombosis, death (cardiac, vascular and all-cause), binary restenosis, repeat revascularisation and bleeding, and composites of these. ARC criteria were used when available to define ischaemic events and bleeding was defined using BARC criteria. The baseline characteristics we studied included standard demographic data, medical history and procedural technical details, but we also included some recent innovations which have the potential to improve risk stratification but which are not in routine widespread clinical use. These were the SYNTAX score, an angiographic risk scoring system; a range of traditional and novel cardiac biomarkers measured in blood taken before and after PCI; and the VerifyNow P2Y12 device, a bedside platelet function test.

The study was designed to be inclusive and, although recruitment was not consecutive and the time frame was broad, the lack of exclusion criteria ensured a cohort of patients likely to represent the general local ACS population. Distinct early and late recruitment periods allowed us to study changes in baseline patient characteristics, therapeutic interventions and clinical outcomes over time. We achieved a high rate of completeness of clinical follow-up to 1 year post PCI (98.5%) and many patients had detailed follow up for years after, with a median clinical follow-up of 3.5 years and mortality tracking of 5.4 years.

## 9.1. Recurrent ACS and death rates

To summarise the main findings concerning recurrent ACS and death: recurrent ACS most commonly presented clinically as NSTEMI and occurred in 6.7% at 1 year following PCI. The rate gradually decreased over the 1$^{st}$ year and stabilised over long-term follow-up to approximately 1.8% per year. Definite, probable or possible stent thrombosis was diagnosed in 18 of the 111 total cases of recurrent ACS (16%). Stent thrombosis usually presented clinically as either STEMI or out of hospital cardiac arrest and more than half the patients with stent thrombosis died. All-cause and cardiac death rates were 2.9% and 2.0% at 1 year. Cardiac death rate gradually decreased over the first year, after which the rate stabilised for the duration of follow-up. The all-cause death rate decreased after about 3 months following PCI, to remain constant for the remainder of the study. Non-cardiac death was more common than death of cardiac cause overall, although cardiac death was more common up to 1 year. The commonest individual causes of death over a median of 5.4 years of follow-up were: metastatic cancer (27%), recurrent acute MI (18%), cardiac failure (15%), sepsis (13%) and

ischaemic stroke (8%). Just over half of all cardiac deaths were due to an acute MI and at least half of fatal MIs were due to a stent thrombosis.

The rates of adverse events in OPERA compare favourably with those from published contemporary clinical trial data. There are 2 large-scale international antiplatelet therapy trials in ACS patients undergoing coronary intervention which recruited patients at around the same period as OPERA: the TRITON-TIMI 38 trial (Wiviott et al, 2007) and the PLATO invasive study (Cannon et al, 2009). Both these trials had higher adverse event rates than those seen in OPERA. TRITON had a particularly high non-fatal MI rate, disproportionate to the death rate which was similar to that in OPERA. In PLATO, both the MI and the all-cause death rates were nearly twice those in OPERA. The high MI rate in TRITON and PLATO may be due to the inclusion of biomarker-only 'procedural MI'; the study protocols did allow for inclusion of biomarker-only MI according to the then current Universal Definition of MI.

The ADEPT-DES registry (Greg Stone et al, 2013) was a major observational study from the U.S. looking at how ischaemic and haemorrhagic adverse events related to the results of platelet function testing in 8582 patients undergoing PCI. DAPT consisted of aspirin and clopidogrel. In this study the MI and death rates at 1 year were 3.1% and 1.9%, both slightly lower than OPERA, and the 1 year definite/probable stent thrombosis rate was extremely low at 0.83% at 1 year. However only about half the patients in this study had presented with ACS. The inclusion of stable patients would be expected to lower the study cohort's risk of recurrent adverse cardiac events, as the MI and stent thrombosis rates in stable patients undergoing elective PCI in the modern era are very low. As an example to illustrate this, the TRIGGER-PCI trial (Trenk et al, 2012) designed to investigate the use of prasugrel in clopidogrel-resistant patients with stable coronary disease was halted early due to low event rates: of 423 patients randomised, only 1 patient experienced the study endpoint (CV death or MI) and the trial was terminated after 6 months.

These outcome comparisons suggest that the ACS patients in the OPERA study were managed at least as well as the patients in these high profile contemporary trials. OPERA patients, being unselected, should have been a higher risk cohort than those in the randomised trials and one might expect worse outcomes rather than better. Baseline characteristics such as age and co-morbidities appeared similar between all 3 cohorts, except for more STEMI patients in PLATO. However, the baseline characteristic we found in OPERA to be the strongest determinate of outcome, the SYNTAX score, was absent from the PLATO and TRITON trials and from the ADEPT-DES study. Nor were any other clinical risk scores provided, making the baseline risk of future adverse events

of patients in these studies harder to determine. The large difference in adverse event rates in these studies, which contain patients of similar characteristics undergoing the same treatment for the same condition in the same era, may also be partially explained by the use of individual and often opaque definitions, particularly for MI and particularly when 'silent' procedural MIs are counted. Furthermore, the outcomes following coronary intervention may be variable between different countries.

Although the OPERA study patients fared well compared to other contemporary ACS patient cohorts, the rate of recurrent adverse events was still rather too high, particularly for patients with a high baseline risk profile. Patients with the lowest SYNTAX scores (lowest quartile, median 2.0) had an estimated rate of recurrent MI, further unplanned revascularisation or all-cause death of 8% at 3.4 years; but patients in the highest quartile (median 22.3) had rates of 26%. If SYNTAX score was 30 or more (5.8% of the cohort) the rate was 38%. For the same 3 groups, estimated cardiac death rates at 5.4 years were <1%, 9% and 19%. Future efforts to reduce adverse event rate will need to focus on these higher risk patients. The single baseline characteristic with the strongest association with recurrent ACS rate was the SYNTAX score with an approximately linear relationship. A history of tobacco smoking and the use of older generation stents were the other 2 independent risk factors for recurrent ACS in the overall cohort. SYNTAX score was also a strong independent predictor of further unplanned revascularisation, cardiac death and all-cause death over long-term follow-up. The score is discussed further in Section 9.3.

## 9.2. Unplanned revascularisation

To summarise the main findings regarding unplanned further revascularisation: the rate of further unplanned revascularisation was 6.9% at 1 year but this rate decreased gradually over the duration of follow-up. There were similar 1 year rates of TLR and of non-TLR at 3.7% and 3.2%. Overall 42% of cases of further revascularisation occurred after the patient presented with recurrent ACS, a scenario more common with TLR than with non-TLR; the remainder re-presented with exertional angina. Further revascularisation was achieved by PCI about 90% of the time, usually with implantation of DES, although a small number of patients required CABG. Nearly all cases of TLR were undertaken to treat 'stent failure'; and most patients with stent failure who survived to reach hospital underwent further revascularisation. Binary restenosis, 3 times more frequent overall than stent thrombosis, was the specific reason for most cases of TLR. Binary in-stent restenosis was found in just under half the cases of recurrent UA and NSTEMI. In contrast to stent thrombosis, restenosis had no apparent impact on the overall mortality rate, nor was restenosis (or the treatment for it) found to be the direct cause of any patient's death.

The type of available stents changed considerably over the course of this study, with BMS used more than DES early in the study recruitment period and 'Xience' everolimus-eluting stents becoming the most frequently implanted device towards the end. There was a definite improvement in the crude overall binary restenosis rate with the introduction of Xience stents, at 3.7% vs. 7.2% compared to other stents, although note the total length of follow-up was shorter with Xience stents which were introduced in the 2nd recruitment phase. BMS restenosis rate was 6.2%. Taxus stents were the worst performers with a restenosis rate of 11.1% in this study.

The estimated 1 year and 2 year cumulative TLR rates with Xience stents in the OPERA study were 2% and 3%, compared to 4% and 6% for all other stent types combined. For BMS the rates at 1, 2 and 3 years were 4%, 5% and 6%. Xience stents came into use after publication of the 'SPIRIT' family of trials. SPIRIT V (Grube et al, 2011) was a post-marketing surveillance study of 2700 patients after implantation of Xience stents, about half of whom had presented with ACS. The 1 year TLR rate was 1.8%, very similar to that found in the OPERA study. Stent type was an important determinate of 1 year outcomes in the OPERA study, with only half the rate of TLR seen with the most modern types of DES than with '1st generation' DES and BMS. Although restenosis and stent thrombosis have become less common early after PCI, they have not been eliminated, occurring in approximately 4% and 2% respectively over total follow-up in the OPERA study in patients receiving Xience stents.

The latest generation of DES inhibit neointima formation in the months following implantation; however, they appear to have a greater propensity to aggressive re-growth of new atheromatous tissue (Neoatherosclerosis) which occurs later than the traditional early neointimal restenosis seen with BMS, but causes eventual catch-up of the overall rate of stent failure. This can be seen in OPERA, in which 6 month TLR rates in patients receiving DES only and BMS only were 1.7% and 3.0%, but cumulative rates at 3 years were 6.4% and 6.0%. Neoatherosclerosis formation can be clearly observed in living patients with restenosis of DES using OCT during coronary angiography (Kang et al, 2011) and the phenomenon is discussed in detail in a review by Park et al, 2012. This problem may also account for the low but persistent risk of stent thrombosis with DES which appears to last for many years after implantation. The stent thrombosis rate in OPERA was too low to allow investigation of this individual adverse endpoint in BMS and DES recipients. However, ACS (all types) rates for BMS, DES (all types) and Xience stents were very similar in the OPERA study: 5.0%, 5.0% and 5.2% at 6 months, and 11.2%, 10.5% and 11.6% at 3 years.

More work, therefore, is required to improve long-term stent safety. It is possible that further technological refinement will result in even better outcomes, although metallic stent technology may be reaching its limit: struts cannot become much thinner without the stent losing radial strength; and polymer drug-elution platforms are now 'biocompatible' with little evidence of an immediate inflammatory response in the vessel wall. The 'bio-absorbable scaffold' is the next stage in the evolution of stent technology and may be less likely to cause neo-atheroma, as these novel polymer devices are designed to gradually and completely dissolve over 2 to 3 years after implantation (Onuma and Serruys, 2011). Certainly they seem to have very low restenosis rates in early trials, but the superiority of these devices over modern metallic stents has yet to be proven. Bio-absorbable scaffold struts are more bulky than those of modern metallic stents and studies so far have shown at least as frequent thrombosis rates with the new scaffold as with the older stent (Serruys et al, 2015).

## 9.3. SYNTAX score

Based on the results of this study, if we were permitted only 1 baseline characteristic to predict the risk of further cardiac adverse events of any sort, this would be the SYNTAX score. It is unusual among clinical risk scoring systems in that it requires the input of no standard baseline characteristics and is purely based on the coronary angiogram. The score was developed to be a relatively objective means of assessing the severity of coronary artery disease in patients taking part in the SYNTAX trial, an important study of PCI vs. CABG in 1800 patients with extensive coronary disease, multi-vessel and/or left main coronary disease (Serruys et al, 2009a). In this trial the score was found to be highly predictive of recurrent adverse events in patients undergoing PCI but not in those undergoing CABG. In the study patients were stratified into low (< 22), intermediate (23 to 32) and high (≥ 33) SYNTAX groups with a median score for the whole cohort of 28. These scores are very high comparison to the OPERA cohort, with a median score of 9 and only 34 patients with SYNTAX ≥ 33, many of whom had a history of previous CABG. They are therefore very different populations. However, within the range of SYNTAX scores we observed in the OPERA study and despite associations between the SYNTAX score and multiple other baseline high risk features, there was a significant independent and probably linear association with SYNTAX score and adverse ischaemic outcomes.

The SYNTAX score was considerably more successful at predicting outcome in OPERA than was the EuroSCORE, which was not an independent predictor of adverse events. The EuroSCORE was devised to assess the risk of early mortality in patients undergoing CABG and is known to be effective for this purpose (Nashef et al, 1999), but the factors associated with poor outcomes following CABG and PCI are different.

Adverse events following CABG are mostly peri-operative and are related to the general frailty of the patient and the presence of co-morbidities such as respiratory and cerebrovascular disease. In contrast, major peri-procedural adverse events following PCI are relatively rare, but later events are more common and are often due to the presence of residual disease following PCI or stent failure, the likelihood of which increase with increasing SYNTAX score. The number and complexity of proximal coronary artery lesions (essentially what the SYNTAX score measures) in patients undergoing CABG is relatively less important, as grafts can be placed onto healthy vessel distal to the stenosis. A recent analysis of causes of death in the SYNTAX trial (Milojevic et al, 2016) showed that, although all-cause mortality was not statistically different between the CABG and PCI groups, the rate of death due to definite MI was much higher in the PCI group compared to the CABG group: 4.1% vs. 0.4%, HR (95% CI) 8.43 (2.99 to 23.67); $p < 0.0001$. In this as in previous analyses, the SYNTAX score was an independent predictor of cardiac mortality after PCI but not after CABG.

Although the SYNTAX score is a good predictor of recurrent adverse events following PCI, it is not clear how patients who are identified as being at high risk of recurrent events by means of the SYNTAX score could have their treatment modified to reduce this risk. Performing more extensive PCI was shown to be detrimental in the SYNTAX trial in which all lesions had to be treated if possible. Improvements in stent design (as discussed above) and pressure wire guidance of PCI (the FAME II study, de Bruyne et al, 2012) are all possible ways in which outcomes may improve. However, part of the risk carried by increasing SYNTAX score is due to its association with other baseline characteristics which themselves are related to increased adverse event rate risk; these would need to be addressed individually where possible to further lower risk.

## 9.4. Bleeding

To summarise the main results regarding bleeding: clinically significant bleeding (BARC 2 to 5) occurred in 3.8% at 1 year. There was a high initial rate due to access site bleeding. The rate dropped after most patients discontinued DAPT, shortly after the 1 year mark, but events continued to occur for the duration of follow-up at a lower rate. The commonest types of bleeding were epistaxis and GI bleeding (mainly lower GI e.g. haemorrhoids). Most cases were clinically trivial or mild and for many patients low-level bleeding was a chronic recurrent problem. Patients with bleeding had a higher mortality rate than those without bleeding, but this finding was confounded by other baseline characteristics which were associated with mortality and bleeding, particularly advancing age and the presence of anaemia pre-PCI. Bleeding was an uncommon direct cause of death, occurring in 5 patients over long-term follow-up. Only 2 or possibly 3 of these cases could be said to be directly related to the intervention or adjunctive

pharmacology (1 arterial access site bleed, 1 GI bleed several days after PCI and 1 case of subdural haematoma at 55 days).

Bleeding induced by antiplatelet and anticoagulant medication is a major concern for ACS patients, particularly those invasively managed due to the risk of stent thrombosis if DAPT has to be discontinued prematurely. Large vascular access site, GI or intracranial bleeds may prove fatal; and many studies have documented alarmingly high mortality rates later on in those who experience non-fatal bleeding. In the OPERA study there was an initial high rate of BARC 2 to 5 bleeding which was mainly due to vascular access site haematomas, the majority of patients having undergone femoral access procedures. After the first few days the bleeding rate dropped and levelled out, and then decreased further at about 13 months, approximately the point after which patients changed from DAPT to single antiplatelet therapy.

Regarding bleeding rates in comparison to other contemporary trials, both the TRITON and PLATO trials thoroughly reported bleeding events as safety endpoints, but neither trial could make use of the BARC criteria which were published following the completion of these studies. However, TIMI major and minor non-CABG related bleeding criteria together probably approximate to the BARC 2 to 5 criteria. Non CABG-related TIMI major and minor bleeding occurred in the PLATO invasive clopidogrel arm in 3.9% at 1 year; and 3.8% in the clopidogrel arm of TRITON at 15 months. The BARC 2 to 5 bleeding rate in OPERA at 1 year was much the same, 3.8%. In ADEPT-DES, ‘clinically relevant’ bleeding at 1 year was reported in 6.2%, a higher rate than the other studies.

The most important baseline risk factor for bleeding in OPERA was increasing age, with BARC 2 to 5 rates at 3.4 years of 0% in the under 40s compared to 21.4% in the over 80s. Anaemia at baseline was also an independent marker of future bleeding problems. Another factor, the use of ACE-inhibitors, was associated with a lower risk of bleeding although this appeared to be confined to men only. Several risk scores for bleeding based on multiple baseline risk factors have been developed although recent studies to validate them have shown poor predictive value (Dobies et al, 2014). From the information available in the OPERA study, formal bleeding risk scores would seem unnecessary, with most of the predictive power coming from the 2 parameters age and anaemia. The BARC 2 to 5 bleeding rates at 30 days, 1 year and 3.4 years in patients who were both anaemic (Hb < 12 g/dl) and aged 70 or over were 7.5%, 13.8% and 25.0%, with excess bleeding rates compared to the other groups seen both early and late in follow-up (Figure 6-5). This should therefore be the group to concentrate on if manoeuvres to reduce bleeding rates are to be considered.

The fatal bleeding rate (BARC 5) in OPERA was 0.3% at 1 year (3 cases). By comparison, fatal bleeding occurred in 0.1% at 15 months in the clopidogrel arm of TRITON (5 cases). Fatal bleeding was, oddly, not reported as a separate endpoint in the PLATO Invasive study, although it was in the PLATO entire cohort study: 0.3% in the clopidogrel arm (23 events) at 1 year. In OPERA, the specific causes of the 1 year haemorrhagic fatalities were:

- An 80 year old man died within 24 hours of PCI from the combination of exsanguination from a punctured femoral bypass graft (a route of last resort to allow treatment of intractable angina when no other access sites could be found) and myocardial ischaemia. This patient was given a IIbIIIa inhibitor following PCI in view of poor coronary flow. Other than this tragic event there were no other fatalities from access site bleeding, the main effects of which were increased discomfort and delayed discharge
- One patient died of a massive GI bleed. This occurred in a 79 year old lady 4 days post PCI, after her discharge home. She was anaemic at baseline (Hb 10.7 g/dl) but was not taking a prophylactic PPI. She was given a IIbIIIa inhibitor during PCI and she had an unusually high level of clopidogrel inhibition and correspondingly very low platelet reactivity: %inhibition of 80%, PRU of 63.
- One patient died at 55 days from chronic subdural haematomas diagnosed in the weeks following PCI. This was a 77 year old lady with diabetes and chronic renal failure who was given a IIbIIIa inhibitor at the time of PCI. She had had trouble with hypoglycaemic attacks and was at risk of head injury from these.

All these patients were elderly, had several other risk factors for bleeding, and had all been given IIbIIIa inhibitors in addition to DAPT. The only haemorrhagic death which may have been avoidable in retrospect was the GI bleed, which was due to endoscopically proven peptic ulceration and may have been prevented by use of a PPI. There is no reason why PPI prophylactic therapy should not be part of standard care in elderly patients who require DAPT. For elderly patients generally, it may be possible to reduce bleeding by limiting the intensity and/or duration of DAPT – however, there is little or no evidence to support these strategies, and reducing the degree of platelet inhibition would likely result in increased rates of recurrent ischaemia. The ideal duration of DAPT in elderly patients, a group with both high bleeding and thrombotic risk, is unknown.

Strategies to reduce bleeding rates are of course desirable in order to reduce patient discomfort and length of hospital stay, and possibly reduce the overtly haemorrhagic death rate, although this appears to be very low in most studies. There remains the question of the apparent association of bleeding with subsequent mortality. Although this finding was replicated in the OPERA study, detailed examination of the circumstances of each death of a patient in whom BARC 2 to 4 bleeding had previously been recorded revealed no case in which bleeding played a significant role in the death. All these patients died of either advanced organ failure or metastatic cancer. It seems that non-fatal bleeding is therefore a marker of increased risk of death due to its association with serious co-morbidities and advanced age, rather than as a direct precipitant.

## 9.5. Platelet reactivity, anaemia and adverse events

To summarise the main findings regarding the associations between platelet function testing and adverse outcomes: platelet reactivity, both Base and PRU, was highly variable between patients. There was no measureable platelet inhibition from clopidogrel (0% inhibition) in more than a fifth of patients, and more than half of the total cohort had a PRU above the 'ischaemic threshold' of 238 i.e. were 'clopidogrel resistant'. The baseline characteristics most strongly correlated with PRU level were the Base value (direct relationship) and pre-procedural Hb (inverse relationship). The 1 year recurrent ACS rate in clopidogrel non-responders (high PRU) was approximately 2.5 times that in responders, but this difference was only of borderline statistical significance. The 1 year rates of death and of combined death and MI in non-responders were about twice those seen in responders; again these findings were of borderline statistical significance.

Patients with 'hyper-response' (PRU < 179) had a significantly increased risk of bleeding. PRU had a U-shaped rather than a linear association with adverse clinical events: the lowest event rate occurred within a PRU range which corresponded closely with the optimal range previously published by the ARMYDA-PROVE group. No patient in this group experienced a cardiac death during the entire follow-up period. The lowest and highest PRU octiles had equivalent combined adverse event rates.

The Base value carried considerable long-term prognostic information, with a higher rate of ischaemic events over long-term follow-up in patients with high Base platelet reactivity.

Anaemia prior to PCI was a powerful marker of poor prognosis in this study, being associated with bleeding, recurrent ACS and death. One third of patients with Hb

levels < 12 g/dL died during long-term follow-up. However, anaemia often occurred in patients with multiple other high-risk features including age and SYNTAX score. There was a strong interaction between anaemia and platelet reactivity. Anaemic patients with high platelet reactivity did badly following PCI, but anaemia combined with low platelet reactivity was not associated with adverse events. By the same token, high platelet reactivity only seemed to be a poor prognostic sign when accompanied by anaemia.

The OPERA study patients with 'clopidogrel resistance' based on the results of a VerifyNow test had 2.5 times more MACE events than clopidogrel responders, but this difference was of only borderline statistical significance. The lack of statistical significance was likely to be because clopidogrel resistance was remarkably common, seen in more than half the patients (in fact many had no detectable platelet inhibition at all), yet the majority of these patients experienced no ill effects.

Coronary thrombosis is a complex, incompletely understood pathological event and there are probably multiple reasons for the high level of toleration of clopidogrel resistance, but one could speculate the following:

- These patients were all in receipt of an additional antiplatelet agent, aspirin, which is known to be clinically very effective in isolation in the setting of acute MI.
- Most patients were only partially resistant to clopidogrel, with a very broad range of degree of inhibition in the cohort; even partial inhibition would be expected to lower the chances of coronary thrombosis.
- Patients had a very wide range of baseline platelet inhibition to thrombin (the Base value) with an almost normal distribution. Furthermore this measurement correlated well with the risk of longer term, off-clopidogrel ischaemic events. Many patients in the study already had a low risk of recurrent ischaemia by having naturally low platelet reactivity to thrombin, mitigating the consequences of clopidogrel resistance.
- Even in the absence of any antiplatelet agents, only a proportion of coronary stents would be expected to thrombose thanks to the body's natural anti-clotting homeostatic mechanisms. The proportion one would expect to thrombose without antiplatelets is entirely unknown and such a study could never be performed in man.

Among the highest risk patients in this cohort were anaemic patients with high platelet reactivity. Furthermore, platelet reactivity as measured by the VerifyNow BASE test, and to lesser extent the PRU test, was highly variable and inversely proportional to

Hb level: the lower Hb level, the higher the platelet reactivity. While the association between Base and Hb was fairly linear, the PRU-Hb curve had more of an inverted U-shape because of a greater frequency of anaemia in patients with hyper-response to clopidogrel. Anaemic patients had a wide distribution of PRU values but those with high Hb levels had low platelet reactivity range within a narrow range.

This inverse association between platelet reactivity and Hb has been observed before by other investigators but the mechanisms by which it occurs are unknown. One might in fact expect the converse situation: people with strongly reactive platelets should have less blood loss and therefore higher Hb levels. This implies that changes in Hb level induce changes in platelet reactivity. It is plausible that a negative feedback mechanism between Hb and platelet reactivity exists for reasons of maintaining homeostasis. In a state of anaemia, an increase in platelet reactivity would be desirable to reduce bleeding and preserve Hb levels. Conversely, in the pro-thrombotic state of polycythaemia, a decrease in platelet reactivity would lower the risk of arterial and venous thrombosis.

One author who noted the association of PRU with VerifyNow Hb has suggested that this is an artefact caused by the light transmission basis of the test: a higher haematocrit may increase turbidity of the blood sample and decrease light transmission (Kim et al, 2014). This was concluded because there was no apparent Hb/platelet reactivity interaction when using the Multiplate analyzer, a point-of-care test which relies on changes in electrical impedance rather than light transmission. However, 2 other investigators who noted the interaction with the VerifyNow test and Hb went on to confirm the interaction using light transmission aggregometry (LTA) (Frelinger et al, 2013 and Toma et al, 2012). As LTA uses plasma devoid of erythrocytes, the interaction cannot be an artefact caused by changes in light transmission due to haematocrit.

An unexplained lack of mortality benefit has been observed in many trials of dual antiplatelet therapy where platelet inhibition has been intensified either by increasing the dose of clopidogrel or substituting it for prasugrel. The ADEPT-DES registry (Stone et al, 2013) showed that the stent thrombosis rate in patients with high platelet reactivity on clopidogrel was considerably higher than in other patients (adjusted HR 2·49 [95% CI 1·43 to 4·31], p=0·001); yet the adjusted mortality rate between the 2 groups was no different. This was put down to an increased mortality rate in patients with clinically relevant bleeding, although the direct cause of the additional deaths in anaemic patients was not reported. Furthermore, unexpectedly higher mortality rates were seen in all 5 trials of oral IIbIIIa antagonists in the 1990s, drugs which inhibit platelet aggregation very powerfully, the IIbIIa molecule being the final common pathway for aggregation

(Chew et al, 2001). This outcome was not explained by bleeding and put down to an unknown toxic effect of the drugs, which are no longer available. All these studies point to some kind of hazard incurred by very low platelet reactivity.

From the OPERA study results, this hazard does not appear to derive either from premature antiplatelet drug discontinuation (which might occur after a bleeding episode), or from the direct effects of bleeding, although bleeding rates are certainly higher in this group. Anaemia and bleeding have been well described as risk factors for death after ACS and PCI, but death directly caused by bleeding is rare; MI-related death is much more common. If a negative feedback loop between Hb and platelet reactivity does exist, this could account for the paradoxically worse prognosis seen in patients with more intense platelet inhibition on clopidogrel. The more the P2Y12 receptor is inhibited, the higher the chances that bleeding would lead to a drop in Hb level, so triggering a protective increase in baseline platelet reactivity. If baseline reactivity increases, PRU would also increase, as these values are strongly linked (Figure 7-28). This would imply the existence of a mid-range optimal level of platelet inhibition. This is indeed found in the OPERA and some, but not all, similar studies.

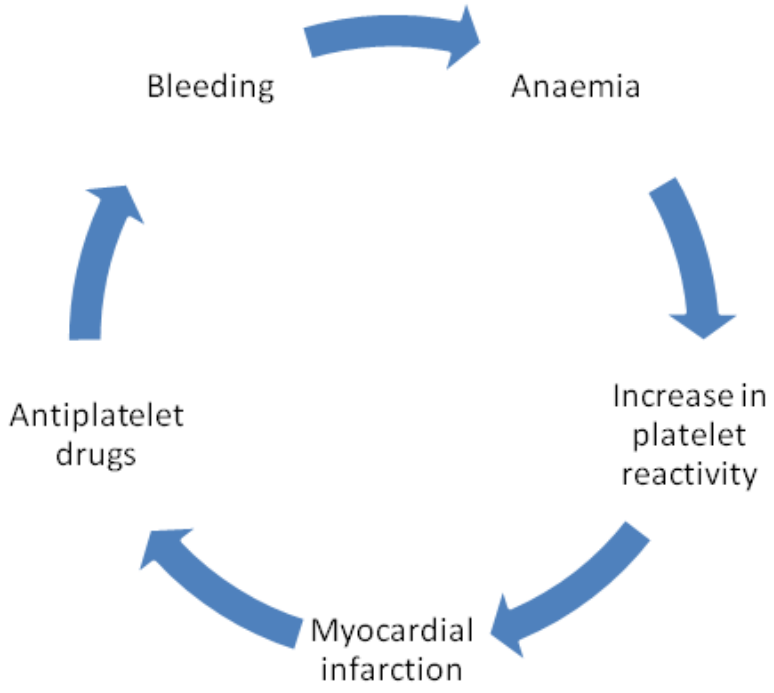



**Figure 9-1 Diagram to show a hypothetical adverse circular relationship between bleeding, anaemia and myocardial infarction which could result in worse outcomes in certain patients**

Although no studies have been published which document changes in individual platelet reactivity in response to changes in Hb level, there is some evidence that platelet reactivity can change considerably in response to a range of other non-

pharmacological stimuli. For example, Vij et al (2009) demonstrated that high altitude produces a marked reduction in platelet reactivity on testing with several agonists (collagen, adrenaline and ADP). Individual patient platelet aggregation rates have been shown to increase after respiratory tract infection (Kreutz et al, 2007). How these stimuli can change platelet reactivity is, however, completely unknown.

*Previous evidence for a VerifyNow optimal range*

Previous platelet function studies using the VerifyNow system have focused on identification of the 'ischaemic' PRU threshold and on the risk of stent thrombosis rather than on bleeding, yet a firm consensus ischaemic has not been decided upon (Tantry et al, 2013). While an association with more intense $P2Y_{12}$ platelet inhibition and bleeding has been previously demonstrated, likewise there is no consensus on a VerifyNow PRU bleeding threshold. Furthermore, most antiplatelet studies exclude patients thought to be particularly at risk of bleeding, a design feature likely to diminish the adverse impact of intense platelet inhibition. However, in addition to ARMYDA-PROVE, 2 further studies have identified a therapeutic PRU range using the VerifyNow device. All 3 studies used Receiver Operator Characteristic curves to estimate bleeding and ischaemic thresholds.

Campo et al (2011) found evidence for a PRU bleeding threshold of 86, based on 19 bleeding events at 1 year in 300 patients undergoing PCI (the ischaemic threshold was 238). This bleeding threshold is substantially lower than that proposed by the ARMYDA-PROVE investigators. However, the design of the former study was to test platelet function at 30 days and only patients with adverse events after this time point were included. In contrast, Patti and the ARMYDA-BLEEDS investigators (2011) derived a different bleeding threshold of 189 from events *up to* 30 days (15 bleeding events) in a similar sized cohort of 310, a similar PRU value to that found by the ARMYDA-PROVE investigators (PRU 179).

While the larger cohort of 732 patients in ARMYDA-PROVE increases this study's discriminatory ability, many of the adverse events in ARMYDA-PROVE were procedure-related: 27 of 36 bleeding events were entry site haematomas and 45 of 57 ischaemic events were peri-procedural MI. The longer-term relevance of this range to an unselected ACS population of patients on DAPT was not known.

## 9.6. Cardiac biomarker elevation and adverse events

To summarise the main findings regarding cardiac biomarkers in the OPERA study: pre-procedural biomarker elevation was common, particularly with TnI, despite a median time from admission with ACS to PCI of 6 days. Post-procedural biomarker elevation in patients who were biomarker-negative at Time 0 hours was common at both the 4 and 12 hour time points, with the highest levels at 12 hours even for the rapidly-excreted biomarker H-FABP. Post-procedural Biomarker elevation was associated with the severity of coronary disease and increased interventional complexity, but not with angiographic complications. Post-procedural biomarker elevation was not an independent predictor of adverse events. Pre-procedural (rather than post-procedural) H-FABP proved to be an independent predictor of adverse outcomes when combined with when platelet reactivity.

Biomarker elevation was frequent in OPERA study patients at all 3 time points it was tested (0, 4 and 12 hours). It is likely that in some cases this was residual elevation from the ACS which led to hospital admission, days before PCI. However, the rapidly excreted biomarker H-FABP was also elevated in many patients, suggesting the possibility of ongoing ischaemia in some despite initial pharmacological therapy. Pre-procedural H-FABP elevation was only of prognostic importance following PCI in patients with a poor response to clopidogrel.

The results of the post PCI biomarker analysis were disappointing from a prognostic point of view, with no independent association with adverse outcome seen. Post-PCI enzyme elevation was usually of low magnitude, suggesting little procedural myocardial damage, and was more likely in the presence of certain mechanical factors such as calcification, longer lesion length and greater number of lesions. These factors would generally lead to longer procedures with more frequent and aggressive balloon dilatation, when the transient myocardial ischaemia inevitably induced by PCI would be expected to be greater.

All the biomarkers tested continued to rise following PCI from 4 to 12 hours. Particularly with the rapidly excreted biomarker H-FABP one might expect an early rise followed by a fall at 12 hours. Some patients showed this pattern, but in some H-FABP continued to rise by 12 hours. Still others had a 12 hour rise but no 4 hour rise. Delayed release of cardiac enzymes in patients undergoing reperfusion to ischaemic myocardium is of interest as it might imply delayed ongoing ischaemia. It seems unlikely that myocardial perfusion normalises immediately following PCI in all patients and it may

be either that the enzyme is ‘washed out’ as perfusion gradually improves (a cardiac biomarker washout phenomenon following reperfusion has been observed in patients undergoing revascularisation in acute ST elevation MI); or the late peak represents ‘reperfusion injury’ in which, theoretically, the oxidative stress induced by restoration of the blood supply can cause further damage to myocytes

Myocardial injury may possibly continue for several hours after stent implantation due continuing ischaemia from mechanical or embolic phenomena, for example partial occlusion of small vessels due to trapped side branches or distal embolisation of atheromatous material. Another possibility is that platelet activation and thrombus formation occurs after the anticoagulation has worn off, with subsequent distal embolisation of thrombus to occlude peripheral branches. Against this is the complete lack of a difference in platelet reactivity in the patients with and without biomarker elevation.

Transient impairment of renal clearance of myoglobin due to contrast-induced renal dysfunction might also be considered a cause of late biomarker elevation. However, there was no significant difference in pre-procedural creatinine between patients with a 4 hour peak and those with a 12 hour peak (95 μmol/L vs. 97 μmol/L, $p = 0.77$), nor was there a significant difference between the volume of contrast used in the same groups (median 215 ml vs. 250ml, $p = 0.57$).

## 9.7. Overall conclusions

1. Patients who present with ACS have an unacceptably high rate of recurrent ACS, despite coronary intervention and other attempts to modulate the disease process. This rate did not diminish during the course of the OPERA study. There is clear evidence of an increase in the rate of recurrent ischaemic events in patients with ACS undergoing PCI who have high platelet reactivity on clopidogrel. Nevertheless, no study has shown clear health benefits of increasing the intensity or duration of long term antiplatelet therapy in unselected patients, there is a lack of benefit seen with prasugrel (a strong $P2Y_{12}$ inhibitor) over clopidogrel (TRITON-TIMI 38), there were high recurrent event rates with ticagrelor (PLATO) and there is a very adverse risk profile associated with bleeding and anaemia, which are more common with prolonged DAPT. The current evidence therefore discourages a strategy of universal long-term intense $P2Y_{12}$ receptor inhibition and reasons for this lack of benefit should be sought.
2. The findings of our study support the existence of an optimal therapeutic VerifyNow PRU in ACS patients following PCI who are unselected with regard to bleeding risk. A PRU level within a fairly narrow range around 200 while on treatment with clopidogrel and aspirin was associated with an extremely low risk of long-term adverse events. The safety and efficacy of PCI and DAPT might in future be further improved if a mid-range 'therapeutic' level of $P2Y_{12}$ inhibition could be reliably achieved, particularly for those patients at increased risk of bleeding, the elderly and the frail. However, it is by no means clear how achieving and maintaining an optimal PRU long term with currently available health resources can be done. Unfortunately, projects attempting to achieve optimal PRU have so far failed to show any clinical improvement with this strategy, although this lack of success could in part be due to poor study design.
3. Post-procedural elevation of cardiac biomarkers was very common and was associated with adverse prognosis, although this was likely due to confounding factors. However, pre-procedural H-FABP was a promising risk marker for 1 year adverse events; adequate response to clopidogrel (PRU <238) appeared to reduce the risk associated with baseline H-FABP elevation to that associated with normal H-FABP levels. H-FABP as a marker of ongoing ischaemia could have a useful role in clinical management of ACS patients, for example to guide the urgency and intensity of treatment in non-STEACS patients. It also has a potential role in identification of a sub-group of patients who would benefit from longer-term DAPT.
4. The SYNTAX score was a valuable risk prediction tool in the OPERA study and it is recommended that this score is calculated in all patients who require revascularisation. The SYNTAX trial has shown that patients with very high scores

have a greatly reduced rate of cardiac death at 5 years when they undergo revascularisation by CABG rather than PCI.

5. Stent design changed considerably during the OPERA study. This was associated with improvements in short term outcomes but longer term benefit was not so obvious, possibly due to the problem of neoatherosclerosis formation. Bioabsorbable scaffolds are currently in development and hold great promise but also have their limitations. Stents and scaffolds are likely to continue change and improve.
6. Platelet reactivity to thrombin (as the VerifyNow Base result) was extremely variable in OPERA patients and high values appeared to be associated with long term adverse prognosis, particularly in anaemic patients. This leads to the speculation that the well-recognised adverse prognosis conferred by anaemia could in fact be caused by an increased propensity to arterial thrombosis, mediated by an increase in platelet reactivity as a direct response to low Hb levels. Platelet reactivity is known to vary in individuals in response to certain factors, but has not yet been shown to alter based on Hb levels. Platelet reactivity as a risk factor for MI has never been studied in the general population.

## 9.8. Suggestions for future research

1. Current antiplatelet therapy regimes in patients with ACS are not fully effective in reducing recurrent cardiovascular events. New therapies must be targeted at patients at the highest risk of recurrent events. Presently, studies could be undertaken in which the markers of risk identified in the OPERA study are used to stratify for long-term antiplatelet therapy. Future studies could look at the benefits of novel antiplatelet therapies such as the PAR-1 inhibitors as alternatives for P2Y12 therapy failure. The most promising novel markers of adverse prognosis from OPERA were pre-procedural H-FABP elevation, high SYNTAX scores and high thrombin-mediated platelet reactivity (VerifyNow Base). All 3 could be determined with relative ease in the cath lab at the time of PCI.
2. Approximately half the cases of further revascularisation in OPERA were to a non target lesion. Presumably these lesions developed after the initial ACS treatment. It would be a useful exercise to re-analyse the initial angiograms and patient characteristics for clues as to why this might happen.
3. In patients receiving P2Y12 inhibitors we should continue to seek ways to tailor platelet inhibition to achieve moderate levels of P2Y12-mediated reactivity, enough to prevent thrombosis but not too much to induce bleeding or anaemia. This would probably require the use of a wider variety of drug doses than is currently available.

4. We have hypothesised from the OPERA data that anaemia may be capable of inducing a rebound increase in non P2Y12 mediated platelet reactivity, in this case in response to thrombin, which accounts for the poor cardiovascular prognosis associated with anaemia. The observation that platelet reactivity and haemoglobin level may be dependent requires further investigation, as does the finding of wide variability in thrombin receptor mediated platelet reactivity, as both of these were linked strongly to prognosis. It would be relatively simple to design a study involving a group of patients with anaemia in which platelet function was tested when anaemic and then again after normal Hb levels were regained. Another study looking at platelet reactivity in relation to subsequent ACS in patients without prior CAD or antiplatelet therapy would also be relatively simple to design, although would be likely to require large numbers.

## 9.9. Strengths and limitations

The strengths of this study include the unselected patient data source, the large size of the cohort, data relating to drug compliance and adherence, 'hard' clinical adverse events arbitrated by an independent panel of experts, highly detailed follow-up data, long length of follow-up and the linkage of data to the Medical Research Information System.

The main limitation of this study is its observational design, meaning that all findings and associations are hypothesis-generating only. Other limitations include the interruption to patient recruitment, the long recruitment time span and the non-consecutive nature of recruitment. There was only 1 PRU test per patient, performed at the time of PCI - this may lead to underestimation of the level of platelet inhibition for patients having emergent PCI; these patients had to be excluded from this part of the study. Next, the 600mg clopidogrel loading dose was achieved by a variety of dosing schedules. However, the effect of this on PRU is likely to be minimal due to the prolonged time from admission to PCI in this study – most patients were effectively on stable DAPT therapy at the time of their VerifyNow test. We did not test for aspirin resistance; however, aspirin resistance is rare compared to clopidogrel resistance and evidence of adverse clinical consequences from aspirin resistance is inconclusive. We did not investigate the newer P2Y12 inhibitors prasugrel or ticagrelor, to which the results of this study cannot be extrapolated. Regarding biomarker analyses, many patients had missing results, mainly at the 12 hour time point. Regarding the SYNTAX score, all scoring was performed by 2 operators (UMS and IRP) and scoring accuracy was not corroborated by an independent observer.

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