Outcomes and monocyte response in percutaneous coronary intervention

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Statement of originality

I confirm that the work in this thesis is my own apart from where referenced or acknowledged.

Epigraph

‘Please hold my hand for every balloon needs a string to stay grounded’ - Wald Wassermann

Abstract

Percutaneous coronary intervention (PCI) is a well-established treatment for coronary artery disease. Despite continuous refinement of the stent-technology and significant improvement of patient outcomes over the years, stent failure continues to be its Achille's heal. Inflammation, the foundation of coronary artery disease, also appears to be related to the small but lifelong risk that accompanies coronary stents. Drug coated balloon (DCB)-only angioplasty is a relatively new technique, which aims to deliver an anti-restenotic drug to the vessel wall without leaving any foreign material behind. Currently, it is recommended by international guidelines for treatment of in-stent restenosis, but not for de novo coronary artery disease.

The main aims of this thesis are: a) assessment of safety and efficacy of DCB-only angioplasty for de novo coronary artery disease and b) assessment of the inflammatory reaction after elective angioplasty with specific focus on monocyte subsets.

In chapter 1, I gave a summary of the historical perspective of PCI from balloon angioplasty to drug eluting stent (DES) and DCB. I focused on the significance of pre-PCI and post-PCI inflammatory status for patient outcomes. Finally, I reviewed Ultrasmall Superparamagnetic Particles of Iron Oxide – enhanced Cardiovascular Magnetic Resonance (USPIO-enhanced CMR), a relatively recent technique which makes possible the in vivo assessment of myocardial cellular inflammation. In chapter 2, I have described the general methods for the retrospective and prospective studies.

In chapter 3, I demonstrated that day-case DCB-only angioplasty is safe in terms of readmission with acute vessel closure (1%). In chapter 4, I demonstrated that paclitaxel DCB-angioplasty is not associated with increased late mortality. I compared 429 consecutive patients treated with paclitaxel DCB versus 1088 consecutive patients treated with non-paclitaxel 2nd generation DES and demonstrated that there was no evidence of late mortality signal.

In chapter 5, I assessed DCB-only angioplasty as part of routine clinical practice, in patients with stable angina. I compared a total of 544 consecutive patients (640 de novo lesions) treated with paclitaxel DCB and 693 consecutive patients (831 de novo lesions) treated with 2nd generation DES and demonstrated that there is no difference between DCB-only angioplasty and 2nd generation DES in terms of all-cause mortality and net cardiac events including target lesion revascularisation.

In chapter 6, I assessed DCB-only angioplasty as part of routine clinical practice, in patients with ST elevation myocardial infarction due to de novo disease. I compared a total of 452 consecutive patients treated with paclitaxel DCB only and 687 consecutive patients treated with 2nd generation DES only and demonstrated that there is no difference between DCB-only angioplasty and 2nd generation DES in terms of all-cause mortality and net cardiac events including target lesion revascularisation.

Chapter 7 investigated the inflammatory response following angioplasty. I prospectively recruited 30 patients undergoing elective angioplasty for de novo disease either with DCB or DES. I demonstrated that intermediate monocytes, a highly proatherogenic monocyte subset, increased significantly two months after elective, uncomplicated angioplasty. The intermediate monocytes increased significantly after DES but not after DCB. Chapter 8 focused on proving the concept that it is possible to detect myocardial inflammation utilising USPIO-enhanced CMR.

In chapter 9, I reflected on the clinical implications of my studies and focused on the need for larger trials in this field.

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Publications

The following publications have been produced as a result of this thesis.

1) **Merinopoulos I**, Wickramarachchi U, Wardley J, Khanna V, Gunawardena T, Maart C, Vassiliou VS, Eccleshall SC. Day Case Discharge of patients treated with Drug Coated Balloon Only Angioplasty for de novo coronary artery disease: A Single Centre Experience. Catheter Cardiovasc Interv. 2020 Jan;95(1):105-108. doi: 10.1002/ccd.28217

2) **Merinopoulos I**, Gunawardena T, Stirrat C, Cameron D, Eccleshall SC, Dweck MR, Newby DE, Vassiliou VS. Diagnostic applications of ultrasound superparamagnetic particles of iron oxide for imaging myocardial and vascular inflammation. JACC Cardiovasc Imaging 2021 Jun; 14(6):1249-1264. doi: 10.1016/j.jcmg.2020.06.038

3) **Merinopoulos I**, Gunawardena T, Wickramarachchi U, Richardson P, Maart C, Sreekumar S, Sawh C, Wistow T, Sarev T, Ryding A, Gilbert T, Perperoglou A, Vassiliou VS, Eccleshall SC. Long-term safety of paclitaxel drug-coated balloon-only angioplasty for de novo coronary artery disease: the SPARTAN DCB study. Clin Res Cardiol. 2021 Feb; 110(2): 220-227. doi: 10.1007/s00392-020-01734-6

4) **Merinopoulos I**, Gunawardena T, Corballis N, Tsampasian V, Eccleshall SC, Smith J, Vassiliou VS. The role of inflammation in percutaneous coronary intervention, from balloon angioplasty to drug eluting stents. Minerva Cardiol Angiol 2022 Jul 5. doi: 10.23736/S2724-5683.22.06091-4

5) **Merinopoulos I**, Gunawardena T, Corballis N, Bhalraam U, Gilbert T, Maart C, Richardson P, Ryding A, Sarev T, Sawh C, Sulfi S, Wickramarachchi U, Wistow T, Mohamed M, Mamas M, Vassiliou VS, Eccleshall SC. Paclitaxel drug coated balloon-only angioplasty for de novo coronary artery disease in elective clinical practice. Clin Res Cardiol. 2022 Sep 14. doi: 10.1007/s00392-022-02106-y

    - Also presented (oral presentation) at BCS conference in 2022 (Runner up for 'Best of the best' clinical abstract) and at ESC conference in 2022

6) **Merinopoulos I**, Gunawardena T, Corballis N, Bhalraam U, Reinhold J, Wickramarachchi U, Maart C, Gilbert T, Richardson P, Sulfi S, Sarev T, Sawh C, Wistow T, Ryding A, Mohamed M, Perperoglou A, Mamas M, Vassiliou V, Eccleshall S. Assessment of paclitaxel drug coated balloon only angioplasty in STEMI. JACC cardiovascular intervention 2023 Apr, 16(7) 771-779. doi: 10.1016/j.jcin.2023.01.380

    - Also presented at BCS conference (poster) in 2022

7) **Merinopoulos I**, Bhalraam U, Holmes T, Tsampasian V, Corballis N, Gunawardena T, Sawh C, Maart C, Wistow T, Ryding A, Eccleshall S, Smith J, Vassiliou V. Circulating intermediate monocytes CD14++CD16+ are increased after elective percutaneous coronary intervention. (To be *submitted to PLoS One)*

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Other related work I participated as a co-author during this PhD

1) Gunawardena TD, Corballis N, **Merinopoulos I,** Wickramarachchi U, Reinhold J, Maart C, Sreekumar S, Sawh C, Wistow T, Sarev T, Ryding A, Gilbert TJ, Clark A, Vassiliou VS, Eccleshall S. J Cardiovasc Dev Dis. 2023 Feb 16;10(2):84. Doi: 10.3390/jcdd10020084
2) Corballis NH, Paddock S, Gunawardena T, **Merinopoulos I,** Vassiliou VS, Eccleshall SC. Drug coated balloons for coronary artery bifurcation lesions: A systematic review and focused meta-analysis. PLoS One. 2021 Jul 9;16(7):e0251986. Doi: 10.1371/journal.pone.0251986.
3) Gunawardena T, **Merinopoulos I,** Wickramarachchi U, Vassiliou V, Eccleshall S. Endothelial dysfunction and coronary vasoreactivity – A review of the history, physiology, diagnostic techniques, and clinical relevance. Curr Cardiol Rev. 2021;17(1):85-100. Doi: 10.2174/1573403X16666200618161942
4) Corballis N, Tsampasian V, **Merinopoulos I**, Gunawardena T, Bhalraam U, Eccleshall S, Dweck MR, Vassiliou V. CT angiography compared to invasive angiography for stable coronary disease as predictors of major adverse cardiovascular events- A systematic review and meta-analysis. Heart Lung. 2023 Jan-Feb;57:207-213. Doi: 10.1016/j.hrtlng.2022.09.018. Epub 2022 Oct 17.

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Chapter 1. Background

The work in this chapter is based on the review articles published by myself, Merinopoulos *et al*. in Current Cardiology Reviews and Journal of American College of Cardiology: Cardiovascular Imaging (1,2). The first part of this chapter summarises the historical perspective that led to the development of drug coated balloon angioplasty. The second part of the chapter focuses on the role of inflammation in atherosclerosis and angioplasty as well as the usage of ultrasmall superparamagnetic particles of iron oxide to image myocardial inflammation.

1.1 Historical perspective of percutaneous coronary angioplasty

The first balloon angioplasty (BA) was performed by Dr Andreas Gruntzig in 1977 (3) when he dilated the left anterior descending artery on a conscious patient. In 1979, the results of the first few cases of BA were published and it was estimated that about 10-15% of candidates scheduled for bypass surgery were suitable for this new technique (4). It soon became apparent that the technique was promising, but not without complications, including acute vessel closure and restenosis which affected up to a third of the patients in the first 6 months requiring repeat procedures or bypass surgery (5).

Bare metal stents (BMS) were soon developed in an attempt to prevent the acute vessel closure or recoil with initial favourable results being first published in 1987 (6). In 1994, it was demonstrated by randomised control trials that elective stent implantation significantly reduced the rate of restenosis and need for repeat coronary intervention and thus drove the era of elective stent implantation (7,8). Of particular note, however, there was no proven prognostic benefit in routine elective stent implantation for patients with stable angina. Use of BMS rapidly expanded over the next few years. It resolved problems such as acute vessel closure due to

dissections and mitigated the issues of acute recoil and constrictive remodelling linked to BA but this was at the expense of increased risk of (sub)acute thrombosis and in-stent restenosis caused by in-stent neo-intimal hyperplasia and activation of vascular and smooth muscle cells (9).

In the early 2000s drug eluting stents (DES) were developed to combine the benefit of a mechanical stent scaffold with the local delivery of an anti-proliferative drug to inhibit in-stent neo-intimal proliferation. They had demonstrably lower rates of in-stent restenosis compared to BMS and their use rapidly proliferated through the cardiology community (9). Despite DES exhibiting a reduced rate of in-stent restenosis compared with BMS, the risk of stent thrombosis still remained, with the added requirement for an extended period of dual antiplatelet therapy for the first 12 months and antiplatelet monotherapy thereafter, which in turn increases the bleeding risk especially in the elderly (10,11).

Thus, coronary artery stents were developed to treat some of the early complications associated with BA and they fulfil this role both acutely (dissection, vessel recoil) or within the first few months post-implantation (restenosis). Whilst the main benefits of their use are seen in the first few months post-implantation, the presence of the permanent metallic scaffolding and the need for longer dual antiplatelet therapy can be associated with an adverse bleeding profile and prognosis in the longer term. Therefore, biodegradable scaffolds were soon developed which would allow coronary positive remodelling and the vessel to return to a more physiological state after complete resorption with an anticipated benefit that this could allow earlier discontinuation of dual antiplatelet therapy when compared to DES; a benefit not supported subsequently by clinical studies (12–14). The main drawback of biodegradable scaffolds is the significantly increased risk of late scaffold thrombosis as demonstrated in randomised clinical trials (15).

Nonetheless, all available stenting options involve a “foreign” implanted scaffold albeit for a short period of time, which necessitates a period of dual antiplatelet therapy commonly varying from one month to one year. Despite the associated increase in bleeding risk, the majority of patients tend to tolerate antiplatelet therapy well in trial data (16,17). However, for patients with hypertension, renal failure, prior history of peptic ulcer or increased age who can have significant bleeding risk (18), even short term dual antiplatelet therapy may convey an extremely risky bleeding profile that outweighs the potential benefit of stent implantation. In addition, for certain patients it may become necessary to stop dual antiplatelet therapy following implantation of a DES unpredictably, such as those diagnosed with neoplasia requiring biopsy, gastrointestinal bleeding or need for urgent surgery (19). Therefore, although the percutaneous options of metallic scaffolding have evolved and improved through the years including transition from BMS to DES and biodegradable scaffolds, the real question is whether the same effect of opening the stenosed vessel without the need of any metallic/ biodegradable scaffolding is achievable.

1.2.1 Concept of drug coated balloon angioplasty

Drug coated balloon (DCB) is a novel treatment strategy allowing delivery of an anti-proliferative drug to the vessel wall without implantation of a stent, ‘leaving nothing behind’. It comprises a semi-compliant angioplasty balloon coated with the anti-proliferative drug and an excipient, an inert carrier molecule that facilitates drug transfer and absorption to the vessel wall (20). Optimal lesion preparation is essential which creates microdissections in the vessel wall and allows better uptake of the drug (21). The balloon is then inflated for 30-60 seconds and the drug absorbed into the vessel wall in a homogenous manner. There is no ‘class effect’ of DCBs as the choice of excipient, anti-proliferative drug used and dose result in different pharmacokinetic profiles. Currently the vast majority of DCBs available use the anti-

proliferative drug paclitaxel although more recently, others using sirolimus have also become available in Europe. Paclitaxel binds to the β subunit of tubulin, arresting the microtubule function and inhibiting cell division. Its lipophilicity and ability to concentrate to the arterial wall make it an optimal agent for DCB (22). Sirolimus reversibly binds to FKBP12, inhibits cell proliferation by forming a complex with the mammalian target of rapamycin and blocks cell cycle progression at the G1 phase (23). Sirolimus has lower lipophilicity compared to Paclitaxel requiring additional technology to ensure that the drug is not lost in transit (time required to deliver the DCB to the coronary vessel) and gets delivered to the vessel wall. The Magic touch DCB uses nanolute technology with sirolimus encapsulated in phospholipid with nanolute technology. The Selution DCB uses a combination of microreservoirs and cell adherent technology (CAT). The microreservoirs are drug delivery systems combining sirolimus with a biodegradable polymer. The cell adherent technology is an ampipathic lipid technology which binds the microreservoirs to the balloon surface protecting them during transit time. Table 1.1 summarises the currently commercially available DCBs.

Device	Excipient	Drug	Dose (µg/mm^2)
Agent	**Acetyl tributyl citrate**	**Paclitaxel**	**2**
Elutax SV	**None**	**Paclitaxel**	**2.2**
Danubio	**n-Butyryl tri-n-hexyl citrate**	**Paclitaxel**	**2.5**
SeQuent Please	**Iopromide**	**Paclitaxel**	**3**
Pantera Lux	**n-Butyryl tri-n-hexyl citrate**	**Paclitaxel**	**3**
Restore	**Shellac**	**Paclitaxel**	**3**
AngioSculptX	**Nordihydroguaiaretic acid**	**Paclitaxel**	**3**
Chocolate Touch	**Undisclosed**	**Paclitaxel**	**3**
Dior II	**Shellac**	**Paclitaxel**	**3**
Essential	**Undisclosed**	**Paclitaxel**	**3**
IN.PACT	**Urea**	**Paclitaxel**	**3.5**
Selution	**Biodegradable polymer**	**Sirolimus**	
Virtue	**Biodegradable polyester-based polymer**	**Sirolimus**	
Magic Touch	**Phospholipid**	**Sirolimus**	
Sequent Please SCB	**Crystaline**	**Sirolimus**	**4**

Table 1.1: Commercially available drug coated balloons. Adapted from Jeger et al (24) and Yerasi et al (25)

1.2.2 Main indications for drug coated balloon angioplasty

DCBs have a class IA indication for treatment of either bare metal stent (BMS) or drug eluting stent (DES) in-stent restenosis (ISR) in the most recent ESC guidelines for coronary revascularisation (26). A recent network meta-analysis showed that treatment of DES ISR with paclitaxel DCB is moderately less effective compared with repeat stenting with DES in reducing the target lesion revascularisation (TLR) at 3-year follow up (HR=1.32, 95% CI 1.02-1.70, p=0.03). Reassuringly, there was no difference in the primary safety endpoint of death, myocardial infarction or target lesion thrombosis (HR=0.80, 95% CI 0.58-1.09, p=0.15) (26). Intravascular imaging to identify mechanical reasons for ISR is recommended irrespective of the final treatment strategy, DCB or repeat stenting. Use of scoring balloon compared to standard balloon, has been associated with better angiographic outcomes in patients undergoing DCB for DES ISR (27). Therefore, optimal lesion preparation with scoring or cutting balloon is recommended in the third report of the international DCB consensus group (24).

At the time of publishing the latest ESC revascularisation guidelines, only small randomised trials had been reported comparing DCB vs DES for de novo coronary artery disease. Since then, the BASKET-SMALL 2 trial demonstrated non-inferiority of paclitaxel DCB vs second generation DES for treatment of de novo small vessel disease with maintained efficacy and safety up to 3 years follow up (28,29). These results were verified in the RESTORE SVD trial, which demonstrated non-inferiority of DCB compared to DES in terms of in-segment stenosis at 9-month follow up (30).

1.3.1 Inflammation and angioplasty

Over the last few decades, it has been appreciated that inflammation has a central role in all stages of atherosclerosis as well as the sequelae of percutaneous coronary intervention (PCI) (31–33). A wealth of studies in animal models, supported by data in humans, have identified

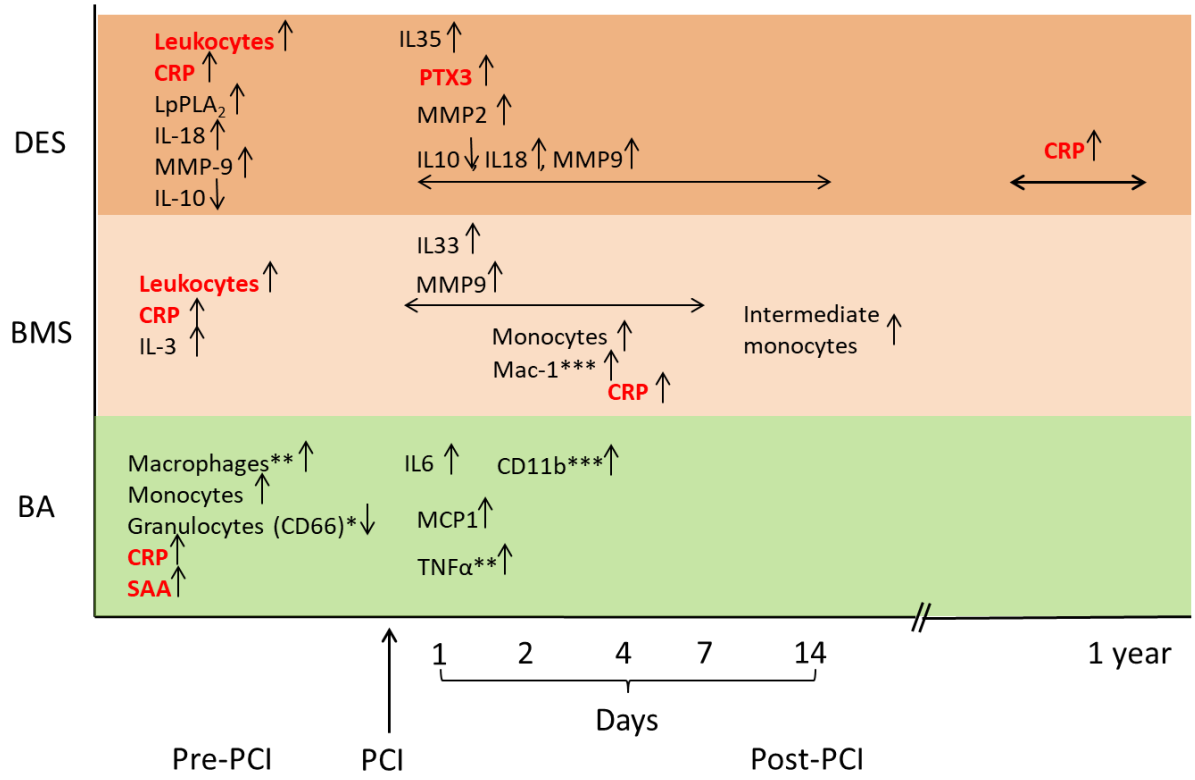
various cytokines, growth factors and other biomarkers that interact with multiple immune cells during the inflammatory process (34–36). Circulating monocytes and their tissue counterparts macrophages have gained great interest in recent years as their multifaceted roles in cardiovascular homeostasis and disease become apparent (37). As insights are gained into the complexities of the inflammatory response to PCI, it becomes evident that a targeted approach is necessary to ensure optimal patient outcomes. In the following sections I will review the importance of pre-PCI inflammatory status as well as the post-PCI inflammatory response and their relationship to patient outcomes.

1.3.2 Pre-PCI inflammatory status

Balloon angioplasty

Liuzzo *et al*. demonstrated that the magnitude of the inflammatory response [as assessed by IL-6, C-reactive protein (CRP) and serum amyloid A protein] post balloon angioplasty is determined to a greater degree by the individual responsiveness rather than by the type of provocative stimuli (38). They showed that increased baseline levels of inflammation in patients with unstable angina determine hyper responsiveness of the inflammatory system even to small stimuli; while plaque rupture per se is not a major cause of the inflammatory response (38). Pre-procedural levels of CRP and serum amyloid A protein have been shown to independently predict clinical restenosis post balloon angioplasty (39). Immunohistochemical analysis of direct atherectomy samples has associated the extent of initial coronary plaque inflammation (macrophages and T lymphocytes) with recurrence of unstable angina after direct coronary atherectomy (40). Macrophages in direct coronary atherectomy samples from patients with unstable angina have also been found to be an independent predictor of restenosis (41). Furthermore, the activation status of blood phagocytes (expression of CD66 by granulocytes and production of IL-1β by stimulated monocytes) can independently predict

restenosis post balloon angioplasty (42). The data suggest that the systemic, as well as the local levels of inflammation pre-PCI, play significant roles in development of restenosis or adverse patient outcomes after balloon angioplasty (Fig 1.1 and 1.2).



*Expression of CD66, ** Atherectomy specimens, *** Expression of biomarker in polymorphonuclear cells
Red characters indicate predictor of MACE, Black characters indicate predictor of restenosis.

Fig 1.1: Timeline of inflammation in angioplasty. Schematic representation of pre- and post-PCI inflammatory biomarkers predicting patient outcomes following angioplasty

Bare metal stents (BMS)

Elevated pre-procedural CRP has been consistently shown in studies to be an independent predictor of death or myocardial infarction after BMS implantation (43–45). Most studies, including a large meta-analysis of 2747 patients undergoing BMS implantation, have also demonstrated that baseline CRP is also an independent risk factor for in-stent restenosis (ISR)

(46). Treatment with statins appears to abolish the increased risk conferred by elevated baseline CRP (43,44). The association between baseline CRP and BMS-ISR, further supports the concept that pre-procedural activation of the inflammatory system can modulate the response of vessel wall to injury (35) (Fig 1.1 and 1.3).

Interleukin-3 (IL-3), synthesized by activated T cells in atherosclerotic plaques, can activate smooth muscle cell migration and proliferation and also increase vascular endothelial growth factor production (47). IL-3 can upregulate adhesion molecules such as P selectin and it is considered a mediator of chronic, rather than acute, inflammation (48). Rudolf *et al*. have demonstrated that IL-3 is an independent predictor of ISR after BMS and that patients with symptomatic stable coronary disease undergoing PCI have higher levels than patients with acute coronary syndrome (ACS) who have higher levels than patients with asymptomatic stable coronary disease (48). These findings suggest that IL-3 is possibly stimulated by the duration and extent of myocardial ischaemia (48).

White blood cell (WBC) count is considered a marker of cellular inflammation and it has been demonstrated that it can predict major adverse cardiovascular events (MACE) in the context of ACS (49). Gurm *et al*. studied the relationship between baseline WBC and long-term mortality in 4450 patients with mainly stable and unstable angina being treated mostly with BMS. They demonstrated that WBC was an independent predictor of mortality in this population as well and were the first to demonstrate a J-shaped relationship between WBC and long-term mortality (50).

<u>Drug eluting stents (DES)</u>

The significance of pre-procedural CRP as a predictor of patient outcomes has been consistently demonstrated by multiple studies in DES era (Fig 1.1 and 1.4). Park *et al*. showed

that baseline CRP was an independent predictor of cardiovascular mortality or MI in a study of more than 1600 patients (predominantly stable and unstable angina) being treated with DES. Baseline CRP did not predict ISR but angiographic follow-up was restricted to 6 months in the study (51). The same group identified baseline CRP as an independent predictor of stent thrombosis as well as death and MI, in their prospective study of more than 2600 stable angina patients with median follow up of 3.9 years (52). In a study of more than 900 patients undergoing elective DES implantation, baseline CRP was an independent predictor of death or myocardial infarction at 2 year, even though CRP did not predict target vessel revascularisation in this study either (53). However, in a study of 167 patients on haemodialysis undergoing elective DES implantation, baseline CRP was an independent predictor of MACE and in-stent restenosis (54). Furthermore, Nicolli *et al*. demonstrated in a small study of 92 patients that baseline CRP was associated with a more aggressive (diffuse) ISR pattern after DES implantation (55). More recently, the long-term prognostic significance of baseline CRP was further evaluated. Oemrawsingh *et al*. demonstrated in more than 400 patients undergoing PCI for stable angina or ACS, that CRP is an independent predictor of mortality or MI after ten years of follow up (56). In conclusion, most of the data demonstrate that pre-procedural CRP is a reliable predictor of hard clinical endpoints including stent thrombosis, with limited value in predicting DES restenosis (57).

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) participates in the oxidative modification of LDL generating pro-inflammatory products. It is secreted by macrophages in the atherosclerotic plaque and considered a biomarker of vascular inflammation (58). It has been shown to be an independent risk marker for coronary artery disease after adjusting for lipid, inflammatory and hemostatic parameters (59). More recently, it was demonstrated that pre-procedural levels of Lp-PLA_2 in patients undergoing elective PCI independently predicted periprocedural myocardial injury (60).

Shah *et al*. have recently demonstrated in their prospective study of more than 4000 patients (all-comers) that baseline WBC remains an independent predictor of MACE and importantly target lesion revascularisation (TLR) as well, in the modern era of DES and pharmacotherapy. The relationship of pre-PCI WBC and MACE was independent of clinical presentation (ACS vs non-ACS) indicating the importance of baseline cellular inflammation even in the context of stable angina (61).

1.3.3 Inflammatory response to PCI

Thirty years ago, Forrester *et al*. hypothesized that restenosis is a manifestation of the healing response to vascular injury post angioplasty. Platelet aggregation, inflammatory infiltrates, cytokines, smooth muscle cell proliferation and extra cellular matrix (ECM) were proposed as major components of that healing process (62). Creation of the largest possible residual lumen in combination with substantial inhibition of intimal hyperplasia was thought to be required to resolve restenosis (62).

Balloon angioplasty

Autopsy studies of patients with balloon angioplasty identified smooth muscle cell proliferation leading to intimal hyperplasia as a main component of restenosis alongside the clinically identified vessel recoil. The degree of medial injury was associated with the degree of restenosis while a change in the composition of ECM (from proteoglycans to collagen), was noted at six months(63). A number of biomarkers have been shown to be part of the post-PCI inflammatory response and to predict patient outcomes (Fig 1.1 and 1.2). Hojo *et al*. demonstrated that interleukin-6 (IL-6), a multifunctional cytokine with central role in inflammation and tissue injury, increases immediately after angioplasty in coronary sinus and is a predictor of restenosis (64). Increased levels of tumour necrosis factor α (TNFα) and

fibronectin (glycoprotein of the extra cellular matrix) have also been demonstrated in atherectomy samples of restenotic lesions post balloon angioplasty (65). Elevated levels of monocyte chemoattractant protein-1 (MCP-1) after elective balloon angioplasty have been shown to be associated with restenosis and to correlate with increased monocyte activity (66) (67). Immunohistochemistry analysis of atherectomy specimens has also shown that restenotic lesions have significantly more macrophages and expression of MCP-1 compared to denovo lesions (68). Taken together these data indicate that macrophages and MCP-1 are implicated in the inflammatory response post-PCI and the development of restenosis. In addition, human studies have implicated that adhesion molecules, important in leucocyte recruitment after vascular injury, play an important role in post-PCI inflammatory response and restenosis. Balloon angioplasty results in neutrophil activation with upregulation of CD11b and downregulation of L-selectin adhesion molecules (69,70). Elevated levels of CD11b 48 hours post elective balloon angioplasty have been associated with restenosis and late lumen loss (71) (72). Furthermore, Inoue *et al*. demonstrated that the percentage increase of CD11b in coronary sinus 48 hours post angioplasty was significantly less after cutting balloon compared to standard balloon, providing a mechanistic link between the controlled vascular injury of cutting balloons and less restenosis (72).

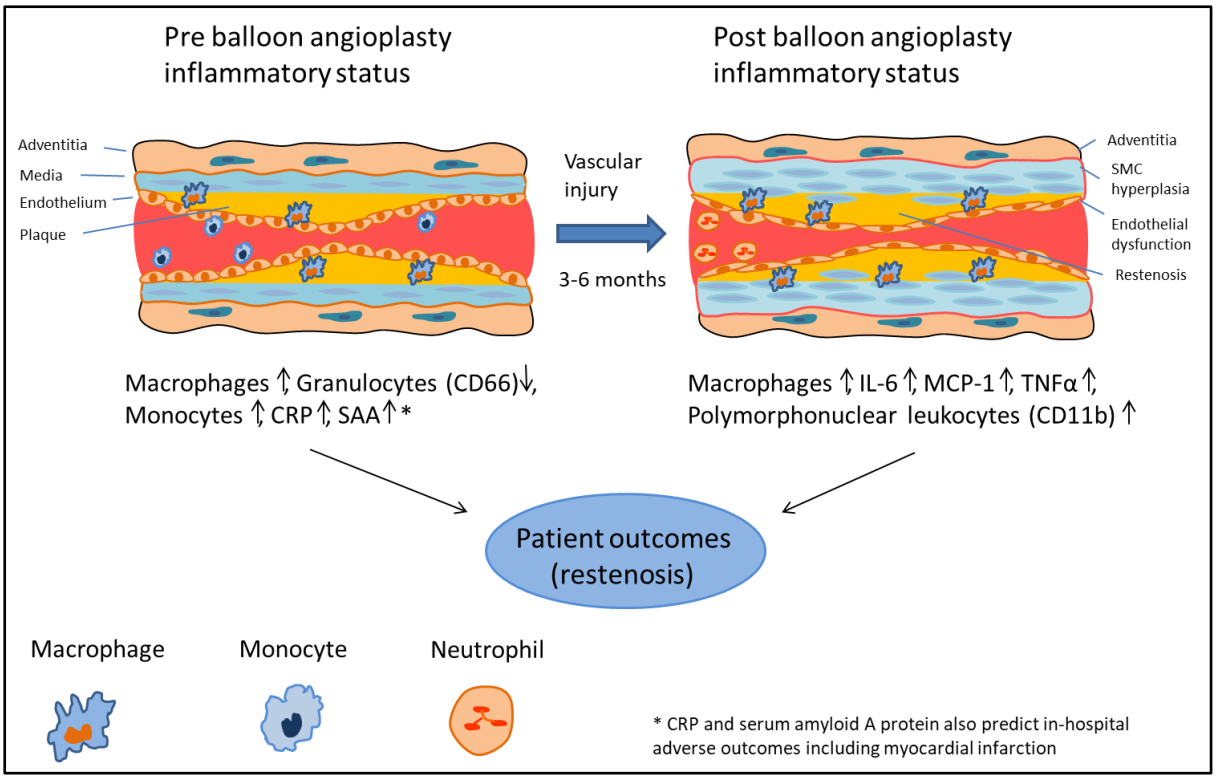


Fig 1.2: Role of inflammation in balloon angioplasty. Schematic representation of how the inflammatory biomarkers pre- and post- balloon angioplasty can predict patient outcomes.

Bare metal stents

Bare metal stents provided an effective solution for the acute limitations of balloon angioplasty, such as limiting dissections and acute vessel recoil. The restenosis rate was also improved relative to balloon angioplasty but remained unacceptably as high as 20-30% in the medium to longer term follow up (73). Human autopsy studies from the era of BMS have described the inflammatory response post stent implantation and linked it to ISR. In the initial reparative phase, denudation of the endothelium and plaque disruption following stent implantation leads to thrombus formation, which covers the stent initially. This thin layer of thrombus gradually gets infiltrated by smooth muscle cells (SMCs) and inflammatory cells such as lymphocytes and macrophages. Increased numbers of SMCs accompanied by macrophages and an

expansion of the ECM lead to the second proliferative phase (74). Histopathological analysis of directional atherectomy specimens from restenotic lesions has also shown that the cellularity of in-stent neointima decreases over time as proteoglycan-rich ECM increases (75). Morphological, human histology studies further linked arterial injury with inflammation and neointimal growth challenging the concept that 'bigger is better' (76,77). Farb *et al*. demonstrated that medial injury or penetration of the stent into a lipid core was associated with increased chronic inflammation leading to neointimal growth and ISR. Macrophages were one of the most predominant cells of the inflammatory response post stenting (76,77)

It is established that monocytes, precursors of macrophages circulating in blood, have strategic roles in all stages of atherogenesis with increasing evidence about the great importance of their various subgroups (78). $CD14^{++}CD16^{+}$ (intermediate) monocytes are independently associated with cardiovascular events in patients referred for elective coronary angiography and in non-dialysis chronic kidney disease patients (79,80). Fakuda *et al*. were the first to identify that the peak monocyte count from peripheral blood, two days after stenting, was the only fraction of leukocytes with significant positive correlation with in-stent neointimal volume at six-month follow-up (81). Their findings demonstrated that monocytes play a central role in the post-PCI inflammatory response (82). Liu *et al*. subsequently demonstrated that the CD14+CD16+CX3CR1+ (intermediate) subset of monocytes 12 days (time point chosen to avoid inflammation from myocardial necrosis) after ST elevation myocardial infarction treated with BMS was an independent predictor for in-stent late lumen loss (83). These limited data indicate that monocytes and most importantly their intermediate subset are closely implicated in the development of BMS-ISR.

A number of inflammatory biomarkers have been shown to increase after PCI with BMS and importantly to be related with patient outcomes (Fig 1.1 and 1.3). CRP is an acute-phase protein produced by the hepatocytes in response to stimulation by IL-6 primarily. It has been shown to

peak 48 hours post stenting for stable angina while normalisation of CRP at 72 hours post procedure is associated with favourable patient outcomes at 1 year follow up (84). Patients who subsequently develop ISR have significantly higher levels of CRP with a later peak indicating a prolonged inflammatory response, compared to patients without ISR (85). Furthermore, the periprocedural (pre- to 24h-post) change in CRP is an independent predictor of long-term MACE, with additional predictive value when compared to the baseline or post-PCI CRP value separately (86) (87). Inoue *et al*. have also demonstrated that at least some amount of the CRP post-stenting is produced locally and that CRP production at the site of PCI is associated with Mac-1 activation (88). Mac-1 (CD11b/CD18) is an integrin responsible for firm leukocyte adhesion to platelets and fibrinogen at injured vessels, which has been shown to increase after elective stenting and be a significant predictor of late lumen loss (89). Interleukin-33 (IL-33) is an alarmin mainly expressed by endothelial, epithelial and smooth muscle cells that guides the immune response after cellular injury and enhances cytokine production (TNF-α, IL-6, IL-1β) from macrophages. Data suggest that a decrease in IL-33 levels after stent implantation is associated with lower ISR rate (90). Matrix metalloproteinases (MMPs) have also been linked to the pathogenesis of ISR. MMPs are proteases that control ECM degradation and facilitate intimal remodelling post angioplasty. Increased level of MMP-9 after stent implantation have been shown to be independent predictor of ISR after BMS insertion (91,92).

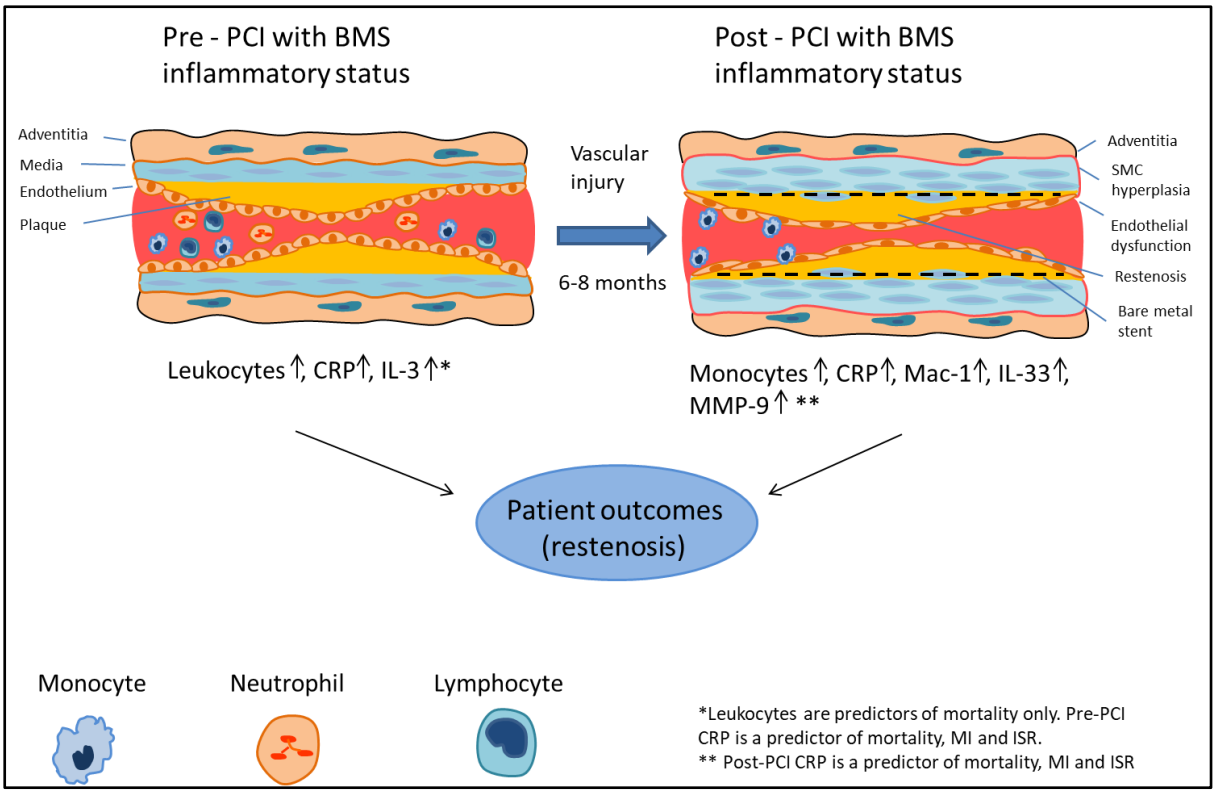


Fig 1.3: Role of inflammation in angioplasty with bare metal stent. Schematic representation of the biomarkers shown to be predictors of patient outcomes in angioplasty with bare metal stent

Drug eluting stents

Drug eluting stents, coated with antiproliferative drugs, were subsequently developed and demonstrated to significantly reduce ISR (73). A human autopsy study from the DES era demonstrated that uneven distribution of drug was associated with ISR while medial injury associated with increased inflammation, angiogenesis and peri-strut haemorrhage was a predictor of DES occlusion. Important differences were identified between DES and BMS restenosis. Even though both BMS and DES had similar macrophage infiltration, macrophage infiltration correlated with neointimal thickness only in BMS but not in DES; indicating suppression of growth factors in DES (93). The neointimal composition of restenotic DES had greater proteoglycan deposition and less smooth muscle cellularity, when compared to BMS

which had greater cellularity and collagen deposition (93). However, neointimal area correlated positively with neointimal vessel and macrophage density but not type of stent, BMS or DES, in another human autopsy study (94). Furthermore, histopathological analysis of directional atherectomy specimens of DES and BMS-restenotic lesions demonstrated significantly increased macrophages in DES compared to BMS (95). Considering all the studies together, the data suggest that macrophages continue to play a significant role in the pathophysiology of DES-ISR.

Despite the reduction in ISR following the development of newer generation DES, late stent failure continues to be a significant concern following stent implantation. In-stent neoatherosclerosis emerged as an underlying pathophysiological substrate leading to very late stent thrombosis and late ISR. A human autopsy study revealed that in-stent neoatherosclerosis occurs both in BMS and DES, but occurs more frequently and significantly earlier with unstable lesion characteristics in DES compared to BMS (96,97). Whilst second generation DES have been shown to have significantly less inflammation score compared to first generation DES in autopsy studies, there was no difference in prevalence of neoatherosclerosis and foamy macrophage clusters (98). The exact mechanism leading to neoatherosclerosis remains to be defined; however it has been proposed that the dysfunctional endothelium following stent implantation results in adhesion and migration of monocytes into the sub-endothelium where they convert to foamy macrophages driving the development of the necrotic core to form fibroatheroma (99).

The inflammatory response after coronary stent implantation has been extensively evaluated in the DES era (Fig 1.1 and 1.4). CRP is the most-studied biomarker. Dibra *et al*. demonstrated that a more intense inflammatory response following elective PCI, as assessed by CRP measurement, was associated with increased ISR risk only for BMS and not for DES (100). Consistent with these results, Gaspardone *et al*. showed that even though BMS, sirolimus

eluting stent (SES), paclitaxel eluting stent (PES) and dexamethasone eluting stent (DEX) elicit an almost identical systemic inflammatory response as assessed by CRP 48 hours post elective PCI, the SES and PES had a significantly lower ISR (101). Therefore, the lower ISR of DES might be related to a blunted local inflammatory response rather than a decreased systemic inflammatory response. In contrast to these studies, Kim *et al*. showed that BMS elicit more inflammatory response post elective PCI compared to DES as assessed by CRP 48 and 72 hours later (102). Kang *et at*. subsequently demonstrated that PES and SES elicit a similar inflammatory response post elective PCI, as assessed by CRP and IL-6, even though SES had a significantly lower volume of neointimal hyperplasia on intravascular ultrasound (103). Even though this study did not identify CRP or IL-6 as significant predictors of neointimal hyperplasia the follow-up study from the same authors demonstrated a significant positive correlation between CRP level at 24h and 72h post-PCI with neointimal hyperplasia on intravascular ultrasound at 9-month follow up (104). In contrast to the previous studies that measured CRP in the short-term period post-PCI, Hsieh *et al*. measured CRP at 9-month follow up after DES implantation in more than 1700 patients. They showed that elevated CRP 9-months post-PCI is an independent predictor of long-term cardiovascular outcomes including ISR(105). Consistent with these results, Shiba *et al*. in their retrospective study of more than 1200 consecutive patients measured CRP at baseline and 8-12 months post-PCI. They confirmed that late-phase CRP is an independent predictor of MACE including TLR in patients treated with DES (106). The concept of residual inflammatory risk (RIR) post-PCI has been evaluated by two recent large retrospective studies. Kalkman *et al*. first showed in more than 7000 patients (mainly stable or unstable angina) that a persistently high RIR post-PCI (predominantly with DES) was associated with significantly higher all-cause mortality and MI at one year (107). The same group corroborated these results, by demonstrating in more than 3000 patients (included in their previous study) with low baseline cholesterol that persistently

high RIR post-PCI remained an independent predictor of major adverse cardiac and cerebrovascular events at one year (108).

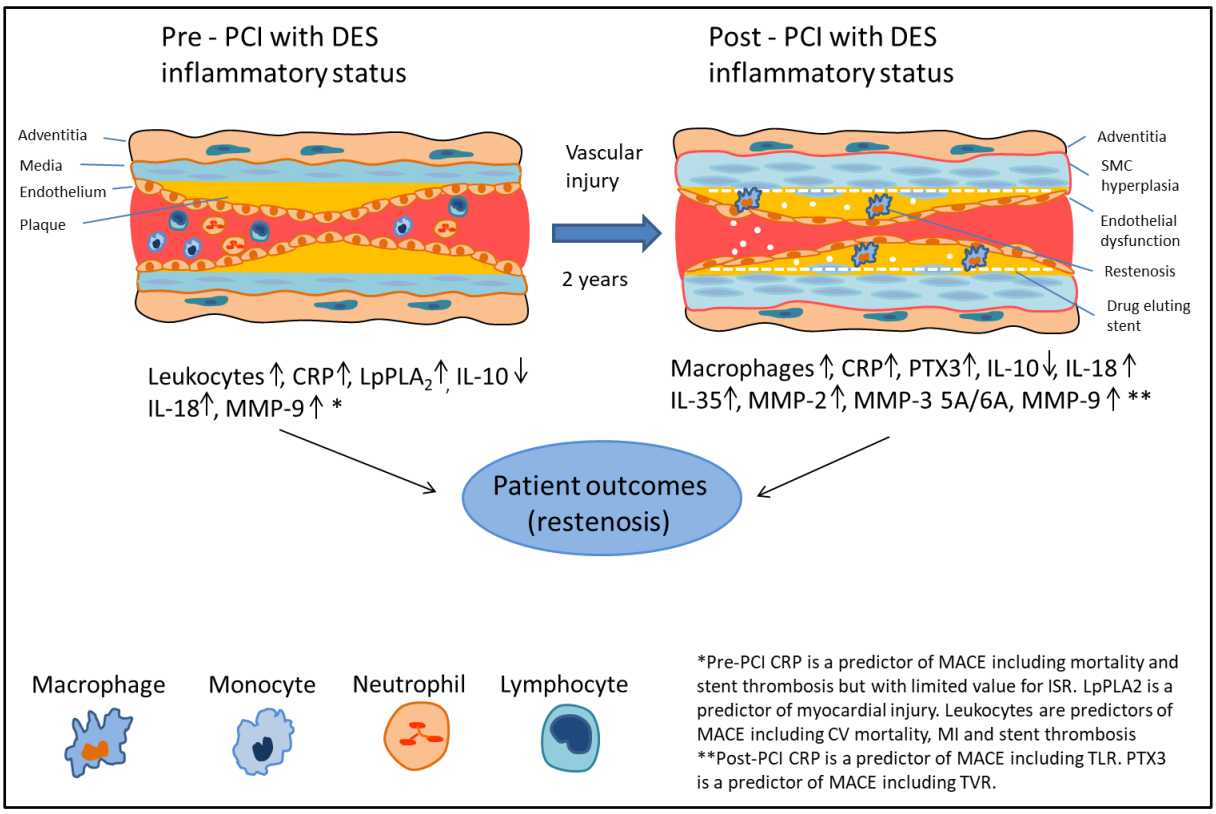


Fig 1.4: Role of inflammation in angioplasty with drug eluting stent. Schematic representation of biomarkers shown to be predictors of patient outcomes in angioplasty with drug eluting stent

Overall, even though there is some disagreement between the studies, most of the data suggest that all stents elicit a similar systemic inflammatory response, as assessed by CRP, irrespective of type of stent. A local, as opposed to systemic, modulation of the inflammatory response is possibly responsible for the better ISR profile of certain stents. However, a persistently high RIR is an independent predictor of poor patient outcomes post-PCI even in patients with low cholesterol. Post procedural late-phase, rather than short-phase, CRP elevation appears to be a more useful biomarker for the prediction of MACE and ISR in the DES era. Of note though,

most of the studies evaluating CRP after DES implantation have included mainly first-generation DES with small number of patients with newer-generation DES (57).

Pentraxin-3 (PTX3), a member of the pentraxin superfamily alongside CRP, has been utilised to assess the local inflammatory response post-PCI. It is produced by macrophages and endothelial cells in response to local inflammation and is highly expressed in the cardiovascular system. There are some data suggesting that BMS have significantly higher levels of PTX3 12 hours after PCI compared to DES; while hsCRP is not significantly different between BMS and DES (109). Haibo *et al*. demonstrated that PTX3 increases significantly 24h after elective DES implantation and that post-PCI PTX3 is an independent predictor of MACE (110). More recently, Kimura *et al*. demonstrated that peak post-PCI PTX3 was associated not only with MACE but also with suboptimal post-stent findings on optical coherence tomography (OCT), linking local inflammation induced by DES implantation with suboptimal stent characteristics and MACE (111).

A variety of pro- and anti-inflammatory cytokines have also been shown to be associated with ISR. IL-18 is a pro-inflammatory cytokine produced by cells participating in the atherosclerotic process such as macrophages, endothelial cells and smooth muscle cells. It plays an important role in neointimal formation, endothelial cell apoptosis and smooth muscle migration (112). IL-10, a crucial anti-inflammatory cytokine produced by T-helper 2 lymphocytes, macrophages and monocytes, plays critical role in plaque stability (112). Elevated levels of the pro-inflammatory cytokine IL-18 and decreased levels of the anti-inflammatory cytokine IL-10 at baseline, 24h and 2 weeks after PCI have been associated with ISR (112). Furthermore, polymorphisms in IL-18 (-137G/C) and IL-10 (+4259GG) have been associated with ISR (112) (113). IL-35 is an anti-inflammatory cytokine, member of the IL-12 cytokine family with immunosuppressive roles. It inhibits atherosclerotic lesion progression via upregulation of anti-inflammatory cytokines, downregulation of the pro-inflammatory cytokines and decreasing the

M1/M2-like macrophage ratio (114). More recently, Liu *et al*. demonstrated that low levels of the pro-inflammatory cytokine IL-1β and high levels of the anti-inflammatory cytokine IL-35 post-PCI, predicted stent strut coverage as assessed by OCT three months later (114). The same study also demonstrated that a) in vitro IL-35 induced activation of the anti-inflammatory M2-like macrophage phenotype, which induce endothelial proliferation and alleviate endothelial dysfunction and b) treatment with IL-35 of an in vivo model resulted in lower percentages of uncovered struts and inhibited inflammatory response (114).

Similar to BMS, MMPs have been shown to predict ISR after DES implantation. Increased levels of MMP-2 24h later and MMP-9 24 hours and 2 weeks after elective PCI with DES have been shown to be independent predictors of ISR (115) (112). More recently MMP3 6A/6A genotype has been found to be a genetic susceptibility factor for ISR after DES implantation (116).

Bioresorbable vascular scaffold (BVS) was developed with the intention to provide vessel patency in the short term and then gradually degrade over time allowing the coronary vessel to return to its natural state. Safety concerns, mainly due to increased risk of stent thrombosis, led to their withdrawal from clinical practice (15). An ex-vivo porcine model study demonstrated that the thick-strut fully bioabsorbable everolimus eluting stent (EES) has significantly more acute thrombogenicity compared to the thin-strut biodegradable polymer metallic everolimus eluting stent (EES). The thin-strut EES also showed greater re-endothelialization at 28 days and reduced inflammatory cell adhesion of monocytes/macrophages at 1 days compared with thick-strut EES (117). Another histopathological study in a porcine model showed Absorb everolimus-eluting BVS has comparable vascular response to XIENCE V with both devices triggering mild to moderate inflammation, even though the inflammation scores were greater in BVS at 6 to 36 months (118). However, studies investigating the inflammatory response after elective BVS with inflammatory biomarkers have demonstrated that Absorb BVS does

not provoke a chronic inflammatory response as assessed by CRP, IL6, monocyte chemotactic protein-1 and soluble CD40 ligand. The neointimal burden did not correlate with levels of inflammatory biomarkers pre-PCI, post-PCI or change (post-PCI minus pre-PCI) (119).

1.4.1 Assessment of myocardial inflammation

Inflammation is the main defense mechanism against infection or tissue injury, and is one of the body's response to extreme deviations from homeostasis induced by various stressful stimuli (120). Earlier, the importance of systemic inflammation in angioplasty was discussed. This section focuses on an emerging non-invasive method to visualize and assess localized, cellular myocardial inflammation.

Macrophages play a central role in these processes by detecting various types of stressors and responding accordingly with signals to orchestrate the inflammatory response (120). Ultrasmall superparamagnetic particles of iron oxide (USPIO) have been used successfully to assess cellular inflammation. These iron-oxide contrast agents are engulfed by tissue-resident phagocytic cells and they generate signal inhomogeneities which can be detected by magnetic resonance imaging (MRI). Indeed, macrophages have an established role in all stages of atherogenesis as they underlie the development of coronary plaques, their progression to vulnerable plaques and eventual disruption (37). It was most recently demonstrated that inhibition of macrophage signaling and function reduced atherosclerosis in a mouse model, opening novel means for treating atherosclerosis (121). In addition, macrophages have divergent functions and also aid the healing process after myocardial infarction (37). Distinct macrophage subtypes with different polarization status are responsible for their diverse properties. Following a myocardial infarction, early pro-inflammatory macrophages (type 1) become polarized toward an anti-inflammatory phenotype (type 2) later on. The balance between these macrophage subtypes plays a crucial role in myocardial repair and function following acute myocardial infarction (122). Therefore, determining the magnitude and nature

of cellular inflammation is of paramount importance in guiding accurate diagnosis, monitoring disease progression, assessing therapeutic efficacy and determining risk stratification of cardiovascular disease. One potential non-invasive method for imaging and assessing cellular inflammation includes the use of USPIO-enhanced MRI.

1.4.2 Iron oxide nanoparticles

Composition

Iron oxide nanoparticles (ION) are formed by small particles of iron oxide with a coating derived from organic compounds. The core iron oxide consists of magnetite (Fe_3O_4) (most commonly) or maghemite (γ-Fe_2O_3 or α-Fe_2O_3), with a core diameter between 4-10 nm (123). The physiological properties of bare iron oxide particles are altered by the organic compound coating, which is often larger than the core iron oxide itself. Bare iron oxide is hydrophobic causing it to aggregate and undergo opsonisation (i.e. binding with plasma proteins) following injection into the bloodstream (124). Coating with organic compounds is therefore essential in order to increase their hydrophilicity and decrease opsonisation and the tendency of the particles to aggregate. These are important factors that determine the manner in which ION interact within the host body. In addition, the coating decreases their toxicity as it prevents the release of iron ions (124). Various compounds have been used for coating but polysaccharide dextran is most commonly used amongst FDA-approved ION-based contrast agents (125).

Physiological properties

The size and coating of ION are two of the most important determinants of their properties (126). According to their size, ION can be classified into:

1) Very small superparamagnetic particles iron oxide with diameter <20 nm,
2) Ultrasmall superparamagnetic particles of iron oxide (USPIO) with diameter 20-50 nm

3) Small superparamagnetic particles of iron oxide with diameter 50-250 nm

4) Micro-sized particles of iron oxide with diameter 1-8 μm (125) (127).

Superparamagnetism is a property of USPIO resulting from their small size and crystalline nature. In the absence of an externally applied magnetic field, the net magnetization is zero as the magnetic orientations of single magnetic domains rotate free from thermal motion and cancel each other out. Application of an external magnetic field reorients the magnetic domains and results in a magnetic moment much greater than that of a paramagnetic substance. Termination of the external magnetic field, however, leads to immediate termination of their magnetic moment unlike larger ferromagnetic substances which retain their magnetic properties in the absence of a continuously applied external magnetic field (123,128,129).

Following intravenous administration, ION contrast agents remain in the intravascular space and ordinarily do not leak into the interstitium, provided that the endothelium is healthy and not affected by a pathological process (123). Resident macrophages of the reticuloendothelial system readily uptake ION from the blood circulation. Large ION (>1 μm in diameter) tend to accumulate in the liver and lungs while USPIO are typically eliminated from blood via uptake by the liver, spleen and bone marrow (130). The half-life of each ION depends on its exact size and the chemical properties of the coating (131). In general, ION of a smaller size with a hydrophilic coating and neutral surface charge can escape immediate recognition from cells of the reticuloendothelial system and have a longer half-life compared to larger ION (125). USPIO for example may have a half-life up to 36 hours compared with a half-life of 2 hours for larger ION (127). The small size and long half-life of USPIO enable them to cross the capillary wall especially at sites where there is loss of endothelial integrity and increased capillary permeability (127,132). Once accumulated in a tissue, ION cause local magnetic field

inhomogeneities that shorten the T2/T2* relaxation processes resulting in signal void (hypointense regions) on T2 and T2* weighted images (128). The effect of ION-based contrasts can be quantified by measuring the decrease in T2* value or an increase in R2* value (R2* = 1 / T2*) which are both frequently reported in the literature (133,134). However, it is necessary to have paired MRI scans (pre- and post- ION-based contrast administration) to be able to measure accurately the change in R2* value from baseline. Given that ION-based contrast results in signal void (hypointense regions), the baseline scan increases the accuracy of image interpretation and artefact elimination. A delay between ION-administration and MRI is also necessary in order to allow enough time for the ION to circulate in the blood and concentrate at sites of interest. According to standard protocols, the second MRI takes place 24 hours after ION-based contrast administration (135). Careful consideration of the physiological properties of each individual ION is essential in order to understand the information that these nanoparticles are providing and to maximize their potential as biomarkers of cellular inflammation (136). Table 1.2 summarizes the characteristics of some ION-based contrast agents commonly used in the studies that we will discuss in this review.

Table 1.2: Summary of discussed ION-based contrast agents

Name	Coating	Size (nm)	Plasma half-life (h)	r1 relaxivity ($mM^{-1} s^{-1}$)	r2 relaxivity ($mM^{-1} s^{-1}$)
Ferumoxytol (Feraheme^{TM})	Carboxymethyldextran	17-31	14-21 (137)	19 (138)	65 (138)
Ferucarbotran (SHU 555A, Resovist)	Carboxydextran	45-60	<1 (136)	7 (139)	82 (139)
Ferumoxtran (USPIO, AMI-227, NC100150, BMS-180549)	Dextran	17-21	24-36 (140)	16 (141)	100 (141)

Table 1.2: Summary of discussed ION-based contrast agents. Relaxivities are given at 1.5T in plasma (142) or Ficoll solution (37), at 37 °C.

There are two proposed mechanisms to explain ION localization to sites of inflammation. According to the first, ION passively migrate across the endothelium at sites of increased permeability or loss of endothelial integrity, such as sites of inflammation (143). ION with smaller size have longer half-time, circulate longer in the blood circulation and therefore have more time to come in contact and potentially migrate across the endothelium. Following migration into the interstitium, ION are engulfed by tissue-resident macrophages via pinocytosis and thus become concentrated at sites of inflammation (127,144). Alternatively, there are data to support a second mechanism, whereby ION are taken up by blood monocytes, stored within the reticuloendothelial system and subsequently transported to areas of inflammation. Montet-Abou *et al.* were the first to demonstrate in a mouse model that monocytes and macrophages labelled *in vivo* prior to myocardial ischemia-reperfusion injury can be tracked to the infarct area (145). Yang *et al.* similarly used a mouse model of myocardial infarction induced by left anterior descending artery ligation seven days after injection of microsized particles of iron oxide to demonstrate that the pre-labelled inflammatory cells mobilized to, and then infiltrated, the MI site (146); however these findings have not been replicated by others recently (147). Finally, human studies of patients with stroke undergoing USPIO-enhanced MRI have suggested that USPIOs are taken up solely by infiltrating macrophages rather than tissue resident macrophages (microglia cells) (148,149). However, the lack of tissue biopsy in this study precludes definitive conclusions to be drawn.

It has recently been demonstrated that the scavenger receptor type A I/II (SR-AI/II) provides the predominant route of ferumoxytol uptake by mouse peritoneal macrophages *in vitro* and *in*

vivo (150). The same study also demonstrated that SR-AI/II mediates uptake of ferumoxytol *in vitro* by M1 and M2 bone marrow derived macrophages (mouse) that express similar levels of SR-AI/II receptors (150), though these results have not been verified by all studies. We speculate that different cell surface receptors expressed by different macrophage subgroups affect the uptake or elimination of USPIO; subsequently leading to different concentration of USPIO within different macrophage subgroups.

1.4.3 Magnetic resonance imaging of ION-based contrast agents

As stated previously, ION cause shortening of T2 and T2*, lending strong applicability to T2* imaging methods. The T2 shortening of USPIO is best visualized using T2*-weighted gradient recalled echo pulse sequences, which are inherently sensitive to field inhomogeneities. While earlier ION studies used qualitative T2*-weighted imaging, recent studies have favored the quantitative T2* mapping approach recommended in the Society for Cardiovascular Magnetic Resonance consensus statement on parametric mapping (151): a multi gradient recalled echo sequence with eight echo times, ranging from 2 to 18 ms. This sequence should preferably be applied with a black-blood preparation to reduce measurement bias and interobserver variability (152). Further, it is worth noting that the consensus suggests that T2* mapping be performed at 1.5T in order to avoid the increased magnetic susceptibility artifacts at higher field strengths (151). Finally, the optimal range of echo times is not only determined by the USPIO agent but also by the uptake of USPIO in the tissue of interest. Tissue-specific echo times selected based on the expected T2* value will increase the accuracy of the decay curves (153).

It is important to consider that, as well as shortening T2 and T2*, ION also cause T1 shortening. This opens up applications for ION as contrast agents in magnetic resonance angiography (154), but it also has consequences in terms of T2* imaging pulse sequence parameters. The

T1 shortening effect of ION is minimal when the nanoparticles are clustered together, as in this case there is a sharp increase in r2 relaxivity, leading to hypointensity on T2*-weighted images; however, in regions where the USPIO are relatively diffuse the T1-shortening effect dominates, diminishing signal attenuation caused by T2 and T2* effects (155). This can lead to USPIO uptake being obscured when the Cardiovascular Magnetic Resonance (CMR) pulse sequence is highly T1-weighted, particularly for agents with lower r2-r1 ratios—see Table 1.2 (156). Fortunately, this effect can be mitigated through use of lower flip angle radiofrequency pulses and longer echo and repetition times.

The majority of CMR studies of ION to date have used Ferumoxytol; however, the use of other agents has little effect on the CMR pulse sequence design, excluding minor adjustments to the flip angle, echo time, repetition time, and range of echo times, based on the different relaxivities of the agent. On the other hand, different nanoparticle blood half-lives may permit further optimization of the post-administration CMR delay; however, this has not been studied in detail, and most studies to date have used a delay of 24 hours.

1.4.4 Diagnostic applications of iron oxide nanoparticles for myocardial imaging

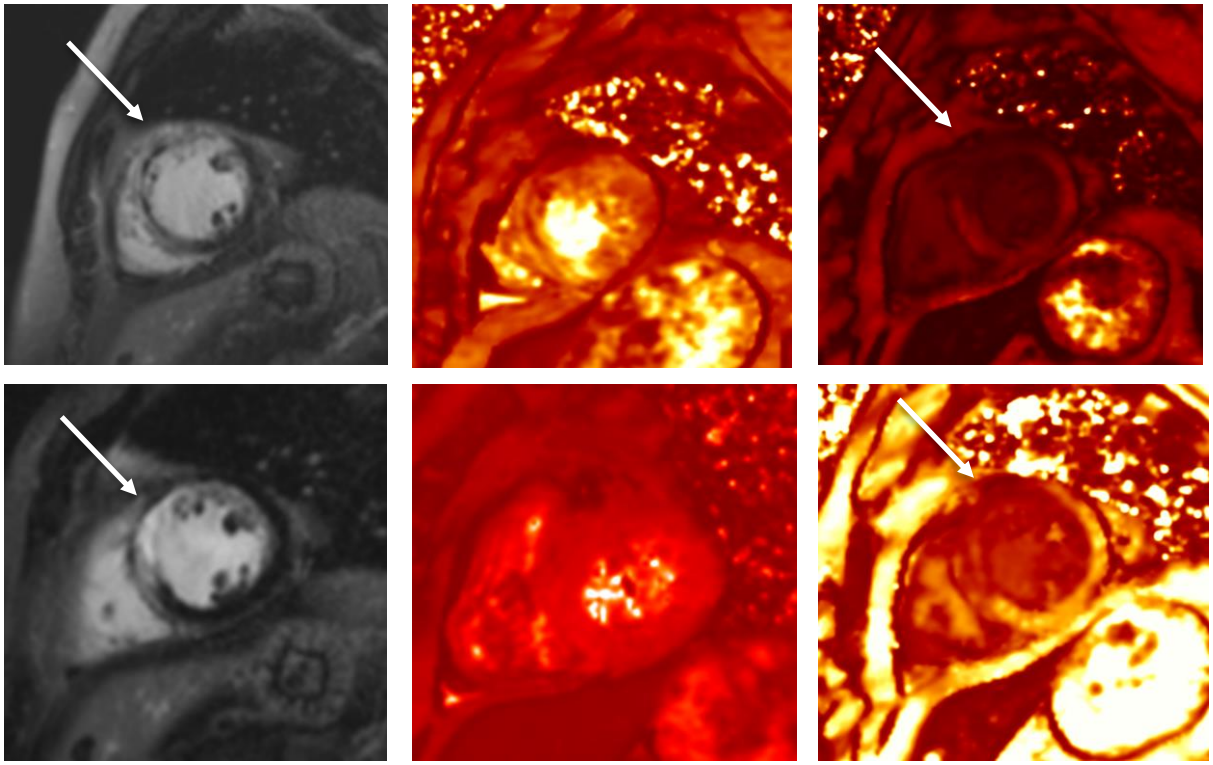
1.4.4.1 Myocardial infarction

Alam *et al.* were the first to publish an open-label pilot proof-of-concept study in 16 patients with STEMI treated with primary percutaneous coronary intervention (PCI) and stent implantation (157). Ten patients had three sequential cardiovascular magnetic resonance (CMR) scans at 3T within five days of admission at baseline, 24 and 48 hours following intravenous ferumoxytol (4 mg/kg; Feraheme, AMAG Pharmaceuticals) administration while six control patients had the same number of CMR scans but no ferumoxytol infusion. They demonstrated a significant increase in R2* in the infarct and peri-infarct area with an interesting

but more modest increase in the remote ‘healthy’ myocardium, while there was no difference in the skeletal muscle, which acted as the control. Figure 1.5 shows an example of a patient one week post anterior myocardial infarction with significant USPIO uptake anteriorly and anteroapically.

Fig 1.5

Panel A



Panel B

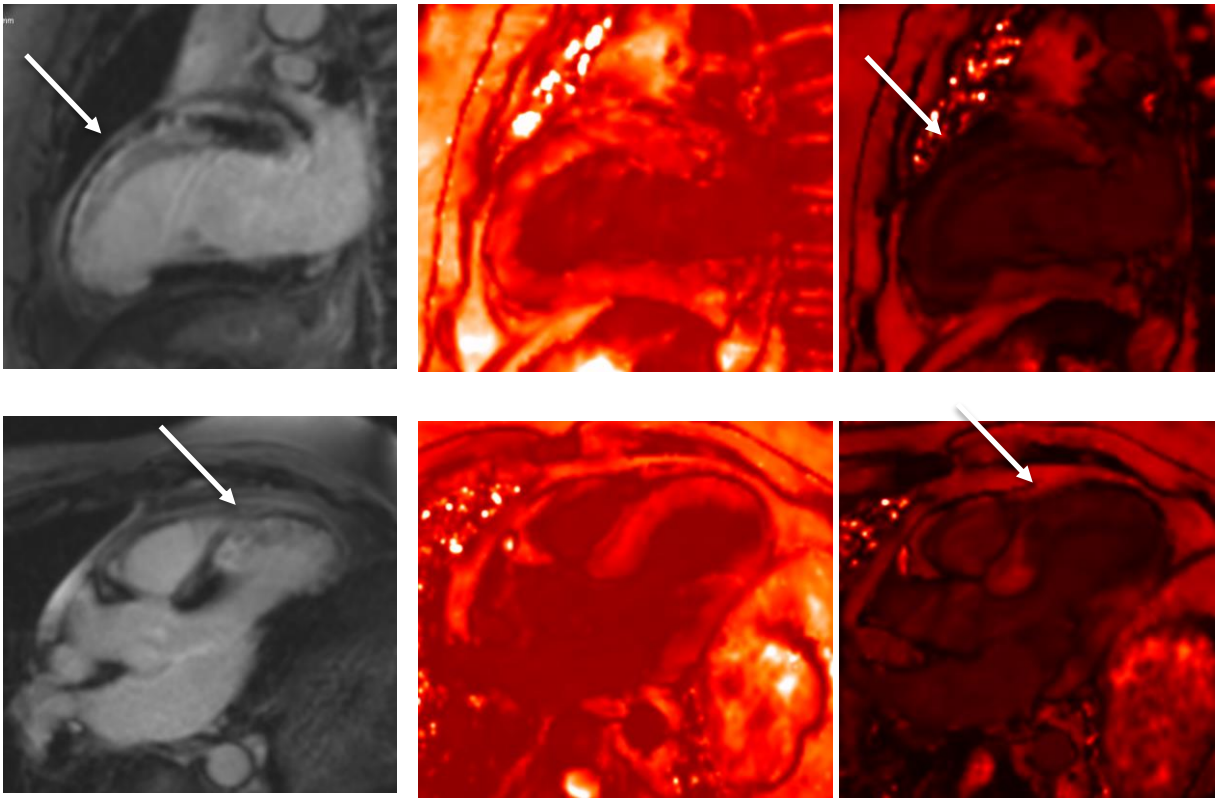


Fig 1.5: Panel A: Patient 1 week post LAD infarction with extensive LGE (arrows) on T1-weighted images (left column), homogeneous myocardial T2* values prior to USPIO (middle column), but intense dark USPIO uptake (arrows) in the region of the infarction, 24 hours post USPIO infusion (right column). Panel B: Same patient with anteroapical LGE (arrows), again homogeneous T2* myocardial values prior to USPIO, but clear USPIO uptake (arrows) on T2* scanning in the region of the LGE 24 hours after USPIO infusion.

LAD: Left Anterior Descending artery, LGE: Late Gadolinium Enhancement, USPIO: Ultrasmall Superparamagnetic Particles of Iron Oxide

The NIMINI-1 (Non-invasive myocardial inflammation imaging based on new molecular magnetic resonance imaging) study also investigated the possibility of visualizing the infarct and peri-infarct areas following acute coronary syndrome using a different USPIO (ferucarbotran, Resovist) with CMR (136). It included twenty patients with acute ST-elevation

(STEMI) or non-ST-elevation myocardial infarction (NSTEMI) who had undergone prompt and successful PCI. The patients underwent a baseline pre-USPIO CMR within seven days of presentation and a second post-USPIO CMR 10 minutes (n=2), 4 hours (n=2), 24 hours (n=10) or 48 hours (n=6) after ferucarbotran administration. The results of that study were less helpful than anticipated as T2* sequences (pre- and post-ferucarbotran administration) did not offer any additional information when compared with standard late gadolinium enhancement (LGE). The absence of an effect was attributed to the low CMR field strength (1.5T), the very small dose of ferucarbotran used (only 0.65 mg Fe/kg) and its short half-life (<15 minutes) (136). The NIMINI-2 study had the same aim as NIMINI-1 trial; however, ferumoxytol was used instead of ferucarbotran (143). It included 14 patients with first STEMI who had undergone successful PCI within 24 hours. The patients had a baseline CMR at 1.5T (median day 3 post-MI) and a second CMR 48 hours post-ferumoxytol administration. The authors detected a substantial drop in absolute T2* values not only in the infarct and peri-infarct areas but also in the remote 'healthy' myocardium with minimal change observed in the skeletal muscle. *Ex vivo* analyses of cultured macrophages and peripheral blood mononuclear cells suggested that ferumoxytol was absorbed by infiltrating myocardial macrophages rather than by peripheral mononuclear cells that later translocated to the heart. However, there might have also been an additional direct ferumoxytol effect, due to USPIO partitioning into areas of myocardium with impaired capillary integrity (143).

Following these promising initial results, Stirrat *et al.* investigated the temporal changes in cellular inflammation and tissue edema post MI (134). They recruited 31 patients with STEMI or NSTEMI and performed repeat T2 mapping and repeat (ferumoxytol) USPIO–enhanced T2* CMR scans. They demonstrated that the infarct area had increased R2* compared with the remote myocardium until two weeks post MI. At the same time, native T2 values were higher

in the infarct area compared to remote myocardium until three months after MI. Biopsies from three patients who subsequently underwent coronary artery bypass graft (CABG) showed co-localization of iron staining with infiltrating macrophages in infarcted but not in non-infarcted areas. These data suggest that the most prominent contributor to USPIO enhancement into tissues is cellular inflammation in the myocardium rather than partitioning of USPIO into the tissues. However, an earlier scan a few hours after the infarction might have helped consolidate this as it is known that ferumoxytol effects are detectable as early as 6 hours after ferumoxytol administration, while differentiated human macrophages do not demonstrate ferumoxytol uptake before 24 hours of incubation. The data also suggest that day 2-3 post MI is the optimum time to image cellular inflammation, as the USPIO uptake in the infarct zone is at its peak. (136). The other important result of this study was that there was no time course variation in USPIO uptake in the peri-infarct and remote myocardium and that the amplitude of R2* change in the remote myocardium was less than that of blood pool. The peri-infarct area appeared to demonstrate increased USPIO uptake compared to remote myocardium early post MI although this observed trend did not reach statistical significance (134). Therefore, despite the intriguing results of the two aforementioned trials (143) (157), it appears likely that the increased signal in the remote myocardium is due to a diluted effect of blood-pool USPIO rather than macrophage uptake of USPIO within these tissues (134).

In further study, Lagan *et al.* investigated five patients with ischemic cardiomyopathy at mean of eight years after MI and four healthy volunteers who underwent CMR (1.5T) at baseline, two days and three days post-ferumoxytol infusion (158). They demonstrated that post-USPIO T2* values were lower in the infarcted myocardium compared to remote myocardium in patients with ischemic cardiomyopathy. This was an interesting result given that the aforementioned trial (134) had not shown a significant difference in the infarcted area when

compared to the remote myocardium beyond two weeks post MI. The reason for the disparity between these two studies remains unclear but merits further clarification and research. It is possible that a difference in the degree of left ventricular systolic impairment of patient groups between these studies provides the explanation. However, we should emphasize that analysis of USPIO-enhanced CMR in patients with ischemic cardiomyopathy can be especially challenging due to increased artifacts in the interface between the blood pool and thin myocardium.

Finally, USPIO-enhanced CMR has also been used for the assessment of myocardial injury and inflammation related to coronary artery bypass graft (CABG) (133). The investigators assessed 87 patients undergoing CABG for stable coronary artery disease, with blood biomarkers of inflammation and myocardial injury, a baseline CMR (pre-CABG) and USPIO (ferumoxytol)-enhanced CMR at 3T within 14 days of surgery. LGE was associated with the delayed 24-hour peak in plasma cardiac troponin I, but not systemic inflammation, myocardial inflammation or bypass time. The pan-myocardial R2* value was increased compared to healthy volunteers while the average R2* value for the three segments with the highest values from the 17-segment model was also increased compared to the pan-myocardial R2* value. However, there was no correlation between USPIO uptake and plasma cardiac troponin I or cardiopulmonary bypass time, indicating that in the complex post-CABG scenario, myocardial injury is not mediated solely by inflammation. Nevertheless, this was the first attempt to assess the elicited myocardial inflammation after CABG. Further studies are warranted to elucidate the determinants and long-term effects of increased cellular inflammation following CABG.

1.4.4.2 Myocarditis

CMR has a central role in diagnosing and monitoring myocarditis. The recently updated Lake Louise criteria demonstrate the additional benefit of the latest CMR sequences, including parametric mapping, and techniques in improving diagnostic accuracy for myocarditis (159). However, the updated CMR-based criteria aim to detect indirect indicators of myocarditis, such as myocardial edema and non-ischemic myocardial injury, rather than directly imaging cellular inflammation. Initial animal data in a rat model of experimental autoimmune myocarditis were very promising. Moon *et al.* were able to demonstrate in this preclinical model that magneto-fluorescent nanoparticle CMR effectively visualized myocardial inflammatory cellular infiltrates and provided more distinct images of inflammation compared to conventional CMR. The authors demonstrated that magneto-fluorescent CMR detected scattered, small and less severe foci of inflammation more accurately compared to conventional CMR and validated their findings with histological data (160).

To date, there is only one clinical study investigating the role of USPIO CMR in patients with acute myocarditis. Stirrat *et al* recruited 14 patients with suspected acute myocarditis and 10 volunteers who underwent T2, T2* mapping and LGE 3T CMR with further T2* mapping CMR 24 hours post-ferumoxytol infusion, at baseline and three months later (161). Of the 14 recruited patients, 9 had confirmed acute myocarditis and were included in the study; two patients were excluded due to takotsubo cardiomyopathy and one for each of polymyositis, lung cancer and incompatible metallic implant. These 9 patients had typical CMR features of myocarditis in terms of LGE distribution and intensely high T2 values in regions of LGE. Despite the aforementioned promising preclinical work, there was no significant difference in USPIO uptake between patients and volunteers even within areas of LGE. The authors concluded that infiltrating macrophages do not contribute significantly to myocardial

inflammation in myocarditis. However, a previous study in patients with viral myocarditis and CMR guided biopsy, showed that LGE correlated well with a predominantly macrophage-rich inflammation (162). Stirrat *et al.* discussed various other possible explanations for these negative results, notably that 1) macrophages are not the predominant cell type in that patient group, and 2) there are inherent difficulties with image interpretation due to blooming artefacts being more dominant in the inferolateral walls, which is the usual site of myocarditis. Irrespective of the exact reason, based on the Stirrat *et al* study, there is no evidence to support clinical use of USPIO CMR in patients with myocarditis.

1.4.4.3 Takotsubo cardiomyopathy

There is currently one published clinical trial investigating the role of USPIO-enhanced CMR in patients with takotsubo cardiomyopathy. Scally *et al.* recruited 55 patients with takotsubo cardiomyopathy and 51 control subjects (163). The patients underwent 3T ^{31}P CMR spectroscopy, T1 mapping, LGE and T2* mapping with repeat T2* mapping 24 hours after ferumoxytol infusion. The patients were assessed at baseline and at 5-month follow up. Compared to control subjects, patients with takotsubo cardiomyopathy had differences in the change of T2* (pre and post-ferumoxytol infusion) and native T1 values during the acute event in both ballooning and non-ballooning segments (Fig 1.6). That difference was no longer significant at 5 months. However, myocardial energetics, assessed by ^{31}P CMR spectroscopy, demonstrated that resting cardiac energetic status was markedly reduced acutely and that there was a continuing trend at 5 months. Previous studies had suggested a possible contributory role of edema and inflammation, as assessed by CMR, to the pathophysiology of takotsubo cardiomyopathy (164,165). However, this study demonstrated for the first time a mechanistic pathway of macrophage-mediated myocardial cellular inflammatory response superimposed on myocardial edema (163). Data on the optimum time for USPIO-enhanced CMR in patients

with takotsubo is limited but given that all patients studied to date were assessed within 14 days of presentation, imaging within the first 2 weeks is suggested.

Fig 1.6

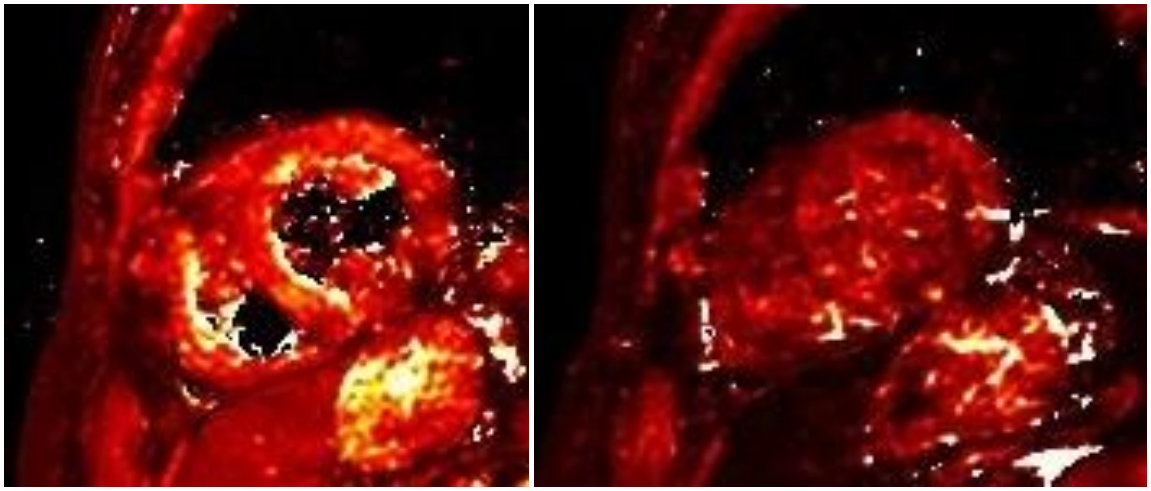


Fig 1.6: Patient with acute takotsubo cardiomyopathy. Mid-cavity short axis views demonstrating a 12 ms change in T2* from pre (left) to post (right) USPIO infusion. In healthy controls the change in T2* is usually 8-9 ms at 3T.

1.5 Assessing coronary vascular wall inflammation

Pericoronary fat attenuation index (FAI) is a new imaging biomarker able to detect coronary vascular inflammation and also predict patient outcomes (166). The coronary arteries are surrounded by adipose tissue which interacts dynamically with the coronary vascular wall. The adipose tissue secretes cytokines that affect the coronary vascular wall while at the same time, inflammatory signals from the vascular wall reach the adipose tissue inducing lipolysis and inhibiting adipogenesis (167). These inflammatory signals are sensed by the adipose tissue with subsequent changes in the profile of the secretory cytokines. The overall effect of vascular inflammation induces a shift in the composition of perivascular adipose tissue towards a more aqueous phase. This change in composition of adipose tissue can be detected by CT coronary

angiography, demonstrating increased attenuation around the coronary artery with a subsequent gradient of decreased attenuation as the distance from the vascular wall increases (168). This change in composition of the perivascular adipose tissue is captured by the fat attenuation index (FAI). It has been demonstrated that FAI, a non-invasive measure of coronary inflammation, is an independent predictor of all-cause and cardiac mortality (169).

Chapter 2. General Methodology

2.1 Introduction

The first part of this chapter describes the general methods used for patient recruitment, biochemical analysis, cardiovascular magnetic resonance (CMR) imaging, statistical analysis, illustration and ethical considerations relating to the 'Macrophage in patients with stable angina undergoing percutaneous coronary intervention' study. The second part of this chapter describes the general methods used for the retrospective analysis of the Norfolk & Norwich University Hospital (NNUH) database including patient selection, patient outcomes, statistical analysis and ethical considerations. To avoid repetition, the methods of the study on day case discharge of patients following drug coated balloon angioplasty are only described together with the study in chapter 3.

2.2 Patient recruitment and follow up

I utilized general outpatient clinics as well as from the elective waiting list at Norfolk & Norwich University Hospital to identify patients with stable angina scheduled for elective coronary angiogram ± angioplasty. All patients provided written informed consent in advance of their procedure. They were recruited in the study if they underwent angioplasty for de novo coronary artery disease during the procedure. Patients were selected to undergo either blood tests only or blood tests and USPIO-enhanced CMR as well. The exclusion criteria for patients undergoing blood test only were significant renal impairment (estimated glomerular filtration rate <30mL/min/1.73m^2 and women of child-bearing potential. The exclusion criteria for patients undergoing USPIO-enhanced CMR were the same as above and in addition 1) previous myocardial infarction 2) atrial fibrillation 3) body mass index >35 4) significant inflammatory condition and 5) known prior allergic reactions to USPIO.

Three healthy volunteers were also recruited to undergo blood tests and USPIO-enhanced CMR. All patients had blood samples taken from the sheath immediately after access was achieved. Five mls of blood were discarded first to avoid hemodilution and 20 mls of blood were subsequently withdrawn. They also had blood samples taken by venesection four hours after completion of the PCI, two weeks later and two months later. All patients were treated either with drug eluting stent or drug coated balloon at the discretion of the consultant interventional cardiologist.

2.3 Blood processing and storage

Blood collected from patients in a 5ml serum separator tube (SST) to yield serum aliquots, a 6ml lithium heparin tube to yield plasma aliquots and two 4ml ethylenediaminetetraacetic acid (EDTA) for cellular analysis. All tubes were inverted 5-8 times to ensure adequate mixing of blood. The samples were processed once the blood had clotted in the SST tube and within 2hours of blood collection. Blood was centrifuged at 3000 RPM for 15 minutes at 4^{o}C. The blood in SST tube separated into two layers. The upper layer was the serum and the lower layer consists predominantly of erythrocytes. The upper layer was pipetted into four 0.5ml aliquots and stored immediately at -80^{o}C in the freezer. The blood in lithium heparin tube separated in three layers. The upper layer was the acellular plasma, the middle layer was the 'buffy coat' or leucocyte fraction and the lower layer consists mainly of erythrocytes. The upper layer was pipetted into four 0.5ml aliquots and stored immediately at -80^{o}C freezer.

2.3.1 Cell fixation and CD14+ cell sorting

Blood from the EDTA was processed in a vented flow hood. The blood was diluted 1:1 with phosphate buffered saline (PBS) and then released very slowly onto 8ml of histopaque, taking extra care not to break the histopaque surface or mix blood with histopaque. It was then centrifuged to 400 RCF for 15min with brakes and acceleration set to zero. Following centrifugation, four layers formed (Fig 2.1). The upper was the serum, the second one was leucocytes, the third one is the histopaque and the lowest one consists mostly of erythrocytes. The layer consisting of leucocytes was pipetted into a separate tube, 50mls of PBS was added in the tube and centrifuged to 1500RPM for 5min. A pellet of cells was generated at the bottom,

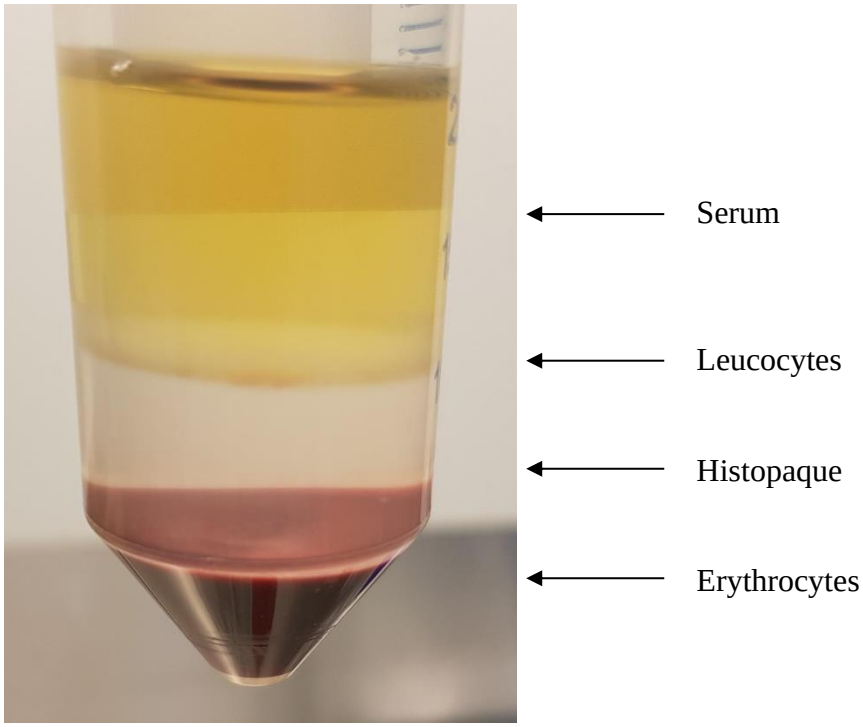


Fig 2.1: Typical example of blood mixed with PBS, released slowly on top of histopaque and centrifuged at 400 RCF for 15 minutes. The upper is the serum, the second one is leucocytes, the third one is the serum and the lowest one consists mostly of erythrocytes.

the supernatant was discarded and the pellet re-suspended in 500µl of Magnetic-Activated Cell Sorting (MACS) buffer. To prepare MACS buffer 5ml of 10x EDTA were mixed with 3.3ml bovine serum albumin (BSA) and 41.7ml PBS and filtered. 300µl of the re-suspended cells were transferred to an Eppendorf tube for sorting while the remaining 200µl were fixed using PFA.

10ml PFA were added to the 200µl of cells suspension and left at room temperature for 15min. Cells were then centrifuged at 1500 RPM for 5min to pellet the cells at the bottom of the tube. The PFA was decanted, the pellet was re-suspended in 25µl of PBS and then centrifuged again at 1500RPM for 5min. The PBS was decanted, the pellet was re-suspended in 10ml MACS buffer and kept in the fridge until cell staining and flow cytometry.

20µl of CD14 magnetic MicroBeads (Miltenyi Biotec, Bergisch, Gladbach, Germany) were added to the 300µl cell suspension and left in the fridge for 15min. The cell-bead solution was subsequently passed through a magnetic column (Miltenyi Biotec, Bergish, Gladbach, Germany) after it had been placed inside a magnetic field and hydrated with 500µl MACS buffer. The column was then washed three times with 500µl MACS buffer each time. The column was then taken out of the magnetic field and the CD14+ cells were then eluted from the column with a final, firm wash with 1ml MACS buffer using the plunger mechanism provided. The solution was centrifuged at 1500RPM for 5min to pellet the cells. The MACS buffer was discarded, the pellet was re-suspended in 350µl lysis buffer and transferred to -80^{o}C.

2.3.2 Cell staining and flow cytometry

Fixed cells were sieved using 70µm cell strainer and centrifuged at 1500RPM for 5min.The supernatant was discarded and the cells re-suspended in 500µl MACS buffer. Some 250µl were

transferred to an Eppendorf tube and 1µl of CD14 and CD16 antibody were added. A further 250µl PBS were added to the solution before flow cytometry analysis using the Cytoflex flow cytometer (Beckman Coulter, Brea California, United States).

FlowJo version 10 was used for analysis of flow cytometry results. All the FCS files from the flow cytometer were uploaded to flowjo and grouped together per patient. We followed the steps below to identify the monocyte populations of interest.

1. Cells visualized on a forward scatter area FSC(A)/ forward scatter height FSC(H) plot, in order to select cells that have an equal area and height and exclude debris and clumps.

2. Selected cells were visualized on a FSC(A)/ side scatter SSC(A) plot. Monocyte populations were selected based on their forward and side scatter properties and lymphocytes, natural killer cells and granulocytes were excluded.

3. Selected cells were visualized on a CD16 / CD14 plot and monocytes were selected based on their characteristic ‘ך ‘ shape.

4. Selected monocytes were re-displayed on a CD16 / CD14 plot to gate the monocyte subpopulations (170).

Percentages of monocyte subpopulations were exported to IBM SPSS Statistics v25 for statistical analysis.

2.3.3 Ribonucleic acid (RNA) isolation and quantification

The cell lysate (see 2.2.1) was allowed to thaw on ice. A total of 350 microliters were transferred on a yellow column (placed in a tube) from the RNA isolation kit (Macherey-Nagel™ Nucleospi™ RNA Mini Kit, Macherey-Nagel, Nordrhein-Westfalen, Germany) and centrifuged at 11000g for 30 seconds in the Eppendorf 5424R benchtop microcentrifuge (Eppendorf, Hamburg, Germany). 100 microliters of binding solution were added to the filtrate and all 450 microliters were transferred to a blue column (placed in a tube) and centrifuged at

11000g for 15 seconds. The blue column was washed with 200 microliters of washing buffer 1 and centrifuged at 11000g for 15 seconds. It was then washed again with 600 microliters of washing buffer 2 and centrifuged at 11000g for 15 seconds. It was then washed again with 250 microliters of washing buffer 2 and centrifuged at 11000g for 2 minutes. Thirty microliters of RNA free water were then added to the blue column (placed in an RNA free Eppendorf tube) and centrifuged 4 minutes later at 11000g for 1 minute.

The NanoDrop 2000 Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States) was used for RNA quantification. The spectrometer was cleaned with 5microlitters nuclease-free water. 1.8 microliters of RNA-free water were used as a blank. 1.8 microliters of RNA were used to estimate the quality and concentration of RNA. RNA purity was measured according to the ratio of absorbance at 260nm to absorbance at 280nm. A 260/280 absorbance ratio between 1.7 and 2.3 was accepted as sufficiently pure. RNA was subsequently stored in freezer at -80^{o}C.

2.3.4 Complementary deoxyribonucleic acid (cDNA) synthesis

The qPCRBIO cDNA synthesis kit (PCR Biosystems, London, UK) was used for cDNA synthesis. RNA was allowed to thaw on ice. For each reverse transcriptase reaction, 4 microliters of cDNA synthesis mix, 1 microliter of RTase enzyme and a corresponding volume of RNA and water were used to a total volume of 15 microliters. The corresponding volumes of RNA and water were calculated so that all reactions per patient (ie baseline sample, 4hour sample, 2 week sample and 2 month sample) to contain the same quantity of RNA. Tubes were gently mixed and centrifuged before loaded to the Thermocycler (Bio-Rad, Watford, UK). They were then incubated at 42^{o}C for 30 minutes, at 85^{o}C for 10 minutes to denature the enzyme and finally cooled at 4^{o}C (Fig 2.2). 30 microlitters of RNA free water were added to the cDNA and all 50 microliters were stored in -80^{o}C.

Fig 2.2: Example of PCR tubes being loaded into Thermocycler to undergo cDNA synthesis

2.3.5 Quantitative polymerase chain reaction (qPCR)

Quantitative PCR (qPCR) was performed using a Roche LightCycler 480 (Roche, Basel, Switzerland), SYBR-green kit and 384-well LightCycler plates (Roche, Basel, Switzerland). I was trained in the technique by Dr James Smith, became independent and run all the analysis. cDNA was allowed to thaw on ice. For each qPCR we used 2.5 microliters SyGreen master

mix solution (PCR Biosystems, London, UK), 0.5 microliters primer mix (forward and reverse primers), 1 microliter RNA free water and 1 microliter cDNA. Each qPCR was repeated three times to verify the results. Negative controls were included for each patient, were all the components of the reactions were added apart from primer mix. The 384-well plate was sealed and centrifuged before loaded into the Roche LightCycler. The program used consisted of 2 minutes of pre-amplification at 95^0C and then 45 amplification cycles of - 95^0C for 15 seconds, 60^0C for 10 seconds and 72^0C for 10 seconds. The plate was then heated to 95^0C for 5 seconds, 65^0C for 1 minute and 97^0C for 30 seconds. Finally, the plate was cooled to 40^0C.

Cycle threshold (Ct) values were estimated for each sample. Values >35 were disregarded as non-specific. The Ct values were converted to values expressing the fold change from baseline according to the delta-delta method, standardized for a housekeeper gene. As each reaction was repeated three times, an average of the three values was calculated.

2.3.6 Biomarker analysis

Biomarker analysis for high-sensitivity CRP (hs CRP), high-sensitivity troponin I (hs Trop I), pentraxin-3, interleukin-6 (IL-6), IL-1β, IL-10 and tumour necrosis factor α (TNFα) was undertaken at the end of the study. I prepared the samples and biobanked them. The analysis was undertaken by Core Biochemical Assay Laboratory in Addenbrooke's hospital. Hs CRP and hs Trop I were measured on the Siemens Dimension EXL autoanalyzer. Pentraxin-3, IL-6, IL-1 β, IL-10 and TNFα were measured using assay kits from mesoscale Discovery. These immunoassay kits are run in the enzyme linked immunosorbent assay (ELISA) format but use electrochemiluminescence detection rather than the production of a coloured product.

2.3.7 Preparation for CMR imaging

A selection of patients underwent CMR imaging. All patients completed a safety questionnaire as per standard hospital procedure to ensure safety of having the CMR. A cannula was inserted prior to CMR (to facilitate gadolinium and ferumoxytol infusions). Blood samples were taken from the cannula at insertion for biomarker and cellular analysis by myself. Clear explanation about the process and breath holding were given to all patients.

2.3.8 Cardiovascular magnetic imaging

A standard baseline protocol was followed in all patients who underwent CMR. I prepared the patient prior to the CMR with the help of a radiographer. The CMR was undertaken by a specialist radiographer. CMR imaging was undertaken on a 3T Discovery 750w GE system (GE Healthcare, Milwaukee, WI, USA) with an 8-channel cardiac coil using a protocol with standardized parameters. A dedicated cardiac array coil was used in all patients. Localiser images were first acquired with half-fourier acquisition single short turbo spin echo (HASTE) and free breath holding. These images were used to guide the acquisition of a vertical long axis cine image with a balanced steady state free precession (SSFP) with breath-holding usually at end expiration. SSFP short axis scout images were then acquired from that vertical long axis image covering the basal level to mid-ventricular level. Breath hold SSFP cines in 2-, 3- and 4-chamber views were then undertaken using the previous images. A stack of short-axis SSFP cine images were then undertaken using the 4-chamber and 2-chamber cine images as a guide, perpendicular to the left ventricular long axis. Finally, 6mm contiguous short axis slices with 1mm gap were acquired from base to apex. Retrospective ECG gating was used for the cine acquisition in the great majority of the patients. In cases of arrhythmia, such as multiple ventricular ectopics, prospective triggering was utilized. T1 and T2 mapping and T2* were then undertaken. Ten minutes following intravenous administration of 0.1mmol/kg of Gd

(Gadovist, Bayer, Berlin, Germany) and normal saline flush, inversion recovery late-enhancement spoiled gradient echo images were undertaken.

After completion of the scan, 4mg/kg ferumoxytol (AMAG Pharmaceuticals) diluted in normal saline for a final concentration of 2-8mg/ml was administered over at least 15minutes. All patients had blood pressure and heart rate monitored at baseline and every 5-10 minutes during the infusion and for 30 minutes after completion of the infusion.

The patients returned 24 hours later for repeat CMR. Following initial localizers and scout images as in first scan, repeat T2* weighted imaging was undertaken.I was trained in analysis of the T2* results by Prof Vassiliou, learning to appropriately draw regions of interest (ROI) and undertake quality insurance. I independently analyzed the CMRs in a blinded fashion.

2.3.9 T2* weighted imaging

Cardiovascular magnetic imaging 42 (CVI42) was used for the T2* weighted imaging analysis. ROI were carefully drawn in the inner third of the PCI area and the remote myocardium, ensuring that the blood pool was avoided. The same process was repeated in the pre-ferumoxytol and post-ferumoxytol scan ensuring that the same corresponding areas were selected between the two scans. Healthy volunteers had ROIs selected in all three coronary artery territories.

2.3.10 Ethical considerations

The ‘Macrophage in patients with stable angina undergoing percutaneous coronary intervention’ study (IRAS: 251729) was approved by the independent East of England – Cambridge central research ethics committee as well as the Health Research Authority and Health and Care Research Wales committee (REC 19/33/0075). The study conformed to the 1975 Helsinki guidelines. All study participants provided written, informed consent.

2.4.1 Structure of the NNUH database

All patients undergoing PCI in NNUH are prospectively entered in an electronic clinical database at the time of their procedure. The NNUH clinical database is a prospectively collected database including every patient treated in NNUH for coronary artery disease. It includes clinical as well as angiographic and PCI characteristics. Domains from the NNUH database are also uploaded to BCIS and MINAP databases. BCIS is a national database which audits all PCI procedures nationally. One limitation of the NNUH database is that it only includes angioplasties undertaken in NNUH. BCIS collect data nationally and therefore allows capture of myocardial infarctions or angioplasties that have happened in different hospitals. For our study, all subsequent angiograms (following the index procedure) were reviewed to verify the angiographic characteristics and clinical records were reviewed to supplement any missing values. This process has increased the robustness of our results.

The NNUH database contains:

a) Patient's demographic information such as sex and date of birth.
b) Fields indicating the reason for the procedure such as emergency/urgent/elective/staged or STEMI/NSTEMI/Stable angina
c) Fields indicating the patient's clinical condition peri-procedurally such as ventilation, cardiac arrest, intubation, cardiogenic shock, inotropic support.
d) Fields regarding angiographic characteristics and procedural details such as vessel (including segment in the vessel) treated, bifurcation disease, vessel calcification, vessel tortuosity, treatment strategy and also devices used (including type of stent or drug coated balloon as well as device length and diameter).
e) Fields regarding the patient's comorbidities such as hypertension, stroke, myocardial infarction, PCI, CABG, heart failure, diabetes, peripheral vascular disease, COPD, renal function, smoking status.

f) Free text fields regarding the full operation report and other comorbidities.

Each entry in the database corresponds to a treated lesion but it is also possible to identify all lesions treated in a single procedure per patient as well as all patients' procedures. The database was initiated in June 2011 and is continuously updated and overseen by a specialist for updates and accuracy.

2.4.2 Data curation

a) Data extraction

Using the appropriate, relevant fields each entry in the database was coded with a 4-digit number as follows:

a) First digit indicates the order of the procedure per patient (1=first, 2=second etc.)
b) Second digit indicates the clinical presentation (1=STEMI, 2=NSTEMI, 3=stable angina 4=staged procedure)
c) Third digit indicates the procedural strategy per patient (1=DCB only, 2=DES only, 3=DCB+DES, 4=other devices)
d) Fourth digit indicates the lesions treated per procedure (1=main lesion, 0=all other lesions).

Using the above coding system the study cohorts in this thesis were identified (STEMI cohort and stable angina cohort). In addition, the patients with repeat PCI were identified and indicated if the repeat PCI was for target vessel revascularization or not. The target vessel was defined as the entire major intervened coronary vessel, including the side branches (171). Dr Tharusha Gunawardena helped with data extraction.

b) Missing data

Variables with missing data such as smoking status or renal function were supplemented using the electronic hospital records by myself.

c) Angiograms review

The angiograms of all index PCI procedures were reviewed by myself to document bifurcation disease and MEDINA classification, TIMI flow pre- and post-procedure, grade of coronary artery dissection in case of DCB treatment. The National Heart, Lung and Blood Institute classification was used to classify coronary dissections (172). In addition, the angiograms of all patients with repeat PCI for target vessel revascularization were reviewed to classify if the repeat PCI was for target lesion revascularization or not. The target lesions was defined as the treated segment including the 5-mm margin proximal and distal to the treated segment (171). A lesion was defined as a bifurcation if there was a side branch more than 2mm in diameter within 5mm of the lesion. MEDINA subtypes 1.1.1, 1.0.1 and 0.1.1 were considered as true bifurcations (173). The vessel diameter was considered as the largest pre/post-dilatation balloon, DCB or DES used and lesion length was based on the DCB or DES length. In cases of discrepancy between my assessment and operator report or in ambiguous cases, Dr Natasha Corballis reviewed the angiogram as well and consensus reached.

2.4.3 Hospital Episode Statistics data

a) Structure of the data

Hospital Episode Statistics (HES) is a database containing information about all admissions, Accident and Emergency attendances and outpatient appointments at NHS hospitals in England provided by NHS Digital. We used the Admitted Patient Care (APC) database which corresponds to the hospital admissions as well as mortality and cause of death data provided

by NHS digital. Each entry in the HES/APC database represents a unique hospital episode and there can be many episodes (with increasing order) within a patient's hospital stay.

The database includes:

a) Sociodemographic information such as: age, sex, type of admission, index of multiple deprivation
b) Up to 20 diagnoses fields regarding the primary diagnosis during the hospitalization as well as secondary/subsidiary diagnoses. The International Statistical Classification of Diseases, Injuries and Causes of Death (ICD) is used. The ICD-10 revision has been used following 1995.
c) Up to 24 procedure fields containing information regarding the patient's main as well as secondary procedures. The Office of Population Censuses and Surveys (OPCS) classification of surgical operations and procedures version 4 is used.

2.4.4 Cohort extraction

Utilizing a one-to-one merge command, I identified all index admissions for our cohorts in the HES/APC database. The diagnoses fields for the index admissions were extracted in a separate database and used to calculate the validated Hospital Frailty Risk Score based on the ICD-10 diagnostic codes (174). Utilizing a one-to-many merge command, I identified all readmissions following the index admission for our cohorts. The readmissions were extracted in a separate database and a new variable was created using the 'date and time wizard' calculating the time to readmission. Causes of readmission were identified from all 20 diagnoses fields and classified according to our defined patient outcomes (Table 2.1). All duplicates were removed and the first readmission for each patient outcome was merged back to our original cohort (with index PCI) utilizing a one-to-one merge command. All repeat PCI procedures as identified from HES/APC data were checked against our NNUH database and all relevant angiograms

were reviewed to document TLR. In case of procedure taking place elsewhere outside NNUH and angiogram not available for review, the TLR variable was indicated as missing. The IBM Statistical Package for the Social Sciences (SPSS) version 25 software was used for cohort extraction, generation of variables and statistical analysis, the R programme (version 3.6.0) was used for statistical analysis while STATA version 17 was used for calculation of the frailty index score.

Outcome	Variable	Codes	ICD-10 / OPCS-4
	Diagnoses		
Cardiovascular mortality	Cardiovascular death	I10X, I214, I219, I249, I251, I255, I259, I340, I350, I489, I500, I619, I629, I639, I64X, I672, I710, I713, I722, J81X, Y608, Q231	ICD-10
Acute coronary syndrome	STEMI	I210, I211, I212, I213	ICD-10
	NSTEMI (including UA)	I214, I219, I200	ICD-10
	Re-infarction	I22, I220, I221, I228, I229	ICD-10
Acute ischemic stroke / TIA	Acute ischemic stroke	I63, I630-6, I638, I639	ICD-10
	TIA	G459, G453	ICD-10
Major bleeding	Hemorrhagic stroke	I61, I610-6, I618, I619	ICD-10
	Subarachnoid hemorrhage	I60, I600-8	ICD-10
	Non-traumatic intracranial hemorrhage	I62, I620, I621, I629	ICD-10
	Hemorrhage not elsewhere specified	R58X	ICD-10
	Gastrointestinal hemorrhage	K920, K921, K922	ICD-10
	Procedures		
	Percutaneous coronary intervention	K49.1-4, K49.8-9, K50.1-4, K50.8-9, K75.1-4, K75.8-9	OPCS-4
	Coronary artery bypass graft	K45.1-9, K46.1-4, K46.8-9, K40.1-4, K40.8-9	OPCS-4

Table 2.1 ICD-10 codes for diagnoses and OPCS-4 procedural codes

ICD-10 and OPCS-4 codes used for patient outcomes

2.4.5 Outcomes

The following outcomes were considered in the retrospective studies in this thesis. All outcomes were identified using the ICD-10 coding system (Table 2.1)

a) Mortality: supplemented by NHS Digital.
b) Cardiovascular mortality defined according to Academic research consortium-2 (171).
c) Acute coronary syndrome encompassing STEMI/NSTEMI/Re-infarction.
d) Acute ischemic stroke including transient ischemic attack.
e) Major bleeding defined as any intracranial bleeding, gastrointestinal bleeding (hematemesis, melaena, gastrointestinal hemorrhage) and hemorrhage not elsewhere classified.
f) Target lesion revascularization defined according to Academic research consortium-2 (171).

2.4.6 Statistical analysis

I undertook statistical analysis in SPSS (version 25) and an independent statistician (Prof Perperoglou) and a data scientist (Dr Bharlaam) also undertook statistical analysis in R (version 4.2). Categorical variables were summarized as counts (percentages) and analyzed using the chi squared test. Continuous variables were summarized using medians and interquartile rage if they were not normally distributed (as assessed by the Kolmogorov and Shapiro test) or mean and standard deviation if they were normally distributed. If not normally distributed the Wilconox rank sum non-parametric test was used, while if normally distributed the independent sample t-test was used to compare the variables. Univariable and multivariable Cox regression models were used to examine associations between variables and patient outcomes. Predictors with p-value <0.05 were introduced into the multivariable Cox regression model. Data are reported as hazard rations (HRs) with 95% confidence intervals. A p-value <0.05 was

considered significant. Cumulative hazard plots were used to compare patient outcomes. Kaplan Meier estimator plots were used to compare DCB vs DES in terms of patient outcomes. Comparisons were performed by the log-rank test. Further information about outcomes and variables included in models are provided in subsequent chapters.

2.4.7 Ethical considerations

Retrospective analysis (IRAS: 195002) of our cohorts of patients has been approved by the independent North West – Haydock Research Ethics committee as well as the Health Research Authority (REC: 17/NW/0278). The Confidentiality Advisory Group (CAG reference: 17/CAG/0145) waived the need for consent as recognized the difficulties in obtaining consent for such a large number of patients retrospectively and granted us permission to obtain HES data from NHS Digital for our analysis (ethics amendment approved 16/12/2019).

Chapter 3. Safety of day case drug coated balloon only angioplasty

The work presented in this chapter is based on the study published by myself, Merinopoulos *et al.* in the Catheter Cardiovascular Interventions Journal (175).

3.1 Introduction

Drug coated balloon (DCB) only angioplasty in de novo coronary lesions, is an alternative to routine elective drug eluting stent implantation (DES) (176–178). In our institution this constitutes 44% of all elective, urgent and emergency PCI. Given the constant pressures on hospital beds, there is an increasing demand for fast and efficient, yet safe turn-around of all elective patients, ideally as day cases. Although there is ample evidence for same day discharge in patients receiving an intra coronary stent, no prior study has reported on this strategy in DCB-only de novo angioplasty (179,180). An important safety consideration, particularly with DCB only angioplasty, is acute vessel closure due to a higher risk of coronary dissection, which will usually be apparent peri-procedurally and will necessitate emergency treatment (181,182). The default position to defer discharge to the following day is therefore readily understandable particularly where an intracoronary stent has not been deployed to scaffold the vessel. In this chapter I report on Norfolk and Norwich University Hospital's experience with same day discharge following DCB-only angioplasty and propose a protocol to achieve this safely.

3.2 Methods

We identified all patients who underwent elective DCB angioplasty at the Norfolk and Norwich University Hospital between September 2017 and April 2018 and were discharged on the day of their procedure. A local protocol had been proposed for guidance (Fig 3.1) but ultimately the decision for same day discharge was left to the Consultant Interventional Cardiologist in

charge of the patient's care. In our institution elective patients can be considered for same day discharge following balloon angioplasty if they fulfil the following criteria:

1) They are pain-free

2) There are no new changes on the post-PCI ECG

3) There is no more than type B coronary artery dissection as defined by Rahman et al. (172), and

4) Absence of high-risk procedural features (such as coronary perforation, occlusion of significant side branch, vascular complications)

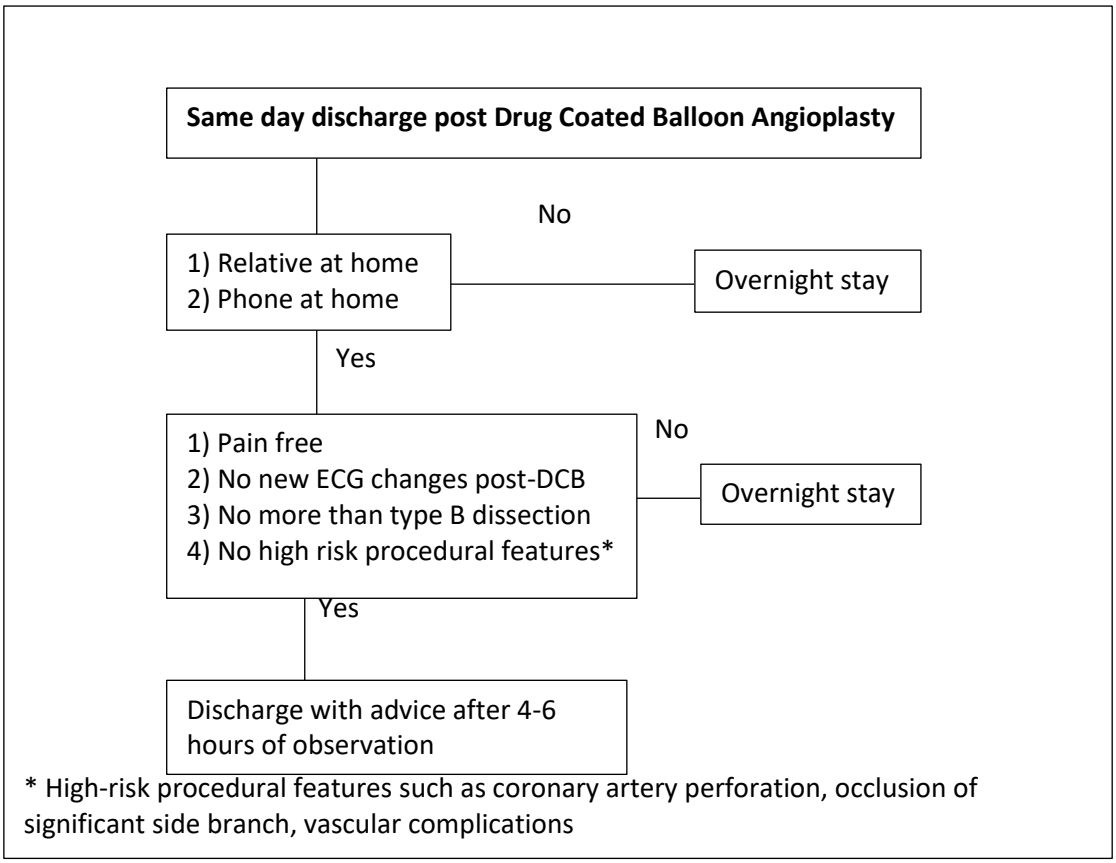


Fig 3.1: Flow diagram for elective drug coated balloon angioplasty patients.

All patients were routinely contacted via telephone post-discharge to identify any complications relating potentially to the procedure. Institutional approval was received for use

of data for the purposes of this manuscript. Survival data was obtained from the Office of National Statistics, a national registry where all deaths are reported.

3.3 Results

One hundred consecutive patients with a total of 113 de novo lesions and 10 in-stent re-stenosis lesions were included (eight patients had in-stent restenosis only whilst two had both in-stent restenosis and de novo disease, giving a total of 105 de novo only lesions in 92 patients). The baseline characteristics demonstrate the unselected nature of the patients; 30% had diabetes mellitus, 41% had a previous myocardial infarction, 56% had undergone previous PCI, 9% had undergone coronary artery bypass grafting surgery (CABG), 55% had hypertension and 69% were current or ex-smokers, as shown in table 3.1. Women were under-represented comprising only 19% of the cohort. However, this is in keeping with the general relative proportion of women undergoing PCI in the UK over the last few years, which is 25% (183).

Table 3.1: Demographics of patients undergoing elective day – case Drug Coated Balloon Angioplasty	
Number of patients	100
Mean Age (years) +/- SD	67 ±10.3
Females	19
Diabetes	30
Previous Myocardial Infarction	41
Previous PCI	56
Hypertension	55
CABG	9
Smoking history (current/previous)	69
PCI: Percutaneous Coronary Intervention, CABG: Coronary Artery Bypass Graft. SD: Standard Deviation	

Table 3.1: Demographics of patients undergoing elective day – case Drug Coated Balloon Angioplasty.

The greater majority of procedures (97%) were completed via the transradial route. The drug coated balloons used were SeQuent Please NEO, 2-4mm in diameter and 10-40mm in length. A total of 140 drug coated balloons were used, 91 with diameter >2.8mm and 49 with diameter <2.8mm, as shown in tables 3.2 and 3.3. I reviewed all the angiograms together with my supervisor (Dr Eccleshall) and graded the severity of the treated lesions according to the ACC/AHA classification system (A-C) (184). A total of 52.0% were type C coronary lesions, 36.6% were type B and only 11.4% were type A lesions. All procedures were also reviewed for any visible dissections which were graded according to the National Heart, Lung and Blood Institute (NHLBI) classification (172). Out of 123 treated lesions, 62 (50.4%) had no angiographic evidence of dissection, 18 (14.6%) had type A dissection, 42 (34.1%) had type B dissection and 1 (0.8%) had type D dissection, which had not been appreciated during the procedure or the time of discharge, as shown in table 3.4.

Table 3.2: Angiographic details of patients undergoing elective day-case Drug Coated Balloon Angioplasty	
Number of lesions	123
Lesion type	
A	14 (11.4%)
B1	23 (18.7%)
B2	22 (17.9%)
C	64 (52.0%)
Bifurcations	28
Heavy calcification	26
Chronic total occlusions	7
Thrombus	1
Small vessel (<2.8mm) Non-small vessel (>2.8mm)	49 91
Vessel treated / out of 123 lesions	
LMS	2 (1.6%)
LAD	57 (46.3%)
Diagonal	7 (5.7%)
LCx	22 (17.9%)
Marginal	10 (8.1%)
Intermediate	3 (2.4%)
RCA	21 (17%)
Vein graft	1 (0.8%)

DCB: Drug Coated Balloon, LMS: Left Main Stem, LAD: Left Anterior Descending, LCx: Left Circumflex, RCA: Right Coronary Artery.

Table 3.2: Angiographic details of patients undergoing elective day-case Drug Coated Balloon Angioplasty.

Table 3.3: Procedural characteristics of patients undergoing elective day-case Drug Coated Balloon Angioplasty	
Access Radial Femoral	 97 3
Number of DCBs used	140
DCB diameter Mean Range	 2.99mm Min = 2, Max = 4mm
DCB Length range	10-40mm
Average DCB Length	23.25mm
Average Fluoroscopy time	14.6minutes
Average Contrast Volume (SD)	129.6 ±48.6ml
Radiation skin dose	1091 mGy
DCB: Drug Coated Balloon, SD: Standard Deviation	

Table 3.3: Procedural characteristics of patients undergoing elective day-case Drug Coated Balloon Angioplasty.

Table 3.4: Core lab analysis elective day-case Drug Coated Balloon Angioplasty	
Dissection type	
No angiographic dissection	62 (50.4%)
A	18 (14.6%)
B	42 (34.1%)
D	1 (0.8%)

Table 3.4: Core lab analysis elective day-case Drug Coated Balloon Angioplasty.

According to the Office of National Statistics, a national body where all deaths are recorded by law, our 30-day mortality was zero. The overall complication rate was 1%. There were no vascular complications and no cases of contrast nephropathy reported. In cases at risk of contrast nephropathy, we routinely undertake all necessary steps to minimise the risk with adequate intravenous pre-hydration and limited use of contrast. Our average contrast volume of 130mls justifies this reassuring result. During our follow-up telephone contact, 99 patients did not report any cardiac related symptoms requiring urgent hospitalisation or urgent investigations. One patient was admitted the day after the procedure with cardiac chest pain, ECG changes and serial troponin rise of 150, 160 and 148 ng/L (normal <14). Urgent angiography revealed TIMI II flow in the target vessel requiring stent implantation. The patient made an uneventful recovery and was discharged home the next day. Retrospective review of the index procedure demonstrated a type D dissection that had not been previously appreciated due to suboptimal imaging.

3.4 Discussion

Acute vessel closure due to coronary artery dissection is one of the most significant complications of balloon angioplasty. Early studies have shown that type A and B coronary artery dissections if left untreated have good long term outcomes (185). This is the first study to report on same day discharge in consecutive patients undergoing DCB-only angioplasty and propose safe criteria to achieve this (Fig 3.1). The case mix of the patients included supports that this can be achieved across all patients with multiple comorbidities and complex lesions.

DCB is an emerging interventional strategy in the extensive armamentarium of Interventional Cardiologists both in the elective and emergency setting (1). The existing pressures on hospital beds nationally exacerbated by the winter crises places greater emphasis on more efficient utilisation of inpatients beds for our elective patients without compromising unduly on patient safety. Our study confirms that day-case DCB angioplasty is safe, with zero 30-day mortality, and carries a low complication rate in an unselected patient population and can improve cost-effectiveness. After one hundred consecutive patients, ninety-seven days in hospital were saved. With an excess bed day of £586 according to Unit Costs of Health and Social Care 2021 we estimate that day-case DCB angioplasty can save about £568 per procedure in the UK, compared to routinely discharging elective patients the following day. However, this might not apply in the majority of centres in UK where standard of care is DES (186). Recent data from the United States demonstrate that same-day discharge after PCI is associated with larger cost savings of $5128 per procedure, (7) while transradial same-day discharge PCI in Canada was associated with savings of Can$ 1,141 mainly due to the extra night for overnight hospital stay (8). Obviously, the economic benefits of day-case DCB angioplasty will be of greater relevance in countries with more expensive overnight hospital stays.

The retrospective nature of our work from a single-centre is a limitation as it can introduce referral bias. However, we are a large tertiary referral centre providing cardiac intervention to

a population in excess of one million and we included consecutive patients to ensure recruitment bias is minimised. The strength of our study is that it represents real world data and that we included and followed up all (consecutive and unselected) patients who met the inclusion criteria during our study duration. Therefore, we believe that our conclusions can easily be generalised to patients undergoing elective DCB-only angioplasty in other institutions.

3.5 Conclusion

Our study has shown that where DCB can be used for the treatment of de novo coronary artery disease, same day discharge of all elective patients according to our protocol can be considered and is cost-effective.

Chapter 4. Long-term safety of drug coated balloon only angioplasty

The work presented in this study is based on the study published by myself, Merinopoulos *et al.* in Clinical Research in Cardiology titled 'Long-term safety of paclitaxel drug-coated balloon-only angioplasty for de novo coronary artery disease: the SPARTAN DCB study' (189).

4.1 Introduction

Drug coated balloons (DCB) are an emerging PCI technology negating the need for stent implantation (20,190,191). Thus far, it has an established role in the treatment of in-stent restenosis (192) with a growing number of studies showing excellent results in de novo coronary artery disease (193–197). The great majority of DCB used are coated with Paclitaxel but encouraging results have emerged over the last year for the use of Sirolimus coated balloons in coronary artery disease (198,199) However, a recent systematic review and meta-analysis of summary-level data raised concerns about the use of paclitaxel containing devices for peripheral arterial disease, suggesting a signal of increased late mortality associated to the paclitaxel dose-time product (200). A subsequent individual patient data meta-analysis confirmed that paclitaxel-coated devices, for peripheral arterial disease, were associated with an absolute 4.6% increased mortality risk (201). Other studies however, with individualised-data analysis of patients treated with paclitaxel DCB for peripheral arterial disease demonstrated no difference in all-cause mortality between DCBs and uncoated percutaneous transluminal angioplasty (202,203). Despite not universal, this concern was sufficient for the FDA to initiate an investigation for the use of paclitaxel containing devices for peripheral arterial disease (204). Currently, there is no data on long-term results of paclitaxel DCB used to treat de novo coronary artery disease. Moreover, the dose of Paclitaxel in coronary DCBs (0.3-0.6mg) is at least an order of magnitude lower compared with Paclitaxel eluting devices

(8.5mg for IN.PACT 6x120 mm balloon for example) for peripheral artery disease (200) (205) indicating that any results from peripheral DCB cannot be extrapolated to coronary DCB. In this chapter I aimed to explore whether there is a signal of increased late mortality in patients treated with paclitaxel DCB for de novo coronary artery disease in up to five years follow up.

4.2 Methods

The full methodology has been described in general methods (Chapter 2.3). In brief, the long-term Safety of PAclitaxel dRug coaTed balloon only ANgioplaSty for de novo coronary artery disease (SPARTAN DCB) study was an investigator-initiated, single-centre, cohort study. Following ethical approval, I retrospectively surveyed the NNUH clinical database to identify all patients treated with either paclitaxel DCB or 2nd generation non-paclitaxel drug eluting stents for stable, de novo coronary artery disease between 1st January 2011 and 31st December 2018. In order to investigate the true potential effect of paclitaxel and to achieve as homogenous a group as possible from our real-world data, I excluded patients being treated for ST Elevation Myocardial Infarction (STEMI) or Non-ST Elevation Myocardial Infarction (NSTEMI). I also excluded patients with prior PCI to ensure homogeneity of our cohort. Similarly, I excluded patients who had repeat PCIs following their index procedure if the PCI strategy was different to the index procedure: i.e. patients treated with DES initially and then later treated with DCB or vice versa were excluded as shown in the Consort diagram (Fig 4.1); however if the patients received a DES or DCB on all occasions they were not excluded. The vessel diameter was taken as the largest pre/post dilatation balloon, DCB or DES used while lesion length was based on the DCB or DES length.

The primary endpoint was all-cause mortality. Survival data were obtained through the UK Health and Social Care Information Service, an independent national body where all deaths in

the UK are recorded by law. Mortality data were obtained six months following the last study patient to ensure a minimum of six-month follow up for every patient.

I undertook the statistical analysis in SPSS (version 25), however the statistical analysis was also confirmed in programme R (version 3.6.0) by an independent professional statistician, Prof Perperoglou. For the main analysis, all-cause mortality was limited to five years post index procedure (if a patient died beyond five years follow up, they were considered alive for the purposes of this analysis) in order to minimise the difference in follow up between DES and DCB group. Kaplan-Meier survival curves were also plotted for those patients alive at two years in order to specifically investigate a late paclitaxel effect. Comparisons were performed by the log rank test. Univariate and multivariate Cox regression analyses were performed to identify predictors of mortality.

4.3 Results

A total of 429 consecutive patients treated with paclitaxel DCB and 1088 consecutive patients treated with non-paclitaxel 2nd generation DES were identified (Fig 4.1). Some 94% of patients in the DCB group were treated with iobromide paclitaxel DCB (67.4% SeQuent Please NEO and 26.6% SeQuent Please), 5% with urea paclitaxel DCB (Falcon) and 1% with other paclitaxel DCB. Some 33.5% of patients were treated with Promus Premier and 26.8% with Promus Element DES, 13% with Synergy DES, 7.6% with Xience Prime, 5.7% with Xience Pro, 6.5% with Onyx DES, 2.9% with Ultimaster DES, 1.8% with Combo dual therapy DES and 2.2% with other second-generation DES. The average age was 66.9 ±10.2 and 66.8 ±10 years old for the DCB and DES group respectively. Male patients accounted for 76.2% of the DCB group and 76.6% of the DES. Table 4.1 demonstrates that the two groups were well balanced for the great majority of baseline patient characteristics. The DES group had a significantly higher incidence of patients with Chronic Obstructive Pulmonary Disease and

smoking history while the DCB group had a significantly higher incidence of patients with atrial fibrillation. Significantly more patients were on dual antiplatelet therapy (DAPT) in the DES group and as expected the mean duration of DAPT was significantly longer in the DES group.

Fig 4.1: Study consort diagram

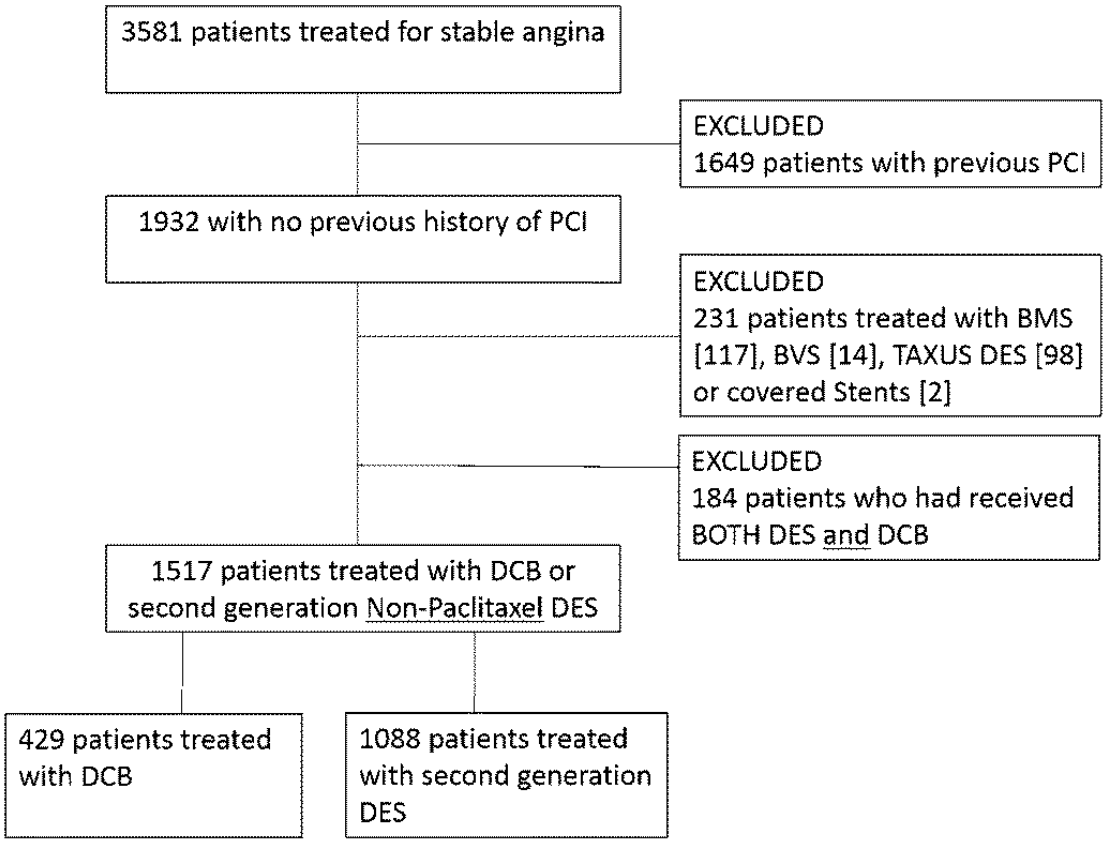


Fig 4.1: Consort diagram indicating how the final population included in the study was identified.

Table 4.1: Baseline patient characteristics of study groups.

	Paclitaxel DCB (n=429)	**Non-Paclitaxel 2nd generation DES (n=1088)**	**p-value**
Age	66.9 ±10.2	66.8 ±10	0.79
Male	327 (76.2)	834 (76.6)	0.86
Hypercholesterolemia	161 (37.5)	456 (41.9)	0.12
Hypertension	236 (55.0)	639 (58.7)	0.19
Peripheral vascular disease	17 (3.9)	48 (4.4)	0.69
Cerebrovascular event	30 (6.9)	54 (4.9)	0.15
Myocardial infarction	52 (12.1)	167 (15.3)	0.11
Coronary artery bypass	35 (8.1)	82 (7.5)	0.68
Heart failure	14 (3.2)	40 (3.6)	0.69
Family history of IHD	133 (31.0)	324 (29.7)	0.64
COPD	14 (3.2)	66 (6.0)	0.02*
Diabetes	98 (22.8)	229 (21.0)	0.44
Smoking (current/previous)	247 (57.5)	696 (63.9)	0.02*
Atrial fibrillation	37 (8.6)	40 (3.6)	<0.01*
eGFR	78.8 ± 20.1	78.5 ± 21.1	0.81
DAPT	397 (92.5)	1050 (96.5)	<0.01*
Mean DAPT duration	73.5 ± 104.7	355.6 ± 60.5	<0.01*

Table 4.1: Baseline patient characteristics of patients treated with DCB or DES. Data are n (%) and *denotes significant result

COPD: Chronic Obstructive Pulmonary Disease, IHD: Ischemic Heart Disease, eGFR: estimated glomerular filtration rate. DAPT: Dual antiplatelet therapy

Table 4.2 shows the characteristics of the target vessels treated with DCB or DES. The groups were well balanced in terms of prognostically significant lesions targeted with no difference in left main coronary artery, left anterior descending artery or multi-vessel PCI.

Table 4.2: Target vessels of study groups

	Paclitaxel DCB (n=429)	**Non-Paclitaxel 2nd generation DES (n=1088)**	**P value**
LMS	10 (2.3)	34 (3.1)	0.41
LAD	229 (53.4)	545 (50.1)	0.25
Cx	76 (17.7)	135 (12.4)	<0.01*
RCA	77 (17.9)	250 (22.9)	0.03*
Graft	4 (0.9)	27 (2.4)	0.06
Multi-vessel PCI	33 (7.7)	97 (8.9)	0.44
Mean Vessel diameter, mm	3.06 ±0.56	3.39 ±0.59	<0.01*
Mean lesion length, mm	26.05 ±11.95	30.03 ±16.52	<0.01*
Large vessels (diameter≥3mm)	320 (74.6)	925 (85)	<0.01*

Table 4.2: Target vessels treated with DCB or DES. LMS: left main stem, LAD: left anterior descending artery, Cx: circumflex, RCA: right coronary artery, PCI: percutaneous coronary intervention. Data are n (%) and *denotes significant result.

The patients were followed up for an average of 31.6 ±16.3 months (interquartile range: 16.8 – 45.3 months) in the DCB group and 44.4 ±18.4 months (interquartile range: 27.1 – 60 months) in the DES group. We obtained mortality data for 1515 patients. It was not possible to obtain mortality status of two patients (one in each group) who were censored at the time of last known alive.

There was no evidence of increased late mortality associated with paclitaxel DCB for de novo coronary artery disease compared with non-paclitaxel 2nd generation DES (Fig 4.2). Interestingly, the Kaplan-Meier curves separate early and then continue to diverge; supporting that DCB-only angioplasty is a safe procedure. Analysis following propensity score matching supported these results (Fig 4.3). The table 4.3 demonstrated the 30-day, 6, 12, 24 and 36 month mortality in the DCB and DES groups. After 36 months of follow up, 9 patients died in the DCB groups vs 50 patients in the DES group. We specifically investigated a possible late mortality effect by analysing separately those patients who were alive two years following the index PCI and there was no evidence of increased late mortality with paclitaxel DCB (Fig 4.4). Univariate Cox regression analysis identified the following adverse prognostic factors: age, hypertension, peripheral vascular disease, previous myocardial infarction, heart failure, smoking, atrial fibrillation and decreasing estimated Glomerular Filtration Rate (eGFR) [and renal failure defined as estimated glomerular filtration rate (eGFR) < 45] (table 4.3). Hypercholesterolemia and family history of ischaemic heart disease were associated with better prognosis on univariate analysis (table 4.4). None of the angiographic characteristics were associated with worse outcome. On multivariate Cox regression analysis only age, worse eGFR and smoking history remained significant poor prognostic factors (table 4.5).

Fig 4.2: Kaplan-Meier estimator plot

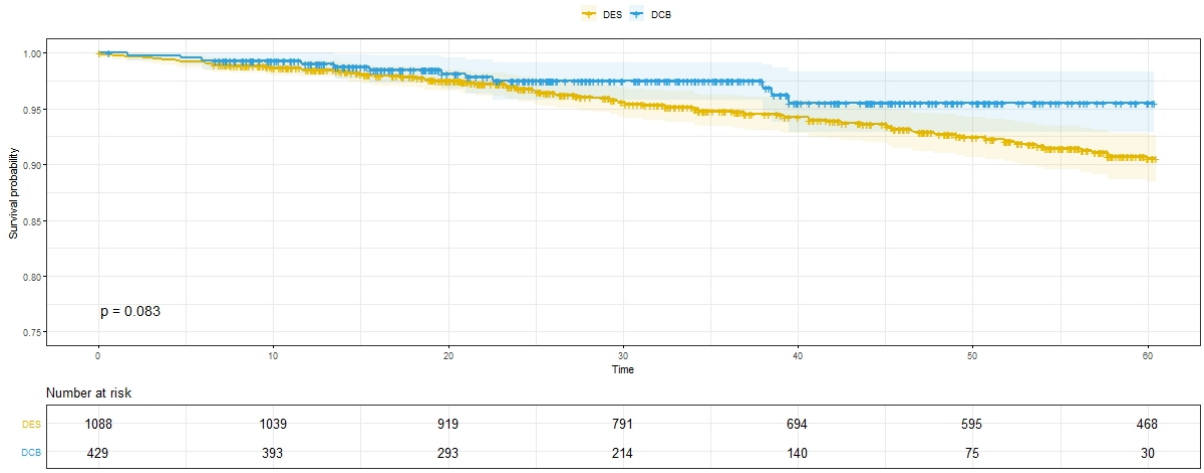


Fig 4.2: Kaplan-Meier estimator plot of all-cause mortality for paclitaxel DCB versus non-paclitaxel 2nd generation DES with numbers at risk are shown below the graph. DCB: Drug coated balloon, DES: Drug eluting stent

Fig 4.3: Kaplan-Meier estimator plot following propensity score matching

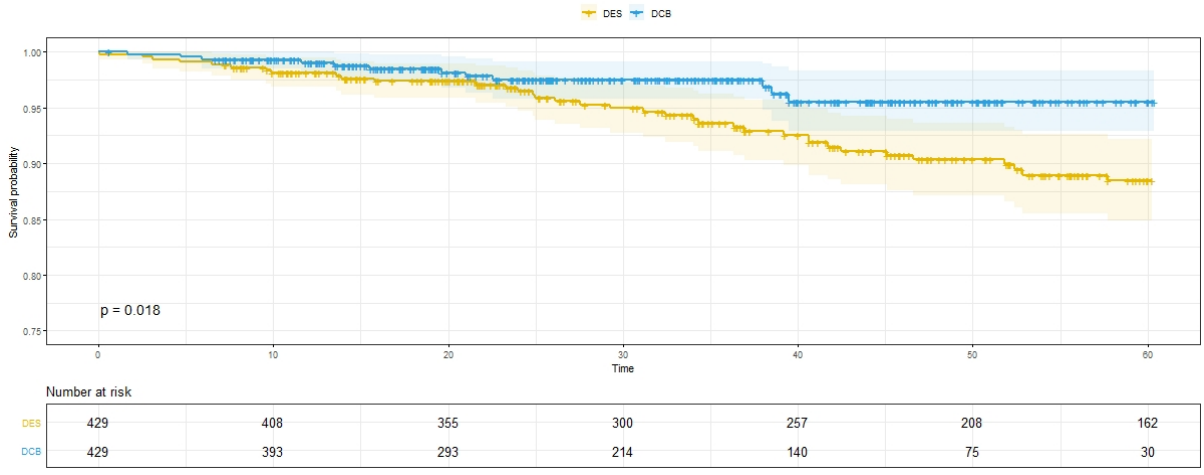


Fig 4.3: Some 429 patients treated with DCB were matched to 429 patients treated with DES using Programme R. Kaplan-Meier estimator plot of all-cause mortality shows no evidence of late mortality associated with paclitaxel DCB. DCB: Drug coated balloon, DES: Drug eluting stent

Fig 4.4: Kaplan-Meier estimator plot of patients alive at two years

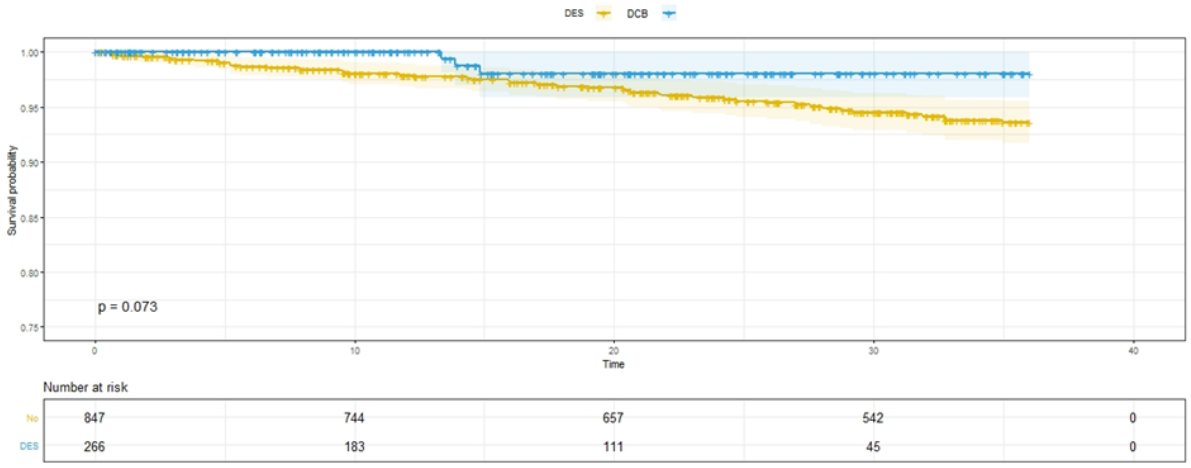


Fig 4.4: Kaplan-Meier estimator plot of patients alive at two years showing no significant difference between DES and DCB with numbers at risk are shown below the graph. DCB: Drug coated balloon, DES: Drug eluting stent

Table 4.3: Mortality rate of study groups.

Mortality (time)	DCB mortality	DCB number at risk	DCB mortality / DCB number at risk (%)	DCB mortality / (DCB number at risk + DCB mortality) (%)	DES mortality	DES number at risk	DES mortality / DES number at risk (%)	DES mortality / (DES number at risk + DES mortality) (%)
30 day	0	428	0%	0%	3	1084	0.3%	0.3%
6 months	3	425	0.7%	0.7%	10	1077	0.9%	0.9%
12 months	4	371	1.1%	1.1%	16	1016	1.6%	1.6%
24 months	9	266	3.4%	3.3%	33	847	3.9%	3.8%
36 months	9	165	5.6%	5.2%	50	723	6.9%	6.5%

Table 4.3: 30-day, 6, 12, 24 and 36 month mortality in DCB and DES groups.

Table 4.4: Univariate Cox regression analysis.

Variable	**P value**	**HR [95% CI]**
DCB	0.08	0.765 [0.56, 1.04]
Female	0.08	1.496 [0.95, 2.35]
Age	<0.01 *	1.115 [1.08, 1.14]
Hypercholesterolemia	0.01	0.566 [0.36, 0.89]
Hypertension	0.01 *	1.808 [1.14, 2.85]
Peripheral vascular disease	<0.01*	2.674 [1.34, 5.32]
Cerebrovascular disease	0.81	0.882 [0.32, 2.40]
Myocardial infarction	0.03 *	1.716 [1.05, 2.80]
Heart failure	<0.01*	3.439 [1.66, 7.12]
Family history of IHD	<0.01	0.434 [0.24, 0.75]
Diabetes	0.23	1.345 [0.83, 2.17]
COPD	0.40	1.426 [0.62, 3.26]
Smoking	<0.01 *	1.965 [1.20, 3.20]
BMI	0.26	0.975 [0.93, 1.02]
Atrial fibrillation	<0.01*	3.151 [1.57, 6.31]
eGFR	<0.01*	0.969 [0.959, 0.979]
Renal failure (eGFR<45)	<0.01 *	4.997 [2.94, 8.49]
CABG	0.27	1.453 [0.75,2.80]
DAPT duration	0.65	1.000 [0.998, 1.001]
LMS	0.11	2.100 [0.85, 5.17]
LAD	0.12	0.714 [0.46, 1.08]
Cx	0.07	1.621 [0.96, 2.71]

RCA	0.78	0.931 [0.56, 1.54]
Graft	0.49	1.495 [0.47, 4.72]
Multivessel PCI	0.72	0.868 [0.40, 1.87]
Vessel diameter	0.59	1.101 [0.77, 1.55]
Lesion length	0.41	0.995 [0.984, 1.007]

Table 4.4: Results of univariate Cox regression analysis

IHD: ischaemic heart disease, COPD: Chronic Obstructive Pulmonary Disease, BMI: body mass index, CABG: coronary artery bypass graft, DAPT: dual antiplatelet therapy, LMS: left main stem, LAD: left anterior descending, Cx: circumflex, RCA: right coronary artery, PCI: percutaneous coronary intervention *denotes adverse prognostic factor.

Table 4.5: Multivariate Cox regression analysis.

Variable	**P value**	**HR [95% CI]**
Age	<0.01*	1.087 [1.06, 1.12]
Heart failure	0.19	1.653 [0.77, 3.55]
eGFR	0.01*	0.985 [0.974, 0.997]
Family history of IHD	0.56	0.843 [0.47, 1.51]
Hypertension	0.11	1.456 [0.91, 2.32]
Hypercholesterolemia	0.11	0.683 [0.43, 1.09]
Peripheral vascular disease	0.45	1.340 [0.63, 2.84]
Smoking	0.01*	1.925 [1.17, 3.16]
Myocardial infarction	0.16	1.439 [0.87, 2.39]
CABG	0.68	0.865 [0.44, 1.71]
Atrial fibrillation	0.32	1.450 [0.70, 3.00]

Table 4.5: Results of multivariate Cox regression analysis. IHD= Ischaemic heart disease, CABG: coronary artery bypass graft *denotes adverse prognostic factor.

4.4 Discussion

Drug coated balloon only angioplasty is recommended by evidence-based guidelines for the treatment of in-stent restenosis while there is also evidence to support their use in small vessel disease and patients with high bleeding risk (26,29,206). Following a recent meta-analysis though, concerns have been raised regarding the safety of paclitaxel devices for peripheral artery disease (200). In SPARTAN DCB study, paclitaxel DCB was not associated with increased late mortality, up to five years of follow up. Instead, there was a trend for better survival when compared with second generation DES.

Our results are consistent with two recent meta-analyses. The recent DAEDALUS study in patients treated with DCB or DES for in-stent re-stenosis showed that there was no significant difference in late mortality associated with DCB. This conclusion is limited however by the fact that follow up was limited to three years and thus might have missed a true late effect (207). In addition, it is difficult to draw definitive conclusions from that study for late mortality relating to paclitaxel, as this was a subgroup analysis and the patient groups were heterogeneous given the previous stent implantations including bare metal stents and paclitaxel DES. A most recent meta-analysis specifically investigating the mortality of paclitaxel DCB for coronary intervention did not show increased mortality with DCB (208). However, this meta-analysis included significantly heterogeneous studies comparing paclitaxel DCB with control treatments such as plain old balloon angioplasty, bare metal stents, paclitaxel and non-paclitaxel drug eluting stent mostly in the setting of in-stent restenosis.

In the SPARTAN DCB study, we included large numbers of patients treated for de novo coronary artery disease and ensured homogeneity of the groups by excluding patients with previous PCI or patients who received both DCB and DES either at their index or subsequent PCIs. As such, our groups of DCB and DES were well-matched for patient characteristics and angiographic findings. We have demonstrated that there is no evidence of increased late mortality associated with paclitaxel DCB compared to non-paclitaxel second generation DES for de novo coronary artery disease up to five years of follow up. In fact, there was actually a trend towards better survival with DCB, a finding consistent with the most recent meta-analysis (208). Furthermore, we specifically investigated a late paclitaxel effect by analysing only patients who were alive at two years, with no evidence of increased late mortality associated with paclitaxel DCB either.

Following a meta-analysis raising concerns about a possible long-term mortality signal due to paclitaxel eluting devices for peripheral vascular disease (200), an intense debate about the

conclusion and various limitations of that study has been triggered in the literature (199,209–211). Subsequent studies showed conflicting results and the FDA initiated an investigation for this matter (204). According to the latest FDA update, clinicians and patients were advised to balance the known benefits of paclitaxel DCB for peripheral arterial disease with the potential for increased mortality when considering their treatment options (212). Despite the similarities in peripheral and coronary DCB, there are also major differences. For example, the dose of paclitaxel in DCBs for coronary artery disease is about an order of magnitude lower compared to the dose of paclitaxel in paclitaxel-coated devices for peripheral artery disease (205) making it therefore unclear whether, even if the results of the DCB for peripheral vascular disease were adverse, how this would translate to the coronary DCB PCI. Furthermore, the underlying mechanism leading to a possible increased late-mortality signal with DCB for peripheral artery disease remains to be defined. Nevertheless, given that the outcomes that were notably concerning included cardiovascular mortality, it is crucial to study the results of paclitaxel DCB for coronary artery disease carefully and provide assurance of safety.

Limitations

The retrospective, non-randomised nature of our work from a single centre can introduce referral bias. However, our institution is a large tertiary referral centre providing cardiac intervention to a population in excess of one million, with the highest implantation of DCBs for coronary artery disease in the UK (213) and I included all consecutive patients fulfilling the criteria. However, these results might not be generalizable to smaller institutions with less experience with DCB only angioplasty. Even though my study is retrospective and non-randomised, the NNUH clinical database was completed prospectively and the two groups were well balanced in terms of patient and angiographic characteristics. Furthermore, the results were consistent following propensity score match analysis. The DES group had significantly longer follow up but this was mitigated by limiting the analysis to five years post index

procedure (if a patient died beyond five years follow up, they were considered alive for the purposes of this study).

4.5 Conclusion

In conclusion, this is the first study to specifically report on the long-term five year follow up of patients undergoing elective DCB PCI for stable, de novo, coronary artery disease and compared with second generation non-paclitaxel stents. This study shows that there is no evidence of increased late mortality associated with paclitaxel DCB for stable, de novo coronary artery disease and therefore, DCB could be considered in this population.

Chapter 5. Drug coated balloon only angioplasty in routine, elective clinical practice

This chapter is based on the study published by myself, Merinopoulos *et al*. in Clinical Research in Cardiology, titled 'Paclitaxel drug coated balloon-only angioplasty for de novo coronary artery disease in elective clinical practice' (214).

5.1 Introduction

Implantation of second-generation drug-eluting stents (DES) is the current guideline-recommended treatment strategy for de novo coronary artery disease (215). Stents were initially developed to treat the limitations of plain old balloon angioplasty related to flow-limiting dissections and acute vessel recoil (25). However, the persistence of stent-related complications, such as stent thrombosis and in-stent restenosis, stimulated the concept of 'leaving nothing behind' (25). Drug coated balloons (DCB) were developed to combine the benefits of local drug treatment without the complications of stent implantation in cases where stenting was not mandated after initial angioplasty (1). Currently, DCBs represent an alternative, emerging treatment strategy with supportive evidence in specific groups such as patients with in-stent restenosis, high-bleeding risk or small vessel disease (216)(217). Randomised data have demonstrated maintained safety and efficacy of DCB vs DES for de novo small vessel coronary artery disease (193,218,219). However, there are no data about the safety of DCB-only angioplasty as part of routine clinical practice and there are limited data about the safety of DCB in de novo large vessels (197). There are no data evaluating if it is possible and safe for DCB-only angioplasty to become part of a routine PCI treatment strategy. Previous work from our group (SPARTAN DCB as shown in chapter 4) demonstrated that there is no evidence of increased late mortality associated with paclitaxel DCB, and indeed better survival with DCB in the propensity score matched cohort (220). However, that analysis excluded patients with previous percutaneous coronary intervention (PCI) and patients with

different PCI strategy in subsequent procedures compared to index (i.e. patients treated with DES initially and then later treated with DCB or vice versa were excluded). Even though that study design was necessary in order to achieve group homogeneity and investigate a true potential effect of paclitaxel, it poses a limitation in terms of generalisability. In the current study we have addressed this limitation by including patients with previous PCI and subsequent PCI irrespective of initial PCI strategy.

In this study, we aimed to explore the safety of DCB-only angioplasty judged by overall mortality, as well as major cardiovascular endpoints, in routine clinical practice for stable, de novo coronary artery disease in all vessel sizes.

5.2 Methods

The full methodology was discussed in chapter 2.4 (page 62). In brief, the paclitaxel drug coated balloon only angioplasty for stable de novo coronary artery disease in routine clinical practice study was an investigator-initiated, single centre, cohort study. Following ethical approval, I retrospectively interrogated the NNUH clinical database to identify all patients whose first entry was for stable, de novo coronary artery disease, up to November 2019. In NNUH, the use of DCB has steadily increased with an associated decrease in second-generation DES use over the last ten years. From 2015 onwards more than 100 patients per year (more than about 40% of patients), with first presentation of stable angina and de novo disease were treated with DCB-only angioplasty (Fig 5.1). I included patients from January 2015 to November 2019 to allow a similar number of patients to be included from each group, without affecting the follow-up period in each group.

Fig 5.1: Yearly usage of DCB and DES

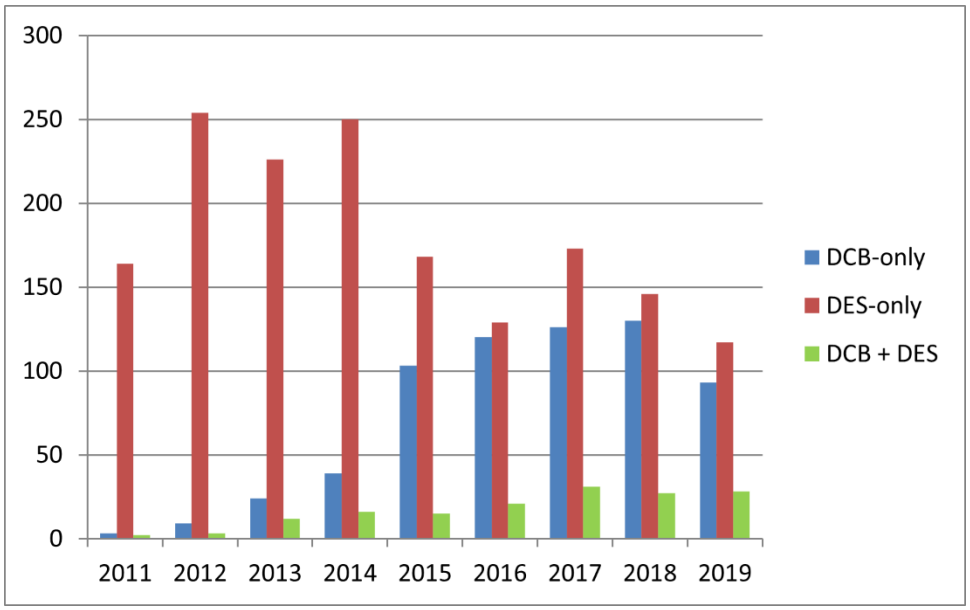


Fig 5.1: Yearly usage of DCB and DES in patients with first presentation with stable angina and de novo disease.

The primary endpoint was all-cause mortality. The secondary endpoints were cardiovascular mortality, acute coronary syndrome (ACS), stroke or transient ischaemic attack, major bleeding and target lesion revascularisation. All deaths were classified as cardiovascular or non-cardiovascular by an adjudication committee according to academic research consortium 2 consensus (171).

5.3 Results

A total of 544 consecutive patients (640 de novo lesions) treated with paclitaxel DCB and 693 consecutive patients (831 de novo lesions) treated with 2nd generation DES were identified (Fig II). The median age was 69 (IQR: 61-75) for both groups. Male patients accounted for 79% of the DCB and 78% of the DES group. The groups were well balanced in baseline patient characteristics as shown in table 5.1. The only difference was that the DES group had significantly more patients with chronic obstructive pulmonary disease.

Fig 5.2: Study consort diagram.

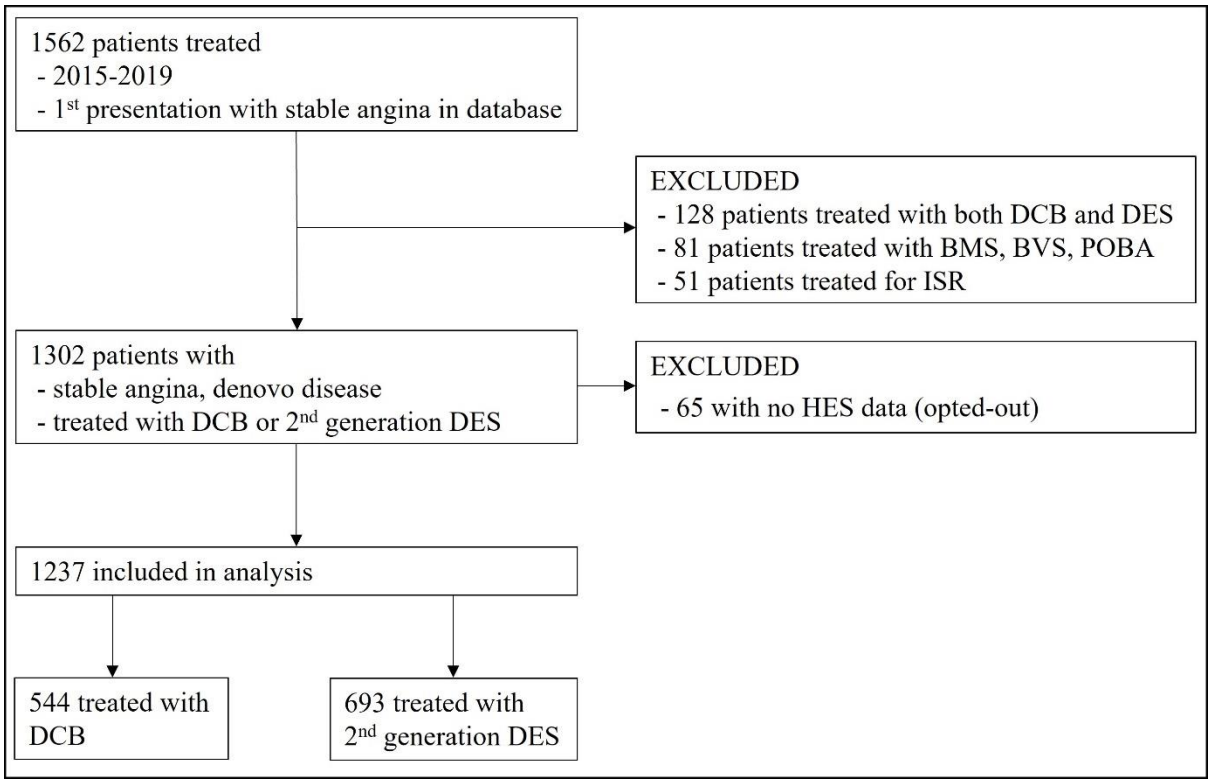


Fig 5.2: Consort diagram indicating how the final population included in the study was identified

The angiographic characteristics of the target vessels treated are shown in table 5.2. The groups were well balanced in terms of prognostically significant vessels targeted (LMS or LAD and multivessel PCI). The DES group had more patients with large vessel treated while the DCB group had more patients with true bifurcations. The DES group had significantly more patients on dual antiplatelet therapy (DAPT) while the duration of DAPT was significantly longer in the DES group as well.

Table 5.1: Baseline patient characteristics.

Characteristic	**DCB, N = 544**	**DES, N = 693**	**p-value**
Age, Median (IQR)	69 (61 – 75)	69 (61 – 75)	0.61[1]
Male, n (%)	429 (79)	541 (78)	0.74[2]
Current/Ex-Smoker, n (%)	336 (62)	455 (66)	0.11[2]
Hypercholesterolaemia, n (%)	186 (34)	224 (32)	0.49[2]
Hypertension, n (%)	307 (56)	397 (57)	0.76[2]
Peripheral vascular disease, n (%)	24 (4.4)	33 (4.8)	0.77[2]
Cerebrovascular disease, n (%)	42 (7.7)	37 (5.3)	0.089[2]
Myocardial infarction, n (%)	93 (17)	123 (18)	0.76[2]
Percutaneous coronary intervention, n (%)	79 (15)	86 (12)	0.28[2]
Coronary artery bypass graft, n (%)	47 (8.6)	56 (8.1)	0.72[2]
Atrial fibrillation, n (%)	56 (10)	52 (7.5)	0.084[2]
Heart failure, n (%)	18 (3.3)	20 (2.9)	0.67[2]
Chronic obstructive pulmonary disease, n (%)	18 (3.3)	44 (6.3)	**0.015[2]**
Diabetes, n (%)	125 (23)	153 (22)	0.71[2]
Family history, n (%)	148 (27)	174 (25)	0.40[2]
eGFR (mL/min/1.73m^2) Median (IQR)	79 (66 – 91)	78 (67 – 91)	0.85[1]
Frailty, n (%)			>0.99[3]
Low	541 (99)	688 (99)	
Intermediate	3 (0.6)	5 (0.7)	
High	0 (0)	0 (0)	

Table 5.1: Baseline patient characteristics of patients treated with DCB or DES. Data are n (%) or median (IQR).

DCB: drug coated balloon, DES: drug eluting stent, eGFR: estimated glomerular filtration rate, eGFR: estimated glomerular filtration rate

[1] Wilcoxon rank sum test[2] Pearson's Chi-squared test[3] Fisher's exact test[4] Wilcoxon rank sum exact test

Table 5.2: Angiographic characteristics of target vessels.

Characteristic	**DCB**, N = 544	**DES**, N = 693	**p-value**
Vessels treated, n (%)			0.006[2]
LMS	15 (2.8)	27 (3.9)	
LAD	309 (57)	376 (54)	
LCx	104 (19)	98 (14)	
RCA	111 (20)	172 (25)	
Graft	5 (0.9)	20 (2.9)	
Multivessel PCI, n (%)	51 (9.4)	83 (12)	0.14[2]
Patients with true bifurcation, n (%)	63 (12)	56 (8.1)	**0.038**[2]
Patients with vessel treated ≥ 3mm	398 (73)	594 (86)	**<0.001**[2]
Dual antiplatelet therapy	518 (95.2%)	681 (98.3%)	**<0.002**[2]
Duration of dual antiplatelet therapy, Median (IQR) days	30 (29, 31)	365 (364, 365)	**<0.001**[1]
Lesions			
De novo lesions treated	DCB (640)	DES (831)	
True bifurcation, n (%)	64 (10)	55 (6.6)	**0.02**[2]
Vessel diameter (mm), Median (IQR)	3.00 (2.75 – 3.50)	3.50 (3.00 – 3.75)	**<0.001**[1]
Lesion length (mm), Median (IQR)	20 (20 – 30)	24 (18 – 38)	**0.043**[1]
Dissection grade post DCB (221)			
A	20 (3.1%)	n/a	
B	278 (43.4%)	n/a	
C	5 (0.8%)	n/a	
D	3 (0.5%)	n/a	

Table 5.2: Angiographic characteristics of target vessels treated with DCB or DES.

DCB: drug coated balloon, DES: drug eluting stent, LMS: left main stem, LAD: left anterior descending, LCx: left circumflex, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction * indicates significant result. [1] Wilcoxon rank sum test[2] Pearson's Chi-squared test[3] Fisher's exact test[4] Wilcoxon rank sum exact test

The median follow-up of patients in the DCB group was 3.7 years (IQR: 2.5 - 4.8) while the median follow-up in the DES group was 3.6 years (IQR: 2.6 -4.9). There was no evidence of increased all-cause mortality (Fig 5.3) associated with paclitaxel DCB for de novo coronary artery disease compared to 2nd generation DES. The mortality rate was 35/544 in the DCB group versus 59/693 in the DES group (HR=1.28; CI: 0.84-1.95; p=0.24). Furthermore, there was no difference in any of the secondary endpoints, cardiovascular mortality, ACS, stroke/TIA, major bleeding or unplanned TLR (Fig 5.4). Univariable Cox regression analysis identified the following adverse prognostic factors for all-cause mortality: increasing age, coronary artery bypass (CABG), heart failure, atrial fibrillation (AF), diabetes, decreasing estimated glomerular filtration rate (eGFR) and frailty. Hypercholesterolaemia was associated with better survival. (Table 5.3). On multivariable Cox regression analysis only age and frailty remained significant predictors of mortality (Table 5.4). Finally, in terms of short-term safety, one patient in the DCB group had acute vessel closure a few hours later and needed to return urgently to the lab. Two patients in the DES group returned urgently to the lab within 72 hours (one with subacute stent thrombosis and one with stent edge disruption requiring further stent). No other patient returned urgently to the lab within seven days in either group.

Following propensity score matching 544 patients treated with DCB were matched to 544 patients treated with 2nd generation DES. Table 5.5 shows the baseline characteristics of the propensity score matched cohort. There was no difference in all-cause mortality (Fig 5.5) or any of the secondary endpoints (cardiovascular mortality, ACS, stroke/TIA, major bleeding or unplanned TLR) (Fig 5.6). Analysis of patients with treated vessel ≥3mm showed that the results were unchanged (Table 5.6 and 5.7). In patients with large vessel treated, on multivariable Cox regression analysis, only increasing age and frailty score were significant predictors of all-cause mortality.

Fig 5.3: Cumulative hazard plot of all-cause mortality.

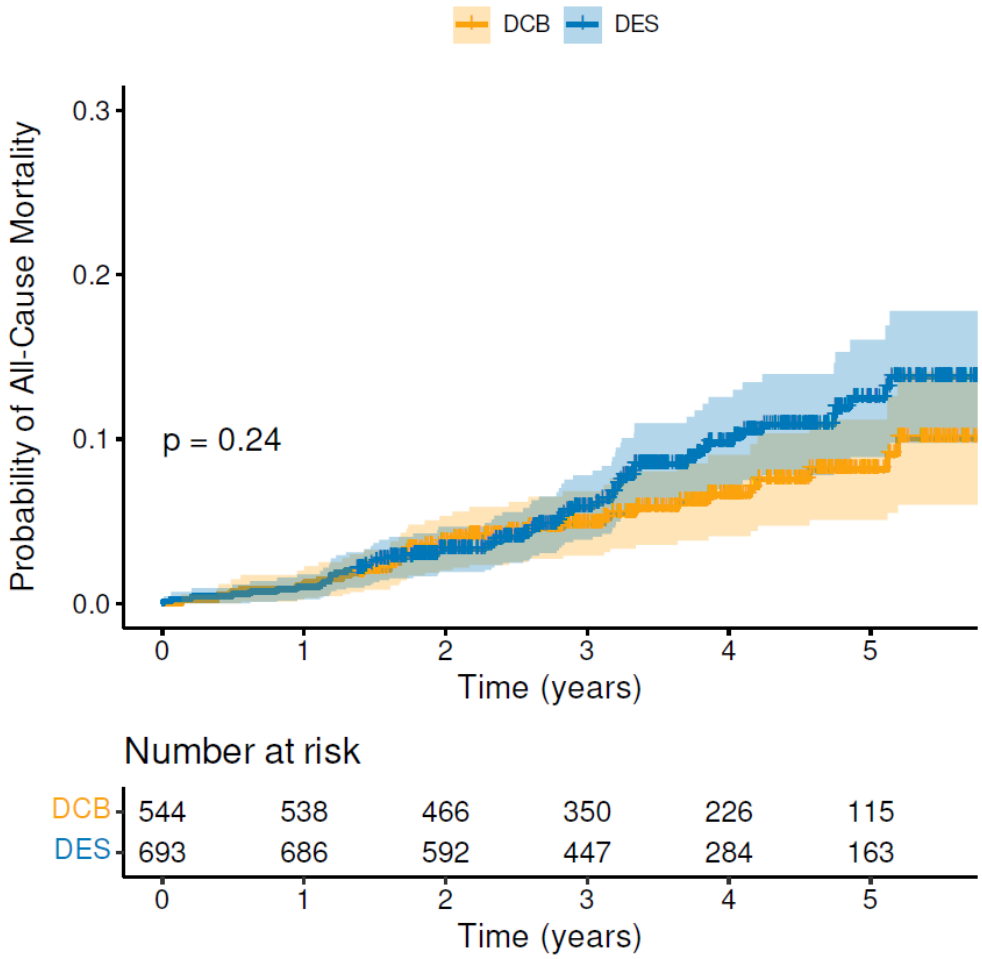


Fig 5.3: Cumulative hazard plot of all-cause mortality for DCB versus 2nd generation DES with numbers at risk shown below the graph. DCB: drug-coated balloon, DES: drug-eluting stent

Fig 5.4: Cumulative hazard plots for major cardiovascular endpoints

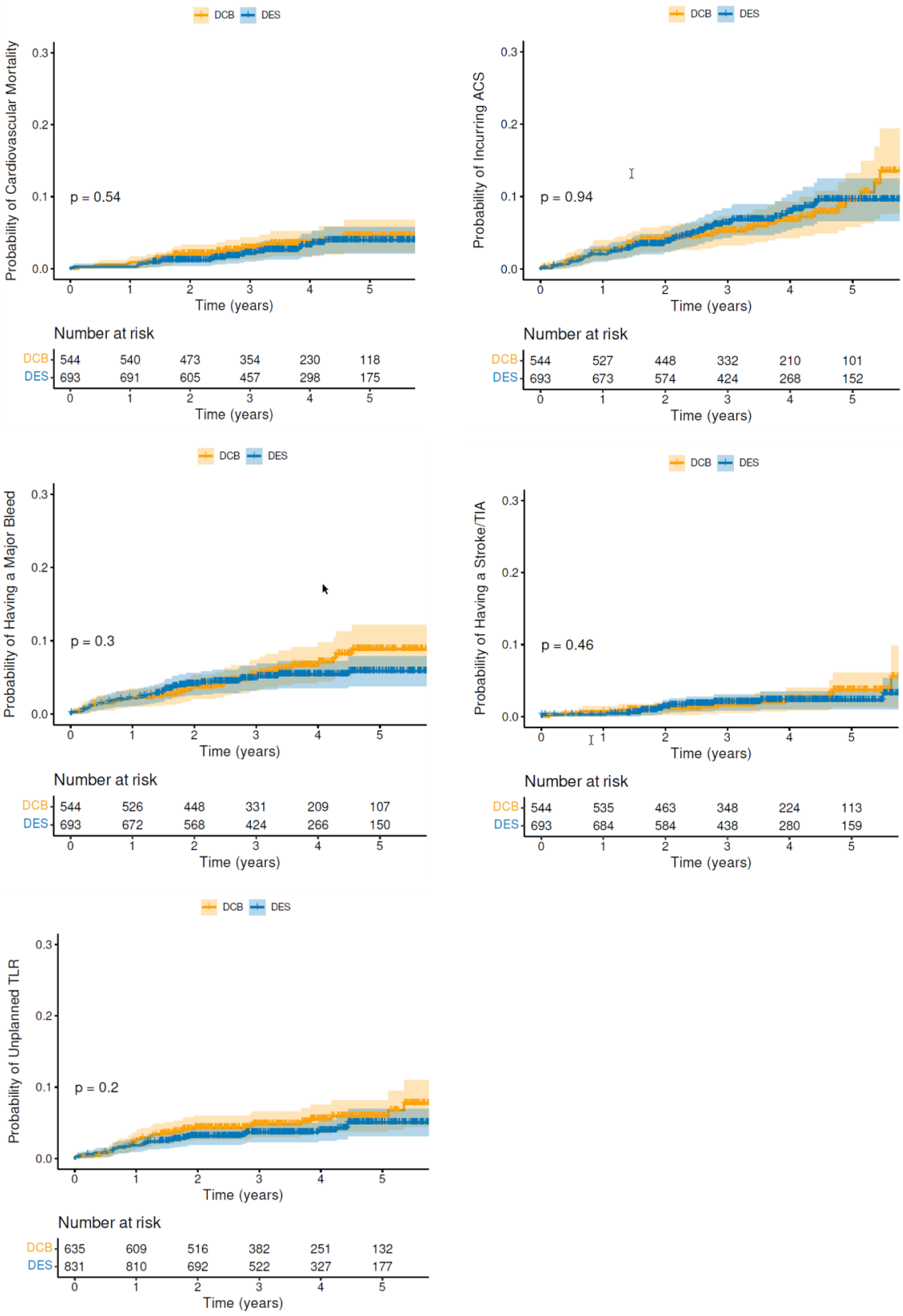


Fig 5.4: Cumulative hazard plots for major cardiovascular endpoints for DCB vs 2nd generation DES.

Fig 5.5: Cumulative hazard plot of all-cause mortality in propensity score matched cohort.

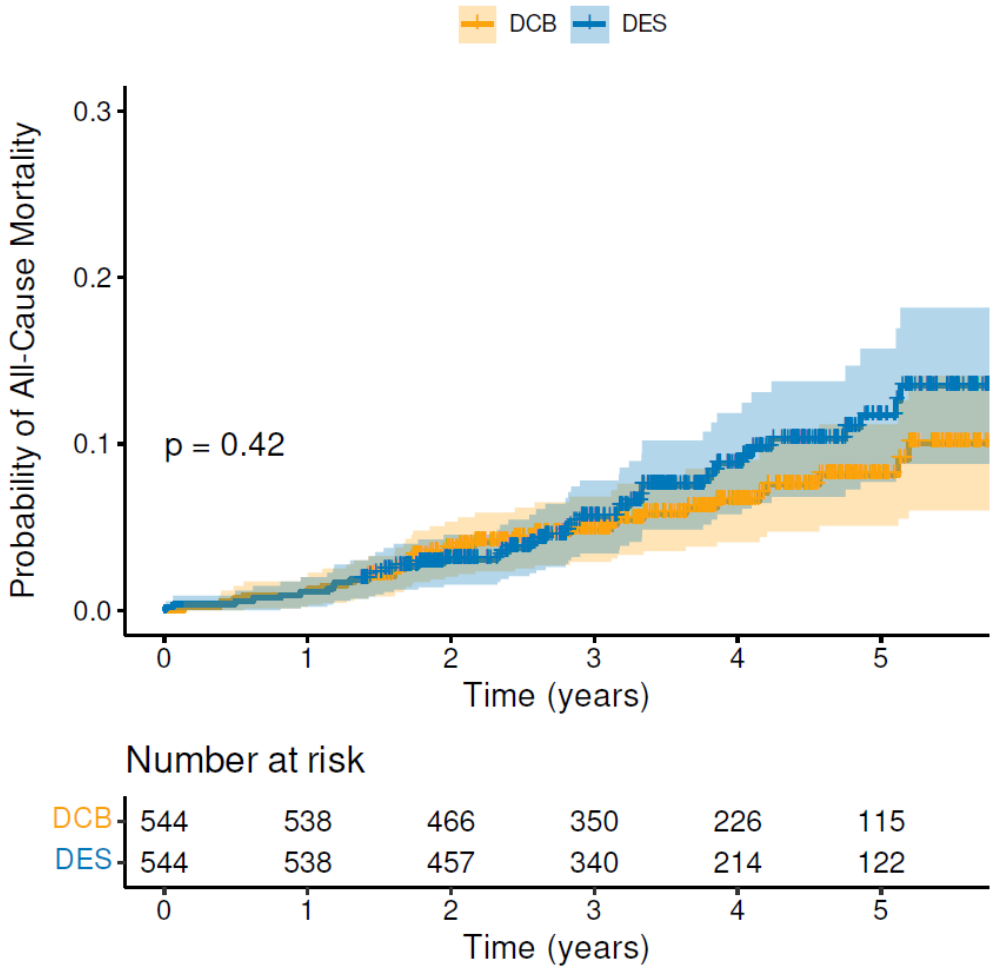


Fig 5.5: Cumulative hazard plot of all-cause mortality in propensity score matched cohort, for DCB vs 2nd generation DES with numbers at risk shown at the bottom of the graph. DCB: drug-coated balloon, DES: drug-eluting stent

Fig 5.6: Cumulative hazard plots for major cardiovascular endpoints in propensity score matched cohort.

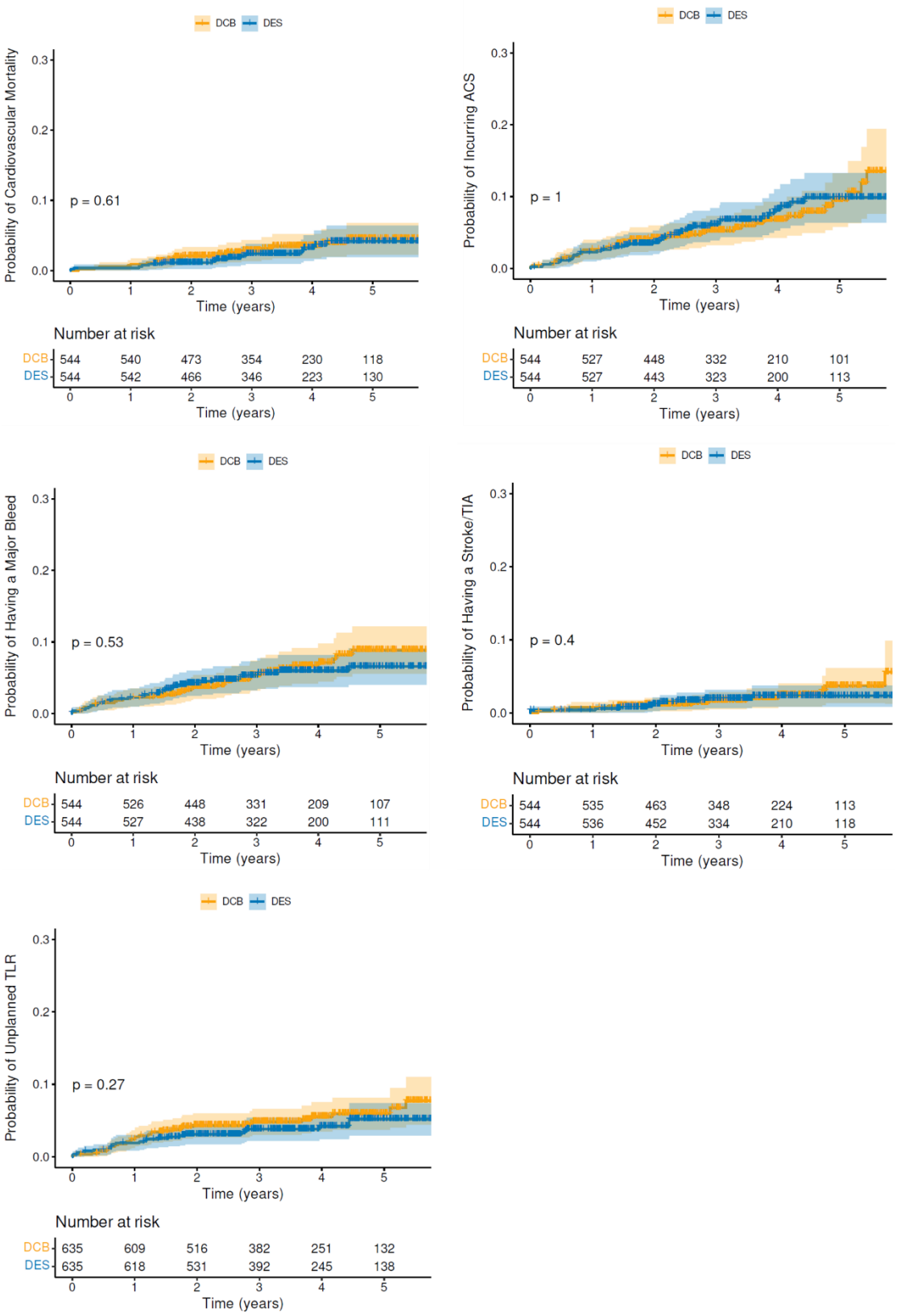


Fig 5.6: Cumulative hazard plots for major cardiovascular endpoints for DCB vs 2nd generation DES in propensity score matched cohort

Table 5.3: Univariable Cox regression analysis for all-cause mortality.

Mortality (Univariate)	**N**	**Forest Plot**	**HR (95% CI)**[1]	**p-value**
DCB/DES [DES]	1,237		1.28 (0.84 to 1.95)	0.24
Age	1,237		1.10 (1.07 to 1.12)	**<0.001**
Gender [Female]	1,237		1.56 (1.00 to 2.45)	0.050
Smoking Status [Current/Ex-Smoker]	1,230		1.26 (0.81 to 1.95)	0.31
Hypercholesterolaemia	1,237		0.44 (0.26 to 0.74)	**0.002**
Hypertension	1,237		1.42 (0.93 to 2.17)	0.11
Peripheral Vascular Disease	1,237		1.51 (0.66 to 3.45)	0.33
Cerebrovascular Event	1,237		1.22 (0.56 to 2.63)	0.62
Myocardial Infarction	1,237		1.32 (0.81 to 2.15)	0.26
PCI	1,237		1.29 (0.75 to 2.21)	0.35
CABG	1,237		2.02 (1.15 to 3.57)	**0.015**
Atrial Fibrillation	1,237		2.29 (1.31 to 3.98)	**0.003**
Heart Failure	1,237		3.98 (1.92 to 8.24)	**<0.001**
COPD	1,237		2.01 (0.97 to 4.14)	0.060
Diabetes Mellitus	1,237		1.58 (1.02 to 2.45)	**0.040**
Family History of CAD	1,237		0.60 (0.36 to 1.01)	0.055
eGFR	1,237		0.98 (0.97 to 0.99)	**<0.001**
Frailty Score	1,237		1.50 (1.36 to 1.65)	**<0.001**
PCI to Multiple Vessels	1,237		0.84 (0.42 to 1.66)	0.61
Bifurcation Disease	1,237		1.27 (0.68 to 2.39)	0.45
Average Vessel Diameter	1,231		0.91 (0.64 to 1.27)	0.57
Vessel Diameter ≥ 3mm	1,237		1.10 (0.65 to 1.83)	0.73

Table 5.3: Results of multivariable Cox regression analysis for all-cause mortality

PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting, COPD: chronic obstructive pulmonary disease, eGFR: estimated glomerular filtration rate, DES: drug eluting stent. [1] HR = Hazard Ratio, CI = Confidence Interval

Table 5.4: Multivariable Cox regression analysis for all-cause mortality.

All-Cause Mortality (Multivariate)	**N**	**HR (95% CI)**[1]	**p-value**
Age	1,237	1.07 (1.05 to 1.10)	**<0.001**
Hypercholesterolaemia	1,237	0.59 (0.35 to 1.02)	0.057
Coronary artery bypass graft	1,237	1.46 (0.82 to 2.58)	0.20
Atrial Fibrillation	1,237	1.24 (0.69 to 2.24)	0.47
Heart Failure	1,237	1.71 (0.77 to 3.80)	0.19
Diabetes mellitus	1,237	1.35 (0.86 to 2.12)	0.19
eGFR	1,237	1.00 (0.98 to 1.01)	0.38
Frailty Score	1,237	1.34 (1.21 to 1.49)	**<0.001**

Table 5.4: Results of multivariable Cox regression analysis for all-cause mortality

eGFR: estimated glomerular filtration rate.

Table 5.5: Baseline patient characteristics of propensity score matched cohort.

Characteristic	**DCB**, N = 544	**DES**, N = 544	**p-value**
Age, Median (IQR)	69 (61 – 75)	69 (61 – 75)	0.68[1]
Male, n (%)	429 (79)	436 (80)	0.60[2]
Current/Ex-smoker, n (%)	336 (62)	353 (65)	0.23[2]
Hypercholesterolaemia, n (%)	186 (34)	194 (36)	0.61[2]
Hypertension, n (%)	307 (56)	303 (56)	0.81[2]
Peripheral vascular disease, n (%)	24 (4.4)	22 (4.0)	0.76[2]
Cerebrovascular disease, n (%)	42 (7.7)	33 (6.1)	0.28[2]
Myocardial infarction, n (%)	93 (17)	84 (15)	0.46[2]

Percutaneous coronary intervention, n (%)	79 (15)	69 (13)	0.38[2]
Coronary artery bypass graft, n (%)	47 (8.6)	40 (7.4)	0.43[2]
Atrial fibrillation, n (%)	56 (10)	45 (8.3)	0.25[2]
Heart failure, n (%)	18 (3.3)	13 (2.4)	0.36[2]
Chronic obstructive pulmonary disease, n (%)	18 (3.3)	22 (4.0)	0.52[2]
Diabetes, n (%)	125 (23)	120 (22)	0.72[2]
Family history, n (%)	148 (27)	143 (26)	0.73[2]
eGFR, Median (IQR)	79 (66 – 91)	79 (68 – 91)	0.57[1]
Frailty, n (%)			>0.99[3]
Low	541 (99)	540 (99)	
Intermediate	3 (0.6)	4 (0.7)	
High	0 (0)	0 (0)	

Table 5.5: Baseline patient characteristics of propensity score matched cohort. DCB: drug coated balloon, DES: drug eluting stent, eGFR: estimated glomerular filtration rate, [1] Wilcoxon rank sum test[2] Pearson's Chi-squared test[3] Fisher's exact test[4] Wilcoxon rank sum exact test

Table 5.6: Univariable Cox regression analysis for all-cause mortality in patients with vessel ≥3mm.

Mortality (Univariate)	**N**	**HR (95% CI)**[1]	**p-value**
DCB/DES [DES]	992	1.18 (0.74 to 1.89)	0.49
Age	992	1.10 (1.07 to 1.14)	**<0.001**
Gender [Female]	992	1.91 (1.16 to 3.14)	**0.011**
Smoking Status	985	1.20 (0.73 to 1.96)	0.47
Hypercholesterolaemia	992	0.51 (0.29 to 0.89)	**0.017**
Hypertension	992	1.35 (0.85 to 2.16)	0.21
Peripheral Vascular Disease	992	1.83 (0.79 to 4.21)	0.16
Cerebrovascular Event	992	1.20 (0.52 to 2.75)	0.68
Myocardial Infarction	992	1.53 (0.91 to 2.57)	0.11
PCI	992	1.05 (0.54 to 2.05)	0.88
CABG	992	2.14 (1.18 to 3.89)	**0.013**
Atrial Fibrillation	992	2.74 (1.51 to 4.99)	**<0.001**
Heart Failure	992	3.97 (1.82 to 8.66)	**<0.001**
Angina Pectoris	992	1.12 (0.66 to 1.91)	0.67
COPD	992	1.83 (0.79 to 4.21)	0.16
Diabetes Mellitus	992	1.62 (0.99 to 2.65)	0.054
Family History of CAD	992	0.62 (0.35 to 1.08)	0.093
eGFR	992	0.97 (0.96 to 0.98)	**<0.001**
Frailty Score	992	1.51 (1.37 to 1.67)	**<0.001**
PCI to Multiple Vessels	992	0.84 (0.40 to 1.74)	0.63
Bifurcation Disease	992	1.39 (0.72 to 2.72)	0.33
Average Vessel Diameter	992	0.76 (0.47 to 1.22)	0.26

Table 5.6: Univariable Co regression analysis for all-cause mortality in patients with vessel ≥3mm.

DCB: Drug coated balloon, DES: drug eluting stent, PCI: percutaneous coronary intervention, CABG: coronary artery bypass graft, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, eGFR: estimated glomerular filtration rate

Table 5.7: Multivariable Cox regression analysis for all-cause mortality in patients with vessel ≥3mm.

All-Cause Mortality (Multivariate)	N	HR (95% CI)[1]	p-value
DCB/DES [DES]			
Age	992	1.08 (1.04 to 1.11)	<0.001
Gender [Female]	992	1.50 (0.90 to 2.50)	0.12
Hypercholesterolaemia	992	0.75 (0.42 to 1.32)	0.32
CABG	992	1.55 (0.84 to 2.84)	0.16
Atrial Fibrillation	992	1.44 (0.76 to 2.74)	0.26
Heart Failure	992	1.35 (0.56 to 3.26)	0.50
eGFR	992	0.99 (0.98 to 1.01)	0.26
Frailty Score	992	1.37 (1.22 to 1.54)	<0.001

[1] HR = Hazard Ratio, CI = Confidence Interval

Table 5.7: Multivariable Co regression analysis for all-cause mortality in patients with vessel ≥3mm.

DCB: drug coated balloon, DES: drug eluting stent, CABG: coronary artery bypass graft, eGFR: estimated glomerular filtration rate

5.4 Discussion

Drug coated balloon-only angioplasty is recommended in evidence-based guidelines for the treatment of in-stent restenosis and new indications are proposed in the recent International DCB Consensus Group recommendations (222) (216). The recent BASKET-SMALL2 trial has demonstrated safety and efficacy of DCB in small vessels up to 3 years follow up and opened up indications for DCB-only angioplasty in de novo coronary artery disease (218). Over the last few years, registry data have demonstrated the safety of DCB-only angioplasty in de novo coronary disease (220) (197) (223). However, the majority of these studies are limited by either long recruitment time or small numbers of patients treated with DCB-only compared to DES.

In addition, very few studies directly compare DCB with DES for stable angina in de novo large vessel disease. Therefore, it is still uncertain if DCB-only angioplasty could be part of routine clinical practice and compete safely with DES in the real world.

My study has demonstrated that DCB-only angioplasty is safe in patients with stable angina and de novo coronary artery disease as part of a routine, clinical practice. In our institution, over the last five years a comparable number of patients with first presentation of stable angina due to de novo coronary disease were treated with DCB-only strategy and DES-only strategy, while at the same time, the number of patients treated with both DCB and DES remained low. There was no evidence of increased all-cause mortality with DCB-only strategy compared with DES-only approach, after > 3.5 years follow-up (median). Furthermore, there was no evidence of a difference in any of the secondary endpoints (cardiovascular mortality, ACS, stroke/TIA, major bleeding or unplanned TLR). My results are consistent with previous registry data that have demonstrated the safety of DCB-only angioplasty and our previous study, SPARTAN DCB, which specifically showed no evidence of increased long-term mortality with DCB (220) (223). In addition, I have demonstrated that the DCB-only strategy can compete with the DES-only strategy safely in routine clinical practice for overall mortality and all major cardiovascular endpoints, including unplanned TLR.

I included large numbers of patients with stable angina due to de novo disease and no restriction in vessel size. Approximately 73% of patients in the DCB group and 86% in the DES group had at least one vessel ≥3mm treated, indicating that the great majority of patients had large vessels treated. When considering only patients with large vessel disease, the results were similar to those observed in the whole population, showing no difference in all-cause mortality between DCB and DES. These results are consistent with previous studies that have demonstrated the safety of DCB-only angioplasty for de novo disease in large vessels (197) (223) (224). A large proportion (49%) of the lesions treated with DCB had residual coronary

dissections, mainly grade B. Consistent with previous work from our group, the rate of acute vessel closure was very low, as only one patient had acute vessel closure within 24h (175).

Limitations

It is possible that the retrospective, non-randomised nature of my work from a single centre could introduce referral bias. However, our institution is a large tertiary referral centre that provides cardiac intervention to a population over one million and has the highest implantation of DCBs for coronary artery disease in the UK (213). Furthermore, I tried to ameliorate referral bias by including all consecutive patients fulfilling our criteria. Given that DCB-only angioplasty has a learning curve, as with most interventional techniques, these results might not be generalisable to smaller institutions with less experience in DCB-only angioplasty. In addition, it is vital to mention that even though my study is retrospective and non-randomised, the clinical database was completed prospectively, and the two groups were well balanced regarding patient characteristics. There were few differences only in terms of angiographic characteristics and recommended DAPT.

5.5 Conclusion

In conclusion, this is the first study to demonstrate that DCB-only angioplasty for stable angina due to de novo disease and predominantly large vessels, is safe compared to 2nd generation DES as part of routine clinical practice. I have demonstrated that routine DCB-only strategy in patients with stable angina due to de novo disease of all-vessel sizes has no increased all-cause mortality or any other major cardiovascular endpoints including unplanned TLR, compared to DES.

Chapter 6. Drug coated balloon only angioplasty in STEMI

This chapter is based on the study published by myself, Merinopoulos *et al*. on Journal of American College of Cardiology: Cardiovascular Interventions, titled 'Assessment of paclitaxel drug coated balloon only angioplasty in STEMI'.

6.1 Introduction

Primary percutaneous coronary intervention (pPCI) is the guideline recommended treatment strategy for patients with ST Elevation Myocardial Infarction (STEMI), with studies demonstrating improved patient outcomes including mortality compared to thrombolysis (225). Stents were initially developed to treat acute complications of balloon angioplasty, such as flow limiting dissections and acute vessel recoil. Since then, implantation of a drug eluting stent (DES) has emerged as the standard of care (225). Despite great advances in stent technology over the years and evolution of 2nd generation DES with significantly better patient outcomes and reduced stent-related events (226), stent-related complications such as stent thrombosis and in-stent restenosis have persisted. This in turn has stimulated the concept of 'leaving nothing behind' PCI (25). Drug coated balloons (DCB) combine the benefits of local drug delivery without the complications of a permanent stent implantation in cases where stenting was not mandated following the initial balloon angioplasty (1).

The safety and efficacy of DCB-only angioplasty has already been demonstrated in in-stent restenosis, small vessel disease and high-bleeding risk cohorts with emerging data in large vessels as well (29,189,214,216,222,227). However, only a few, predominantly small studies have evaluated the safety of DCB-only angioplasty in the setting of pPCI (29,228–230). The present study sought to assess the safety of DCB-only angioplasty in pPCI as compared with newer generation DES.

6.2 Methods

The full methodology has been described in chapter 2.4 (page 62). In brief, the assessment of paclitaxel drug coated balloon only angioplasty in STEMI was an investigator-initiated, single centre, retrospective, propensity matched, cohort study. Following ethical approval, I interrogated the NNUH clinical database for patients treated for STEMI. In NNUH, usage of DCB has steadily increased and usage of second-generation DES has steadily decreased over the last ten years as shown in Figure 6.1. From 2016 onwards more than 100 patients per year with first presentation of STEMI and de novo disease were treated with DCB-only angioplasty, representing at least 35% of all the STEMI patients. Hence, for the purposes of this study I considered patients from 2016 onwards, so that the two groups were more balanced in terms of frequency and follow up. I excluded patients with cardiac arrest, intubation or cardiogenic shock as their outcomes are determined mainly by the severity of the clinical presentation rather than the treatment strategy (Fig 6.2).

Fig 6.1: PCI activity in STEMI

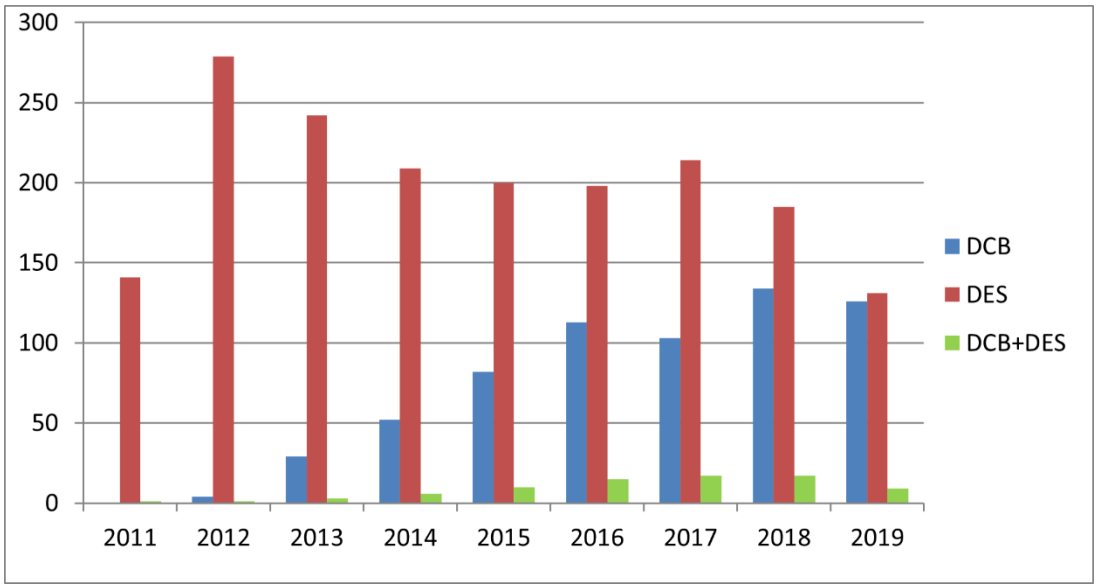


Fig 6.1: Yearly usage of DCB and DES in patients with first STEMI presentation due to de novo disease fulfilling inclusion criteria.

Fig 6.2: Consort diagram

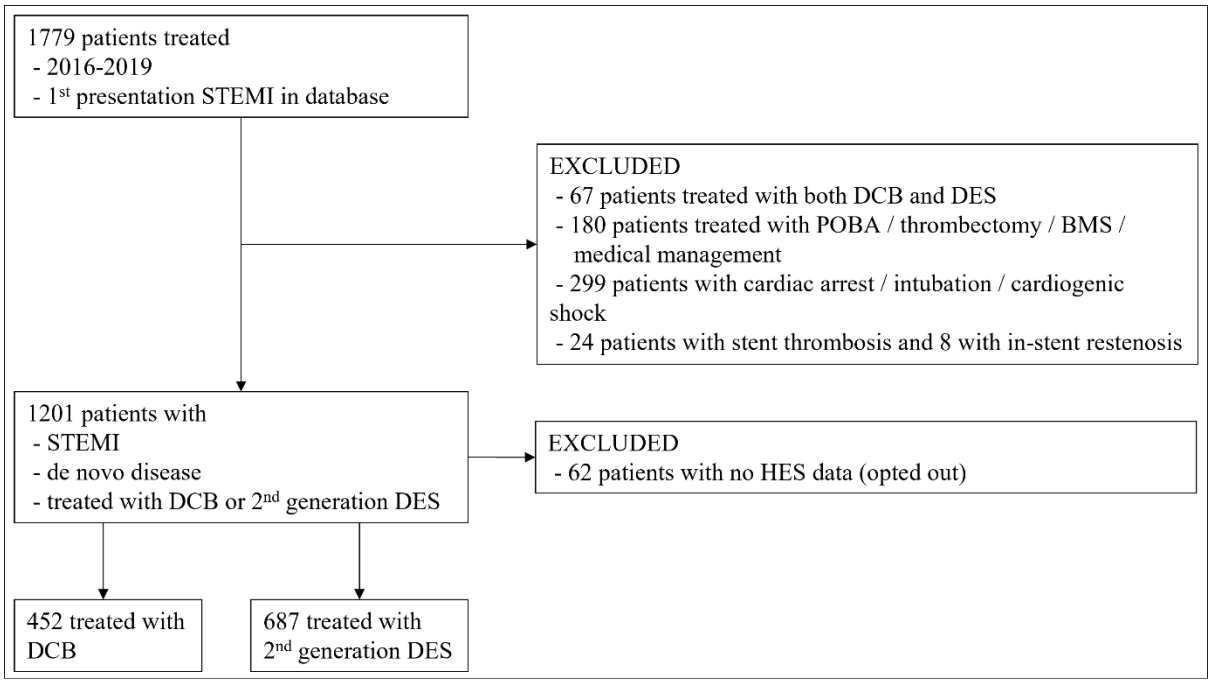


Fig 6.2: Consort diagram indicating how the final population included in the analysis was identified.

STEMI: ST segment elevation myocardial infarction, DCB: drug coated balloon, DES: drug eluting stent, POBA: plain old balloon angioplasty, BMS: bare metal stent

The primary endpoint was all-cause mortality. The secondary endpoints were cardiovascular mortality, acute coronary syndrome (ACS), stroke or transient ischaemic attack, major bleeding and target lesion revascularisation. All deaths were classified as cardiovascular or non-cardiovascular by three blinded adjudicators according to academic research consortium 2 consensus (171). I estimated the well validated 'acuity' score based on gender, age, serum creatinine, white blood cell count, anaemia, clinical presentation and antithrombotic medications (231).

I undertook statistical analysis in SPSS (version 25) and the analysis was verified by an independent data scientist in program R (version 3.6.0). Propensity score matching was done using the MatchIt package for R (v4.5), specifically utilising the optimal pair matching

algorithm (*https://kosukeimai.github.io/MatchIt/reference/method_optimal.html*) to achieve a 1:1 match. This algorithm was chosen over the typical nearest neighbour matching method due to better overall matching performance (by enabling less within-pair distance variation). Variables that were shown to be significant predictors of all-cause mortality in the univariate cox-regression models were used in the propensity score matching process. These were: age, hypertension, peripheral vascular disease, stroke, previous ACS, history of heart failure, atrial fibrillation, family history of coronary artery disease, chronic obstructive pulmonary disease, diabetes, glomerular filtration rate, LMS treatment, bifurcation disease, frailty score, heavy calcification and acuity score. The performance of the match was assessed by visually inspecting the dimensionally reduced jitter plot (Fig 6.3) and density curves of the variables.

Fig 6.3. Propensity score matching performance.

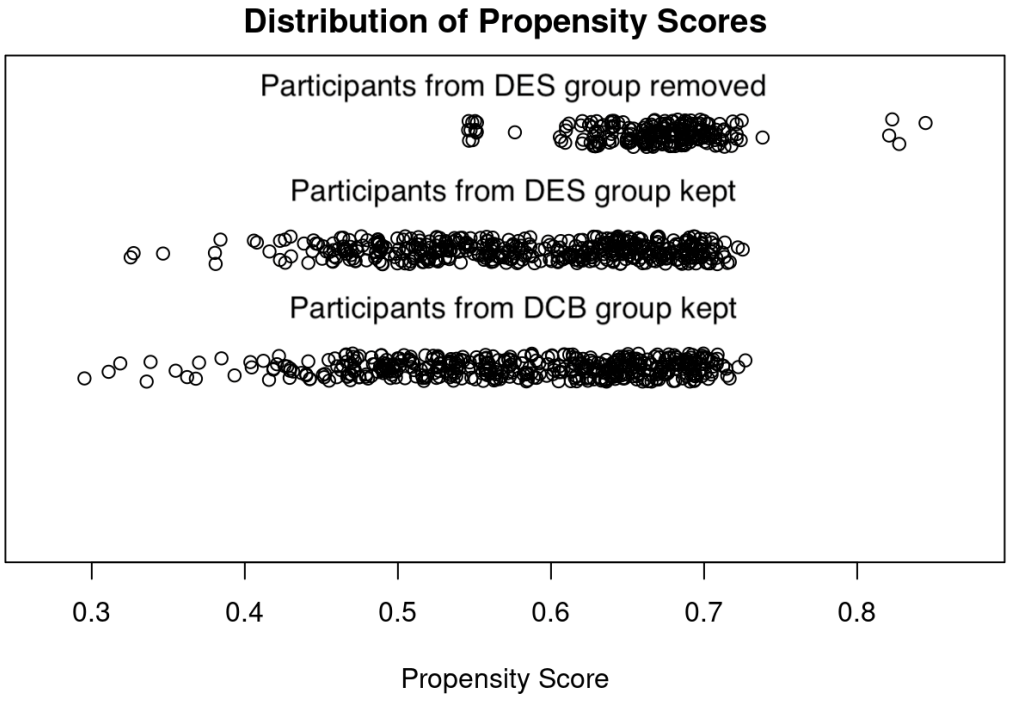


Fig 6.3: Visual performance of the propensity score showed that the matching achieved was extremely satisfactory.

6.3 Results

A total of 452 consecutive patients treated with paclitaxel DCB only and 687 consecutive patients treated with 2nd generation DES only were identified (Fig 6.2). There were 24 patients who required bailout stenting (21 for worsening dissection and 3 for worsening acute vessel recoil following DCB). These patients have not been included in the analysis as they were identified during the index procedure and received a DES – hence excluded. The mean age was 66 (±13) and 66 (±11) years old for the DCB and DES groups, respectively. Male patients accounted for 73% and 74% for the DCB and DES groups, respectively. The groups were well balanced in baseline patient characteristics as shown in table 6.1. There were very few differences; the DCB group had more patients with previous stroke and higher frailty index while the DES group had more patients with history of smoking.

Table 6.2 shows the angiographic characteristics of the target vessels treated. Overall, the groups were well balanced with very few differences. The DCB group had significantly more patients with bifurcation and true bifurcation disease treated. The DES group had a significantly larger median vessel diameter but both groups had median vessel diameter more than 3 mm.

Table 6.1. Baseline patient characteristics

Characteristic	**Overall**, N = 1,139[1]	**DCB**, N = 452	**DES**, N = 687	**p-value**
Age, Mean (SD)	66 (12)	66 (13)	66 (11)	0.97[2]
Sex, n (%)				0.69[4]
Male	839 (74)	330 (73)	509 (74)	
Female	300 (26)	122 (27)	178 (26)	
Hypercholesterolaemia, n (%)	179 (16)	79 (17)	100 (15)	0.18[4]
Hypertension, n (%)	424 (37)	183 (40)	241 (35)	0.065[4]
Peripheral vascular disease, n (%)	14 (1.2)	7 (1.5)	7 (1.0)	0.43[4]
Stroke, n (%)	30 (2.6)	18 (4.0)	12 (1.7)	**0.021**[4]
Previous myocardial infarction, n (%)	65 (5.7)	30 (6.6)	35 (5.1)	0.27[4]
Previous percutaneous coronary intervention, n (%)	54 (4.7)	25 (5.5)	29 (4.2)	0.31[4]
Previous coronary artery bypass graft, n (%)	11 (1.0)	5 (1.1)	6 (0.9)	0.76[3]
Heart failure, n (%)	4 (0.4)	2 (0.4)	2 (0.3)	0.65[3]
Atrial fibrillation, n (%)	91 (8.0)	37 (8.2)	54 (7.9)	0.84[4]
Family history of IHD, n (%)	114 (10)	44 (9.7)	70 (10)	0.80[4]
Chronic obstructive pulmonary disease, n (%)	62 (5.4)	27 (6.0)	35 (5.1)	0.52[4]
Diabetes, n (%)	146 (13)	63 (14)	83 (12)	0.36[4]
Smoking (current / previous), n (%)	689 (63)	254 (58)	435 (67)	**0.006**[4]
Estimated glomerular filtration rate, Mean (SD)	91 (26)	92 (27)	90 (25)	0.23[2]
Frailty Score, Median (IQR)	0.00 (0.00 – 0.70)	0.00 (0.00 – 0.80)	0.00 (0.00 – 0.50)	**0.034**[2]
Acuity score, Median (IQR)	17 (14 – 23)	17 (14 – 23)	18 (14 – 23)	0.87[2]

Characteristic	**Overall**, N = 1,139[1]	**DCB**, N = 452	**DES**, N = 687	**p-value**
Discharge medications				
Aspirin, n (%)	1,100 (97.5)	421 (94.4)	679 (99.6)	**<0.001**[4]
Second antiplatelet, n (%)	1,125 (99.7)	446 (100)	679 (99.6)	0.16[4]
DAPT duration (Mean, SD)	347 (77)	348 (78)	346 (77)	0.73[2]
Anticoagulation	54 (4.8)	23 (5.2)	31 (4.5)	0.64[4]
Beta blocker, n (%)	1,051 (93.2)	414 (92.8)	637 (93.4)	0.71[4]
ACE inhibitor / ARB, n (%)	1,053 (93.4)	415 (93.0)	638 (93.5)	0.74[4]
Statin, n (%)	1,104 (97.9)	437 (98.0)	667 (97.8)	0.84[4]
Aldosterone antagonist, n (%)	155 (13.7)	60 (13.5)	95 (13.9)	0.82[4]

[1] Median (IQR); Range; n (%); Mean (SD)

[2] Wilcoxon rank sum test

[3] Fisher's exact test

[4] Pearson's Chi-squared test

Table 6.1. Baseline patient characteristics of patients treated with DCB or DES. Bold characters indicate significant result.

DCB: drug coated balloon, DES: drug eluting stent, eGFR: estimated glomerular filtration rate

Table 6.2. Angiographic characteristics of target vessels.

Characteristic	**Overall**, N = 1,139[1]	**DCB**, N = 452	**DES**, N = 687	**p-value**
Vessel treated, n (%)				0.075[3]
LMS	7 (0.6)	2 (0.4)	5 (0.7)	
LAD	452 (40)	196 (43)	256 (37)	
LCx	181 (16)	78 (17)	103 (15)	
RCA	496 (44)	175 (39)	321 (47)	
Graft	3 (0.3)	1 (0.2)	2 (0.3)	
LMS treated, n (%)	7 (0.6)	2 (0.4)	5 (0.7)	0.71[3]
LMS/LAD treated, n (%)	459 (40)	198 (44)	261 (38)	0.050[4]
Multivessel PCI, n (%)	43 (3.8)	17 (3.8)	26 (3.8)	0.98[4]
Bifurcation, n (%)	386 (34)	188 (42)	198 (29)	**<0.001**[4]
True bifurcation, n (%)	88 (7.7)	50 (11)	38 (5.5)	**<0.001**[4]
Heavy calcification, n (%)	159 (14)	67 (15)	92 (13)	0.50[4]
Vessel diameter, Median (IQR)	3.50 (3.00 – 4.00)	3.50 (3.00 – 3.50)	3.50 (3.00 – 4.00)	**<0.001**[2]
Lesion length, Median (IQR)	25 (20 – 32)	25 (20 – 30)	24 (18 – 32)	0.93[2]
Culprit Vessel ≥3mm, n (%)	958 (85)	363 (81)	595 (87)	**0.008**[4]
TIMI flow pre-PCI, n (%)				0.28[4]
0/1	852 (75)	330 (73)	522 (76)	
2/3	286 (25)	121 (27)	165 (24)	
TIMI flow post-PCI, n (%)				0.75[3]
0/1	9 (0.8)	4 (0.9)	5 (0.7)	
2/3	1,130 (99)	448 (99)	682 (99)	
Coronary Dissection, n (%)				>0.99[3]
No angiographic dissection	317 (70)	317 (70)	0 (NA)	
Type A	72 (16)	72 (16)	0 (NA)	
Type B	62 (14)	62 (14)	0 (NA)	

Characteristic	**Overall**, N = 1,139[1]	**DCB**, N = 452	**DES**, N = 687	**p-value**
Bifurcation treatment strategy, n (%)				**<0.001**[3]
DCB MB Only	148 (38)	148 (79)	0 (0)	
DCB SB Only	20 (5.2)	20 (11)	0 (0)	
DCB MB & SB	17 (4.4)	17 (9.1)	0 (0)	
DCB MB & POBA SB	2 (0.5)	2 (1.1)	0 (0)	
DES MB	169 (44)	0 (0)	169 (85)	
DES MB & SB	8 (2.1)	0 (0)	8 (4.0)	
DES MB & POBA SB	6 (1.6)	0 (0)	6 (3.0)	
DES SB Only	15 (3.9)	0 (0)	15 (7.6)	

[1] Median (IQR); Range; n (%); Mean (SD)

[2] Wilcoxon rank sum test

[3] Fisher's exact test

[4] Pearson's Chi-squared test

Table 6.2. Angiographic characteristics of target vessels treated with DCB or DES.
DCB: drug coated balloon, DES: drug eluting stent, LMS: left main stem, LAD: left anterior descending, Cx: left circumflex, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction, bold characters indicate significant result

The median follow-up for the DCB group was 2.9 years (interquartile range: 2 – 4.2) while for the DES group it was 3.4 years (IQR: 2.3 – 4.3) ($p<0.001$). The incidence of death was 49/452 (10.8%) in the DCB group and 62/687 (9%) in the DES group (hazard ratio (HR)=0.77; CI: 0.53-1.12; p=0.18). Kaplan Meier estimator plot showed that there was no significant difference in all-cause mortality associated with paclitaxel DCB compared to DES (Fig 6.4). Furthermore, there were no significant differences in any of the secondary endpoints, cardiovascular mortality, ACS, stroke, major bleeding or unplanned TLR (Fig 6.5). The median length of hospitalisation post-pPCI was 2.22 days (IQR: 1.63, 2.87) for the DCB group and

2.19 days (IQR: 1.57, 2.69) for the DES group. There were six in-hospital deaths (1.3%) in the DCB group and five in the DES group (0.7%). The difference was not statistically significant (p=0.56). There were no planned or unplanned in-hospital TLR in the DCB group, while there were five unplanned in-hospital TLR in the DES group. Three patients had acute stent thrombosis while another two patients had ongoing chest pain requiring stent optimisation. Furthermore, there was no difference in all-cause mortality or unplanned TLR within 30 days. The 30-day mortality was 2% vs 1.5% (p=0.49) while the 30-day unplanned TLR was 0.2% vs 0.7% (p=0.41) for the DCB and DES group respectively. Analysis of net adverse cardiac events at the short term after propensity score matching, did not show any significant differences between DCB and DES at 30 days or 1 year.

Univariable Cox regression analysis (table 6.3) identified the following adverse prognostic factors for all-cause mortality: increasing age, hypertension, peripheral vascular disease, stroke, previous myocardial infarction, coronary artery bypass graft (CABG), heart failure, atrial fibrillation (AF), chronic obstructive pulmonary disease (COPD), diabetes, decreasing estimated glomerular filtration rate (eGFR), frailty, vessel treated and true bifurcation. On multivariable Cox regression analysis (table 6.4), only age, history of heart failure, frailty and family history of IHD remained independent predictors of mortality.

Propensity score matched analysis for all positive variables in univariable Cox regression analysis demonstrated no difference in mortality between DCB and DES (Fig 6.6). There were no significant differences in any of the net adverse cardiac events including unplanned TLR (Fig 6.7). Multivariable Cox regression analysis for the propensity matched population identified frailty score, acuity score, history of heart failure and family history of IHD as independent predictors of mortality (Table 6.5).

Subgroup analysis according to vessel size (more or less than 3mm) or bifurcation disease demonstrated that the results were consistent in these subgroups.

Fig 6.4: Kaplan Meier estimator plot of all-cause mortality.

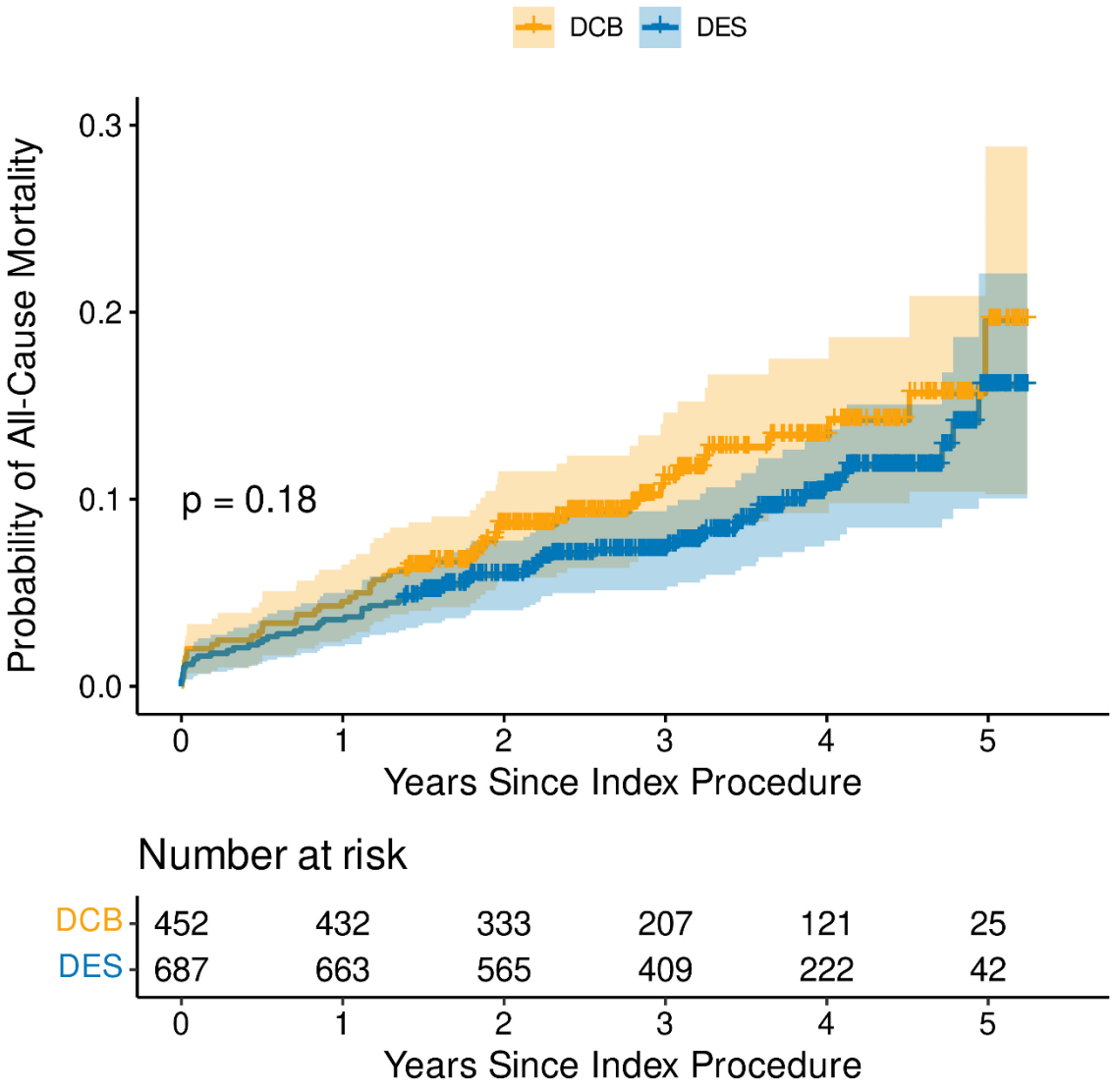


Fig 6.4: Kaplan Meier estimator plot of all-cause mortality for DCB vs 2nd generation DES with numbers at risk shown below the graph. DCB: drug coated balloon, DES: drug eluting stent

Fig 6.5: Kaplan Meier for net adverse cardiac events in full cohort

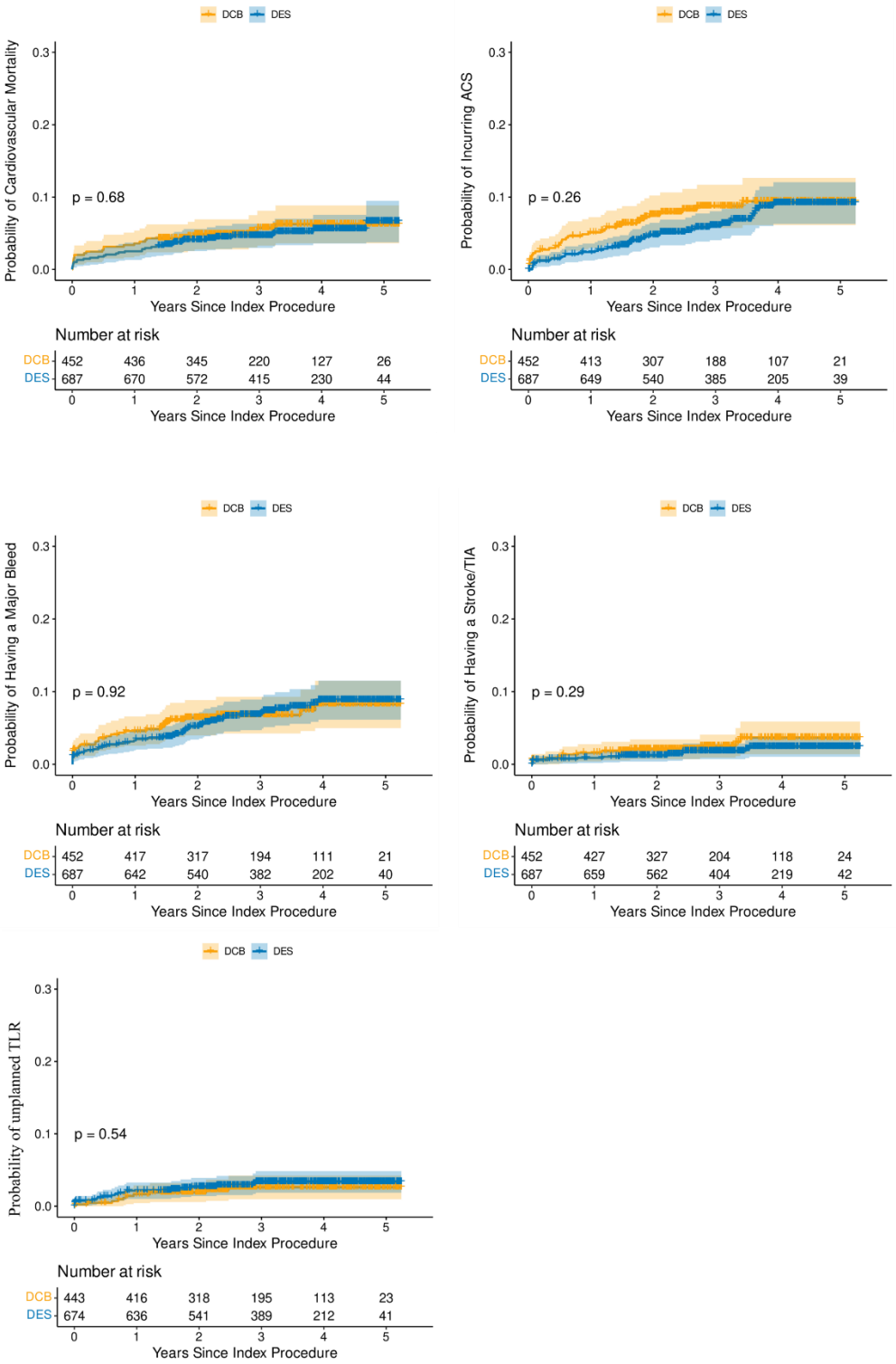


Fig 6.5: Kaplan Meier estimator plot showing net cardiac events for DCB vs 2nd generation DES in full cohort. DCB: drug coated balloon, DES: drug eluting stent, TIA: transient ischaemic attack, TLR: target lesion revascularisation

Table 6.3. Univariable Cox regression analysis.

Mortality (Univariate)	**N**	**HR (95% CI)**[1]	**p-value**
DES		0.78 (0.53 to 1.13)	0.18
Age (per year)	1,139	1.09 (1.07 to 1.11)	**<0.001**
Female		1.29 (0.87 to 1.93)	0.21
Hypercholesterolaemia	1,139	0.75 (0.43 to 1.32)	0.32
Hypertension	1,139	1.50 (1.03 to 2.18)	**0.033**
Peripheral vascular disease	1,139	5.84 (2.56 to 13.3)	**<0.001**
Stroke	1,139	3.98 (2.01 to 7.88)	**<0.001**
Previous Myocardial infarction	1,139	2.49 (1.42 to 4.36)	**0.001**
PCI	1,139	1.40 (0.65 to 3.01)	0.39
CABG	1,139	4.95 (2.02 to 12.1)	**<0.001**
Heart failure	1,139	31.6 (11.4 to 87.4)	**<0.001**
Atrial fibrillation	1,139	2.46 (1.48 to 4.08)	**<0.001**
Family history	1,139	0.37 (0.15 to 0.90)	**0.029**
COPD	1,139	3.57 (2.13 to 5.98)	**<0.001**
Diabetes	1,139	2.13 (1.38 to 3.31)	**<0.001**
Current / ex-smoker	1,090	0.98 (0.66 to 1.45)	0.90
eGFR (per ml/min/1.73m^2)	1,138	0.97 (0.96 to 0.98)	**<0.001**
Frailty	1,139	1.24 (1.18 to 1.30)	**<0.001**
Acuity score	1,102	1.12 (1.09 to 1.15)	**<0.001**
LMS treated	1,139	8.88 (3.62 to 21.8)	**<0.001**
Multivessel PCI	1,139	1.00 (0.37 to 2.73)	>0.99
Vessel diameter (per mm)	1,133	0.93 (0.68 to 1.27)	0.64
Lesion length (per mm)	1,133	1.01 (0.99 to 1.02)	0.38
Vessel ≥3mm	1,133	0.85 (0.52 to 1.39)	0.52
TIMI flow post-PCI	1,139	0.91 (0.13 to 6.55)	0.93
Bifurcation	1,139	1.39 (0.95 to 2.03)	0.088

Mortality (Univariate)	**N**	**HR (95% CI)**[1]	**p-value**
True bifurcation	1,139	2.69 (1.64 to 4.42)	**<0.001**
Heavy calcification	1,135	2.50 (1.66 to 3.78)	**<0.001**

[1] HR = Hazard Ratio, CI = Confidence Interval

Table 6.3. Results of univariable Cox regression analysis for all-cause mortality.
PCI: percutaneous coronary intervention, CABG: coronary artery bypass graft, COPD: chronic obstructive pulmonary disease, eGFR: estimated glomerular filtration rate, TIMI: thrombolysis in myocardial infarction. Bold characters significant result

Table 6. 4. Multivariable Cox regression analysis.

All-Cause Mortality (Multivariate)	**N**	**HR (95% CI)**[1]	**p-value**
DCB/DES [DES]	1,138	0.90 (0.80 to 1.02)	0.11
Age (per year)	1,138	1.01 (1.00 to 1.01)	**0.020**
History of stroke	1,138	1.36 (0.93 to 1.98)	0.11
History of Heart Failure	1,138	11.6 (4.29 to 31.5)	**<0.001**
Family History of Coronary Artery Disease	1,138	0.66 (0.53 to 0.80)	**<0.001**
Estimated glomerular filtration rate (per ml/min/1.73m^2)	1,138	1.00 (1.00 to 1.00)	0.13
Frailty Score	1,138	1.06 (1.02 to 1.10)	**0.001**

[1] HR = Hazard Ratio, CI = Confidence Interval

Table 6.4. Results of multivariable Cox regression analysis.
DCB: drug coated balloon, DES: drug eluting stent, bold characters indicate significant result

Fig 6.6: Kaplan Meier estimator plot in propensity score matched groups.

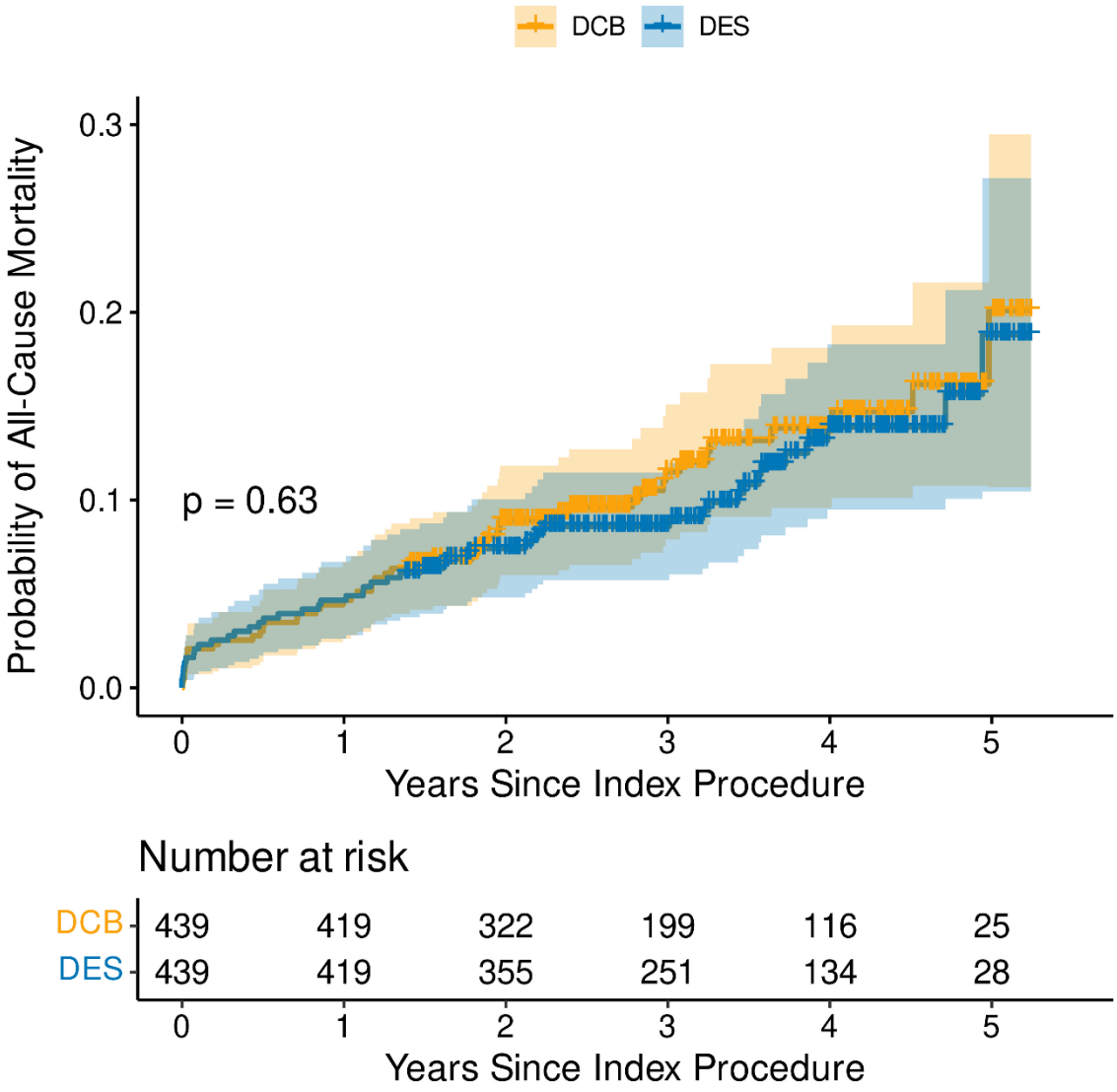


Fig 6.6: Kaplan Meier estimator plot in propensity score matched groups showing no difference in mortality between DCB and DES.

DCB: drug coated balloon, DES: drug eluting stent

Fig 6.7: Kaplan Meier plots for net adverse cardiac events in matched cohort.

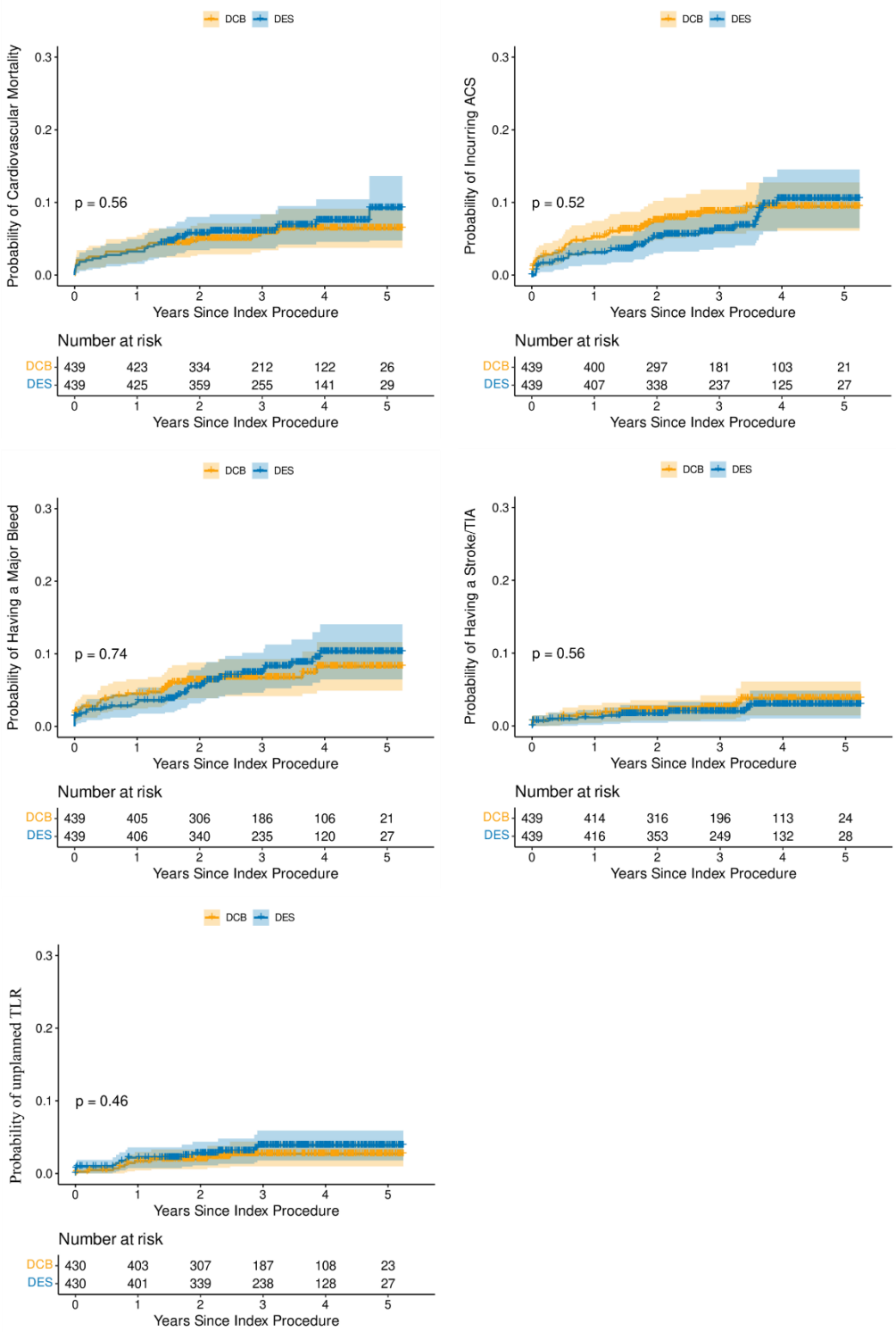


Fig 6.7: Kaplan Meier estimator plot showing net cardiac events for matched cohort for DCB versus 2nd generation DES. DCB drug coated balloon, DES: drug eluting stent, TIA; transient ischaemic attack, TLR: target lesion revascularisation

Table 6.5. Multivariable Cox regression analysis for propensity matched population.

All-Cause Mortality (Multivariate)	N	HR (95% CI)[1]	p-value
DCB/DES	878	0.89 (0.78 to 1.01)	0.080
History of Heart Failure	878	8.94 (3.28 to 24.4)	**<0.001**
Family History of Coronary Artery Disease	878	0.68 (0.53 to 0.86)	**0.002**
Bifurcations	878	1.14 (0.99 to 1.30)	0.067
Frailty Score	878	1.06 (1.02 to 1.10)	**<0.001**
Acuity Score	878	1.01 (1.00 to 1.02)	**0.031**

[1] HR = Hazard Ratio, CI = Confidence Interval

PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DES: drug eluting stent * indicates significant result

6.4 Discussion

This is the largest cohort analysis assessing the safety of DCB-only angioplasty compared with 2nd generation DES for pPCI showing no difference in all-cause mortality between DCB and DES for STEMI and no difference in the propensity score-matched analysis. Furthermore, there was no difference in any of the net adverse cardiac events including unplanned TLR.

pPCI has significantly improved the outcomes including survival of patients presenting with STEMI (232,233). Even though pPCI with 2nd generation DES has emerged as the standard of care, stent related events have persisted despite great advances in stent technology (222,234,235) DCB-only angioplasty, an emerging treatment strategy providing local drug delivery to prevent restenosis without the placement of a permanent stent, has not been fully evaluated compared to 2nd generation DES. The recent REVELATION trial demonstrated safety and efficacy of DCB compared to DES for STEMI in terms of fractional flow reserve (236). In addition, there are only few, small studies that have assessed safety of DCB compared to 2nd generation DES, demonstrating that DCB-only strategy is feasible in STEMI

(229,230,237). This is the largest analysis reporting on the most relevant, hard endpoint of all-cause mortality and also all net adverse cardiac events including unplanned TLR.

I have demonstrated that DCB-only angioplasty is safe in patients with STEMI and de novo disease compared to 2nd generation DES. In our institution, over the last six years a comparable number of patients with first presentation of STEMI due to de novo coronary artery disease were treated with DCB-only strategy and DES-only strategy, while at the same time, the number of patients treated with both DCB and DES remained low. In the short term, DCB-only angioplasty appears safe with no difference in in-hospital outcomes and reassuringly no cases of acute vessel closure. Furthermore, there was no difference in mortality or any of the net adverse cardiac events including unplanned TLR, after >3 years (median) follow up. These results were similar following propensity score matched analysis and consistent with previous smaller studies (229,230,237). Subgroup analysis demonstrated consistent results in both small or large vessels and in bifurcation disease. Further studies will need to verify these results in such subgroups. Given the equipoise of our results, it is important to note that in the context of STEMI, a DCB-only strategy may provide an advantage in cases of a) uncertain vessel size resulting in inadequate stent apposition b) uncertainty about antiplatelet compliance or bleeding risk and c) may simplify treatment of complex bifurcation lesions (216). In our institution, all interventional cardiologists have become very experienced in DCB-only angioplasty and use it when they feel this will provide a very good result. Following optimal lesion preparation, DCB is considered if there is no more than type B dissection and no more than 30% vessel recoil (assessed visually). (216).

We included a large number of consecutive patients with STEMI due to de novo disease and no restriction in vessel size. More than 80% of the culprit vessels in both groups had diameter ≥ 3mm, indicating that the great majority of patients treated had large vessel disease. The median vessel diameter was 3.5mm in both groups. Furthermore, the groups were well

balanced in terms of baseline patient characteristics. The only differences were that the DCB group had more patients with previous stroke while the DES group had more patients with history of smoking. In terms of angiographic characteristics, the DCB group had more patients with bifurcations and true bifurcations treated indicating that complex disease was treated, and possibly reflects operator bias towards a DCB-only approach in bifurcations.

Limitations

The retrospective, non-randomised nature of our cohort from a single centre is a possible source of bias. However, our institution provides cardiac intervention as a large tertiary centre to a population in excess of 1.5 million people for pPCI, and has one of the highest implantation of DCBs for coronary artery disease in the UK (213). In addition, we included all consecutive patients fulfilling the inclusion criteria ameliorating referral bias. DCB-only angioplasty, as most interventional techniques, is accompanied by a learning curve; therefore, our results might not be generalizable to smaller institutions with less clinical experience in DCB-only strategy. The decision to use DCB or DES was at the discretion of the treating interventional cardiologists who used what they felt would provide best result for the patient. Therefore, as this was not a randomised study, it is a limitation.

Our data did not allow us to use the ARC-HBR criteria which were published a few years after the start of our cohort (238). However, we have calculated and run the analysis using the 'Acuity' score. Finally, even though this study is retrospective and non-randomised, our clinical database was completed prospectively and the high-rate of DCB implantation in our institution resulted in groups well-balanced in terms or patient and angiographic characteristics. Lastly, despite our efforts we were not able to review the follow up angiograms of 9 (1.9%) patients in the DCB group and 13 (1.9%) patients in the DES group who have had re-PCI elsewhere.

6.5 Conclusion

In conclusion, this is the largest cohort analysis comparing DCB-only angioplasty to 2nd generation DES in STEMI reporting on all-cause mortality and all net adverse cardiac events including unplanned TLR. Using propensity matching, I have demonstrated that DCB-only angioplasty is safe with no difference in mortality or any of net adverse cardiac events including unplanned TLR, compared to DES in STEMI.

Chapter 7. Inflammatory response after elective percutaneous coronary intervention

This chapter is based on the study by myself, Merinopoulos et al. submitted for publication in Heart titled 'Circulating intermediate monocytes CD14++CD16+ are increased after elective percutaneous coronary intervention'.

7.1 Introduction

It is well established that inflammation plays a central role in the pathogenesis of atherosclerosis but also in the sequelae of percutaneous coronary intervention (PCI) (31,32). It is increasingly recognised that periprocedural inflammation is associated with worse adverse cardiovascular events (239). Various inflammatory biomarkers and mediators elicited following PCI, such as C-reactive protein (CRP), pentraxin-3 (PTX3), interleukins (IL), tumour necrosis factor α (TNFα) and leucocytes, have been shown to be associated with worse patient outcomes (36). Anti-inflammatory and immunomodulatory medications are now being trialled to improve prognosis with encouraging results (240,241). Monocytes play a crucial role in all stages of atherogenesis, from the initial formation of atherosclerotic plaques to the acute inflammatory phase following plaque destabilisation and finally during myocardial healing and remodelling following myocardial infarction (78). The relationship of circulating monocytes with in-stent neointimal hyperplasia after bare metal stent implantation was first demonstrated almost 20 years ago (81).

Over the last decade, the nomenclature of distinct monocyte subtypes has been standardised into classical CD14++CD16- monocytes, intermediate CD14++CD16+ monocytes and non-classical CD14+CD16++ monocytes (242). The classical monocytes are the most abundant subset both in blood (about 80-85% of circulating blood monocytes) but also in atherosclerotic

plaques. They express CCR2, CD62L and CD64 and are considered inflammatory mediators (78). The non-classical monocytes express high levels of CX3CR1 but not CCR2 or CD62L, have patrolling properties and also have important role in angiogenesis (78). The most recently described intermediate monocytes can be differentiated from non-classical monocytes as they express CCR2. They are the main producer of reactive oxygen species while the receptors they express indicate their pro-atherosclerotic capabilities (78,79). As the classification of the subsets of monocytes was standardised only in 2010, it is difficult to draw definitive conclusions about the role of various monocytes subsets from older studies.

Linked to inflammation, stent characteristics represent another important mechanism involved in adverse reaction to stents (35). Foreign body reactions to the metal platform as well as hypersensitivity reactions to the polymer contribute to the inflammation elicited after PCI and have been associated with adverse cardiac events (35). Previous work has demonstrated that the inflammatory reaction in terms of platelet and neutrophil activation is less after balloon angioplasty compared to bare metal stent implantation (243). Drug coated balloon (DCB) is an emergent technology allowing drug delivery to the vessel wall without implantation of a permanent scaffold (1). There is currently no data comparing 2nd generation drug eluting stents (DES) with DCB in terms of the elicited inflammation.

In the present study, we aimed to investigate the cellular as well as the humoral inflammatory response following PCI in the modern era. We assessed the effect of PCI in the acute and short-term on serially measured monocyte subsets and humoral mediators of inflammation. We included patients treated with 2nd generation DES or DCB aiming to compare these PCI strategies in terms of their elicited inflammatory response.

7.2 Methods

Study population

In this prospective study we recruited patients undergoing elective PCI for de novo coronary artery disease, either with DES or DCB utilised at the discretion of the operator. Adult patients (>18 years old) with stable angina were included in the study. We excluded patients with significant renal impairment (estimated glomerular filtration rate <30mL/min/ 1.73m^2) or any significant inflammatory condition on immunosuppression as well as pregnant women. This was an exploratory, pilot study, therefore no formal study sample size was estimated. Patients treated with DCB should not have more than type B dissection or >30% recoil as per study protocol (in accordance with international consensus (24)). All patients provided written, informed consent prior to being recruited in the study. The study is compliant with the Declaration of Helsinki with regards to an investigation in humans and it was approved by the East of England – Cambridge Central Research Ethics Committee (REC: 19/EE/0075). Patients were identified from outpatient clinics and also the elective PCI waiting list of Norfolk & Norwich University Hospital. Baseline clinical characteristics were collected from all patients.

Blood sampling and processing

Patients underwent blood tests at baseline (pre-PCI), four hours, two weeks and two months later. The baseline blood tests were taken from the radial artery sheath prior to intervention and subsequent blood tests were taken by venepuncture. Blood collected from patients in a 5ml serum separator tube (SST) to yield serum aliquots, a 6ml lithium heparin tube to yield plasma aliquots and two 4ml ethylenediaminetetraacetic acid (EDTA) for cellular analysis. The samples were processed once the blood had clotted in the SST tube, within two hours of blood collection and subsequently stored at -80^{0}C until biomarker analysis at the end of the study.

Cytometric analysis

Human peripheral blood mononuclear cells were isolated and fixed initially until cell staining and flow cytometry within two weeks from blood collection. The antibodies used in this study were FITC anti-human CD14 and APC anti-human CD16 both from Biolegend. Flow cytometry was performed using the CytoFLEX Flow Cytometer (Beckman Coulter, Brea California, United States) and analysis was carried out using FlowJo version 10 software.

Reverse transcriptase quantitative polymerase chain reaction

CD14 magnetic beads were used to isolate CD14+ leucocytes. Ribonucleic acid (RNA) was isolated from CD14+ leucocytes and quantified. Complimentary deoxyribonucleic acid (cDNA) was synthesized, and quantitative polymerase chain reaction (qPCR) was performed for IL-10, CCL2, CXCR4, TNFα, TREM1, PTX3, CD36, IL-18 and IL-1B at baseline, two weeks and two months post PCI. Cp values were estimated for each sample (values >35 were disregarded as non-specific) and converted to values expressing the fold change from baseline according to the delta-delta method, standardised for a housekeeper gene. Each reaction was repeated three times and an average of the three values was calculated. Real time quantitative PCR was performed on a Roche Lightcycler 480 (Roche, Basel, Switzerland) using SYBR-green technology (PCR biosystems, UK). Table 7.1 shows the sequences of the primers used.

Biomarker analysis

Biomarker analysis for high-sensitivity CRP (hs CRP), high-sensitivity troponin I (hs Trop I), pentraxin-3, IL-6, IL-1β, IL-10 and TNFα was undertaken at the end of the study. Hs CRP and hs Trop I were measured on the Siemens Dimension EXL autoanalyzer. Pentraxin-3, IL-6, IL-1 β, IL-10 and TNFα were measured using assay kits from mesoscale Discovery. These

immunoassay kits are run in the enzyme linked immunosorbent assay (ELISA) format but use electrochemiluminescence detection rather than the production of a coloured product.

Statistical analysis

A visual density-curve inspection and Shapiro-Wilk tests were used to determine normality. If normally distributed, continuous variables were expressed as mean ± standard deviation. Continuous variables that were not normally distributed were expressed as median (interquartile range). Wilcoxon rank-sum and Mann-Whitney U test were used to compare differences in variables between DCB and DES. Differences across the timeframes were assessed pairwise with the Friedman test. The nested p-values were calculated with the Durbin-Conover test after applying Holm adjustments. Categorical variables were compared using the chi-square test. As pairwise analysis can only be performed with complete data, participants with missing data were excluded listwise. All statistical analysis was performed using R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

Table 7.1: Primer sequences used for RT-qPCR.

Primer	**Forward**	**Reverse**
GADPH (Housekeeper)	5’ – ACAGTTGCCATGTAGACC	5’ - TTGAGCAGGGTACTTTA
MMP9	5’ - AAGGATGGGAAGTACTGG	5’ - GCCCAGAGAAGAAGAAAAG
IL-10	5’ - GCCTTTAATAAGCTCCAAG	5’ - ATCTTCATTGTCATGTAGGC
CCL2	5’ - AGACTAACCCAGAAACATCC	5’ - ATTGATTGCATCTGGCTG
CXCR4	5’ - GGTGGTCTATGTTGGCGTCT	5’ - CTCACTGACGTTGGCAAAGA
TNFα	5’ - CTCAGCCTCTTCTCCTTC	5’ - AGAAGATGATCTGACTGCC
TREM1	5’ - ACAGATATCATCAGGGTTCC	5’ - CCTAGGGTACAAATGACCTC
PTX3	5’ - AGAGAGAGTTGAGACCAATC	5’ - AAACAATTGTCCCTCTGTTC

CD36	5’ - AGCTTTCCAATGATTAGACG	5’ – CAACTGGCATTAGAATACCTC
IL18	5’ – CAGCCGCTTTAGCAGCCA	5’ - CAAGGAATTGTCTCCCAGTGC

Table 7.1 shows the primer sequences used for RT-qPCR

7.3 Results

We recruited 30 patients from June 2020 until July 2021. Two patients did not return for repeat blood tests, two patients had elevated baseline (pre-PCI) troponin (Fig 7.1) and were thus excluded as per our *a priori* protocol. Following exclusion of these patients 26 patients were included in the final analysis. Their baseline characteristics and medications are shown in tables 7.2 and 7.3 while the procedural characteristics in table 7.4.

Fig 7.1: Consort diagram

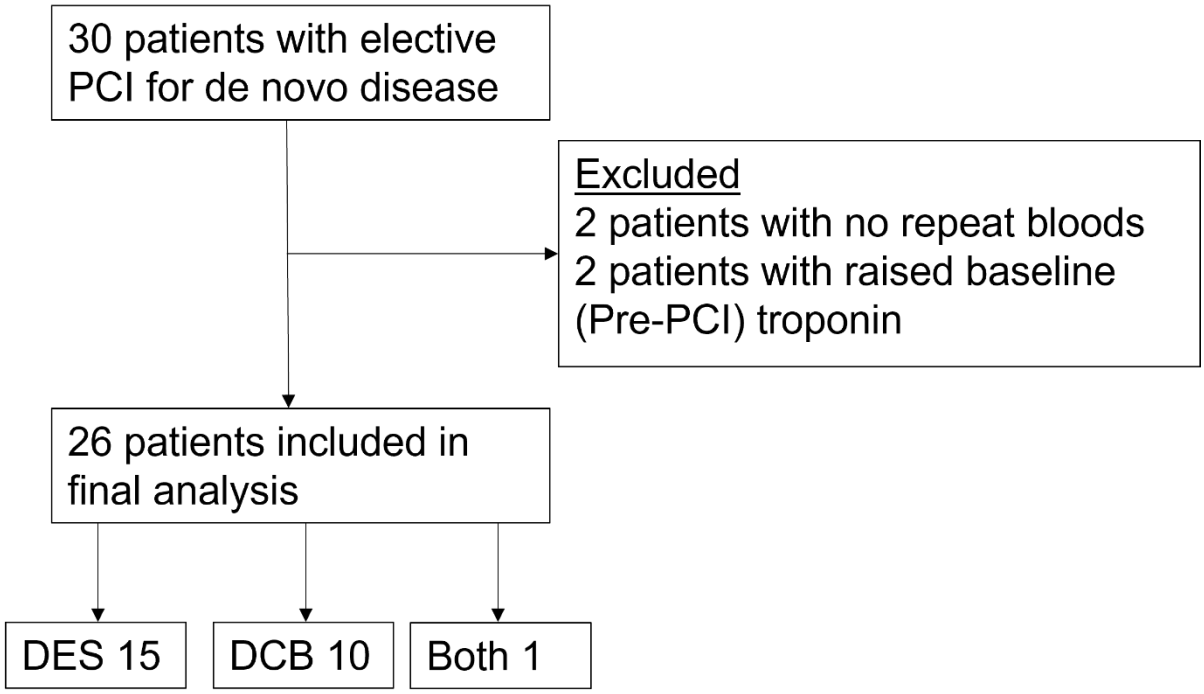


Fig 7.1: Consort diagram showing the flow of the patients in the study

Table 7.2: Baseline patient characteristics.

	All patients (N=26)	**DCB group N=10)**	**DES group (N=15)**	**P value**
Age (years)	69.5 (10.3)	71.6 (11.1)	68.4 (10.1)	0.46
Male	23 (88.5%)	9 (90%)	13 (87.7%)	0.8
Body mass index	27 (3.8)	26.8 (3.8)	26.7 (3.7)	0.92
Previous MI	11 (42.3%)	3 (30%)	8 (53.3%)	0.25
Previous PCI	13 (50%)	5 (50%)	8 (53.3%)	0.87
Diabetes	5 (19.2%)	2 (20%)	3 (20%)	1
Stroke	0	0	0	n/a
Hypertension	12 (46.2%)	6 (60%)	6 (40%)	0.32
Peripheral vascular disease	0	0	0	n/a
Atrial fibrillation	3 (11.5%)	1 (10%)	2 (13.3%)	0.8
Hypercholesterolaemia	20 (76.9%)	7 (70%)	12 (80%)	0.56
Chronic kidney disease (eGFR<60 ml/min/m^2)	5 (19.2%)	3 (30%)	2 (13.3%)	0.31
Mean eGFR ml/min/m^2	78.5 (21.1)	70.5 (18.1)	84.1 (22.5)	0.12
Rheumatoid arthritis	0	0	0	n/a
Asthma	2 (7.7%)	1 (10%)	1 (6.7%)	0.76
Chronic obstructive pulmonary disease	2 (7.7%)	1 (10%)	1 (6.7%)	0.76
Family history of IHD	9 (35%)	1 (10%)	8 (53.3%)	**0.03**
Ever smoker	11 (42.3%)	5 (50%)	6 (40%)	0.62

Table 7.2: Baseline patient characteristics of patients in final analysis

Table 7.3: Baseline medications.

Medications	**All patients (N=26)**	**DCB group (N=10)**	**DES group (N=15)**	**P value**
Aspirin	25 (96.2%)	9 (90%)	15 (100%)	0.21
Clopidogrel / Ticagrelor	26 (100%)	10 (100%)	15 (100%)	n/a
Statin	24 (92.3%)	9 (90%)	14 (93.3%)	0.76
Betablocker	18 (69.2%)	7 (70%)	10 (66.7%)	0.86
Nitrate	8 (30.8%)	4 (40%)	4 (26.7%)	0.48
ACE/ARB	17 (65%)	6 (60%)	11 (73.3%)	0.48
Calcium channel blocker	8 (30/8%)	4 (40%)	4 (26.7%)	0.48
Ivabradine	1 (3.8%)	1 (10%)	0	0.21
Ranolazine	6 (23.1%)	4 (40%)	2 (13.3%)	0.13

Table 7.3 showing the baseline medications

Table 7.4: Procedural characteristics.

	All patients (N=26)	DCB group N=10)	DES group (N=15)	P value
Radial access	24 (96%)	10 (100%)	14 (93.3%)	0.41
Number of devices (stents or drug coated balloons) used				
1 device 2 devices 3 devices	19 (76%) 5 (20%) 1 (4%)	6 (60%) 3 (30%) 1 (10%)	13 (86.7%) 2 (13.3%) 0 (0%)	0.24
Mean device diameter (mm)	3.2 (0.55)	3.1 (0.46)	3.3 (0.59)	0.31
Mean device length (mm)	29.3 (14.3)	34 (20.1)	26.2 (8.2)	0.27

Table 7.4: Procedural characteristics of patients in final analysis

Cytometric analysis of monocyte subsets

Analysis of the full cohort showed that the intermediate monocytes decreased significantly from baseline to four hours, recovered at two weeks and increased significantly at two months post PCI. In detail, the intermediate monocytes (CD14++CD16+) were 9.07% (7.27-15.9) at baseline, 4.62% (2.39-9.75) at four hours, 12.4% (9.47-16) at two weeks and 21.3% (9.65-25) at two months (Fig 7.2). Consequently, the opposing trend was seen in classical monocytes (CD14++CD16-). In detail, the percentage of the classical monocytes were 82.4% (75.5-88.7) at baseline, 91.9% (80.3-96.7) at four hours, 82.9% (74.3-86.2) at two weeks and 72.3% (66.9-82.5) at two months (Fig 2). The percentage of classical monocytes at two weeks and two months were significantly reduced compared to four hours but not the baseline. The non-classical monocytes (CD14+CD16++) did not change significantly at any point in time (Fig 7.2). Analysis of the DCB and DES groups separately demonstrated that there are few differences in the pattern of monocyte response post-PCI between DCB and DES. In the DES group the intermediate monocytes decreased significantly at four hours, recovered at two weeks and increased significantly at two months when compared to baseline (Fig 7.3). In the DCB group there was no difference in the intermediate monocytes at any point when compared to baseline, but they were significantly increased at two weeks and two months when compared to four hours (Fig 7.3). In the DES group the classical monocytes increased significantly at four hours while in the DCB group there was no significant change from baseline at any point (Fig 7.3). Raw data are presented in table 1 in the appendix.

Fig 7.2: Monocyte response after elective percutaneous coronary intervention.

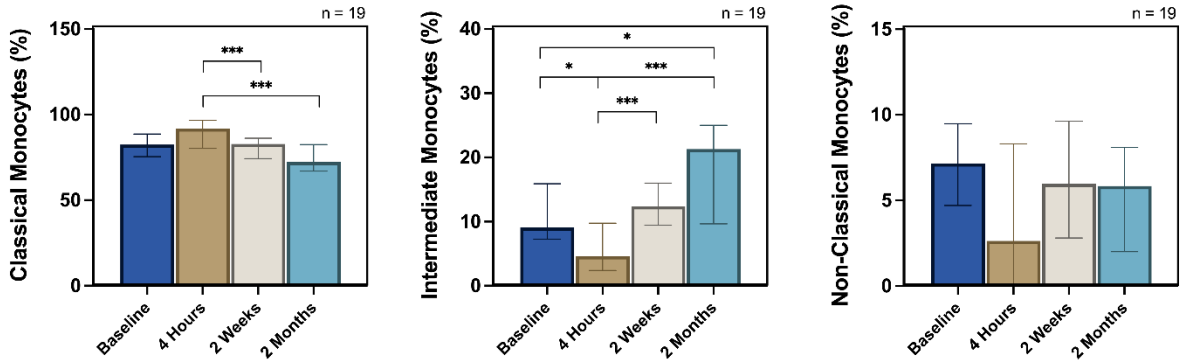
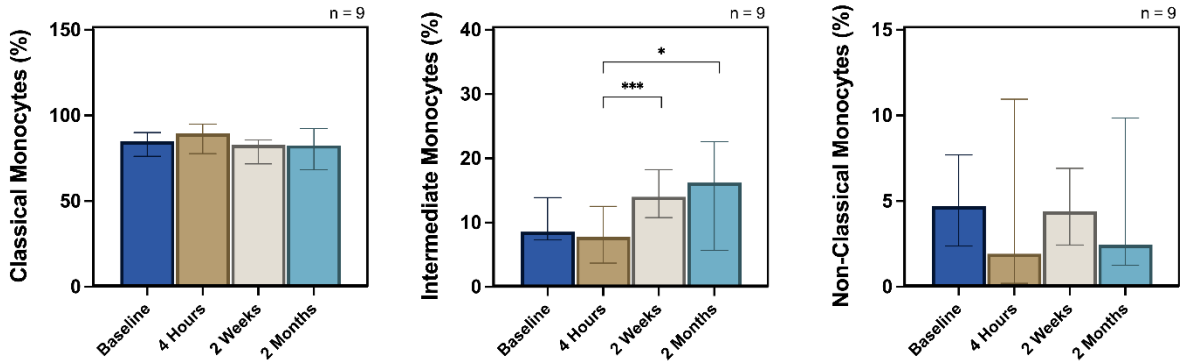


Fig 7.2 demonstrates the monocyte response for the classical, intermediate and non-classical monocyte subsets after elective percutaneous coronary intervention. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Fig 7.3: Monocyte response after elective angioplasty with drug coated balloon (A) or drug eluting stent (B).

A)



B)

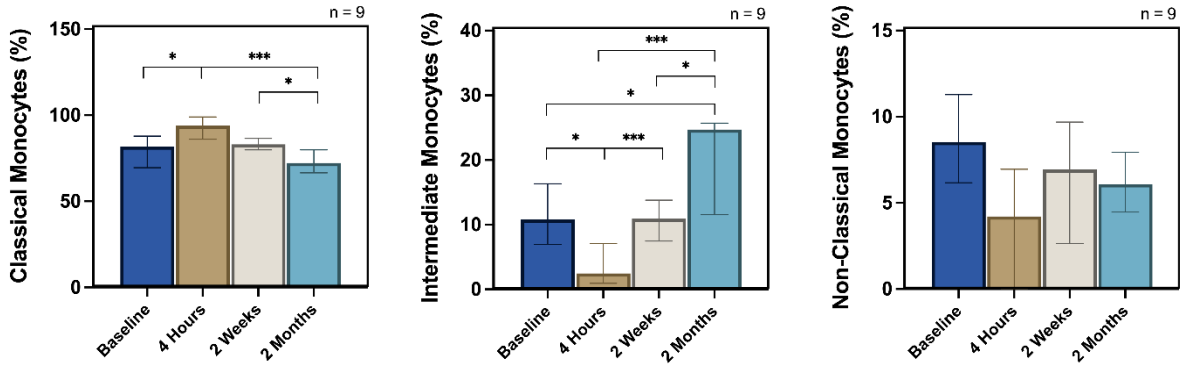


Fig 7.3 demonstrates the monocyte response for the classical, intermediate and non-classical monocyte subsets after elective angioplasty with drug coated balloon (A) or drug eluting stent (B). * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Gene expression of CD14+ monocytes

Analysis of the full cohort showed that CD14+ leucocytes had a) significantly decreased expression of CXCR4 at two months b) significant increased expression of pentraxin 3 at two weeks and two months c) significantly decreased expression of IL-18 at two weeks and d) significantly decreased expression of IL-1B at two months (Fig 7.4). Analysis of the DCB and DES groups separately, demonstrated some differences between the groups. In the DCB group, there was a significant decrease of IL-10 expression at two months while there was no significant difference in the DES group. In the DES group, there was a significant decrease of the expression of IL18 and IL-1B at two weeks and two months, while there was no difference in the DCB group (Fig 7.5). Raw data are presented in table 1 in the appendix.

Fig 7.4: Gene expression of CD14+ leucocytes following elective percutaneous coronary intervention.

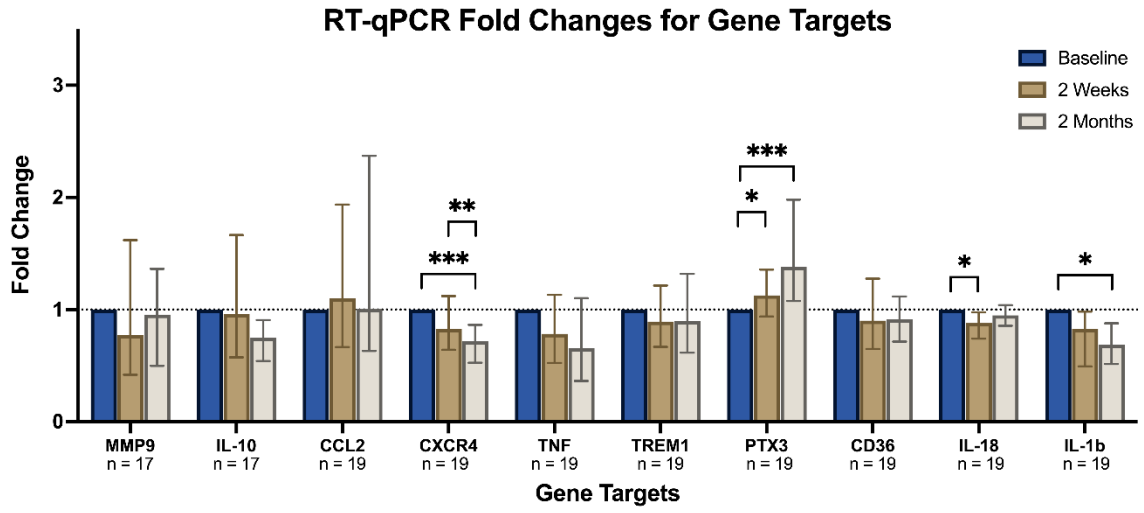
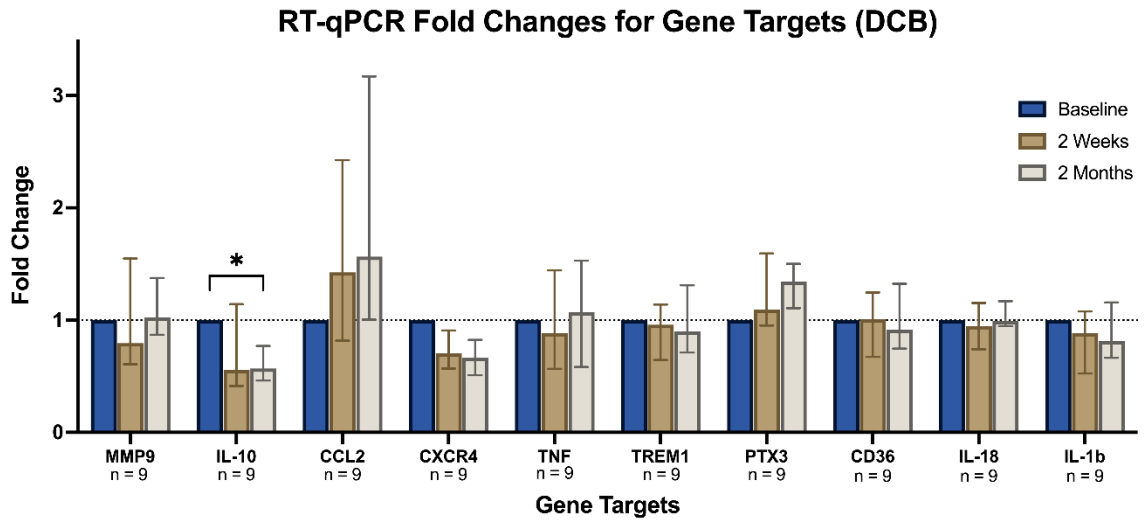


Fig 7.4 shows the change in gene expression (fold change compared to baseline) of CD14+ leucocytes following elective angioplasty. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Fig 7.5: Gene expression of CD14+ leucocytes following elective angioplasty with drug coated balloon (A) or drug eluting stent (B).

A)



B)

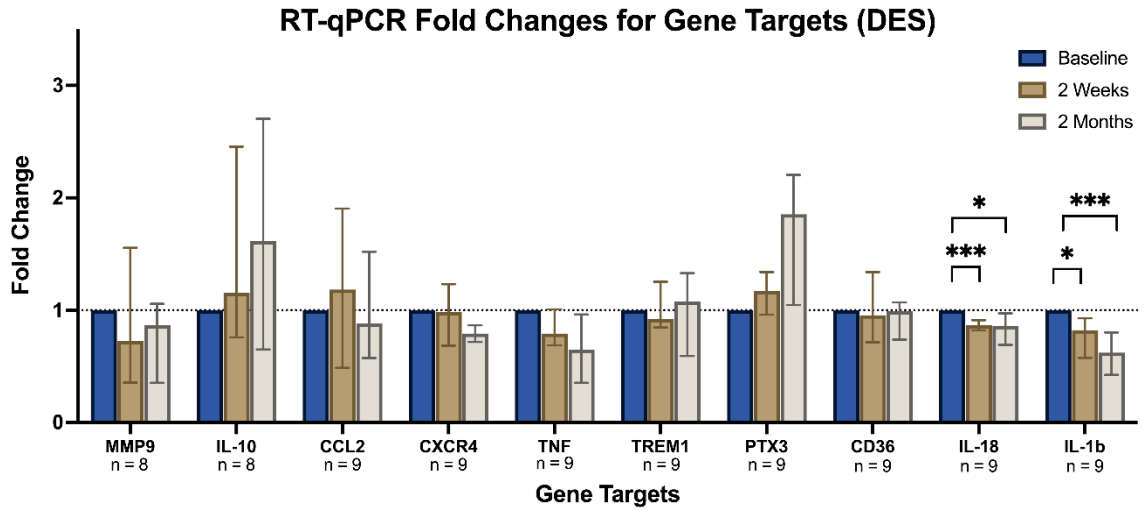


Fig 7.5 shows the gene expression (fold change compared to baseline) of CD14+ leucocytes following elective angioplasty with drug coated balloon (A) or drug eluting stent (B). * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Biomarker analysis

Analysis of the full cohort showed that a) both IL-6 and TNF-α peaked at four hours and remained significantly elevated post-PCI until two months later, b) hsTroponin I peaked at four hours and remained significantly elevated until two weeks later c) Pentraxin 3 was significantly elevated only at four hours and d) there was no significant difference at hsCRP or IL-10 at any point in time (Fig 7.6). Analysis of the DCB and DES group separately demonstrated only few differences between the groups. In the DCB group, hsTroponin I was significantly elevated only at four hours while in the DES group it remained significantly elevated until two weeks later. In the DCB group, pentraxin 3 remained significantly elevated until two months later while in the DES group it was only significantly elevated at four hours (Fig 7.7). Raw data are presented in table 1 in the appendix.

Fig 7.6: Inflammatory biomarker response following elective percutaneous coronary intervention

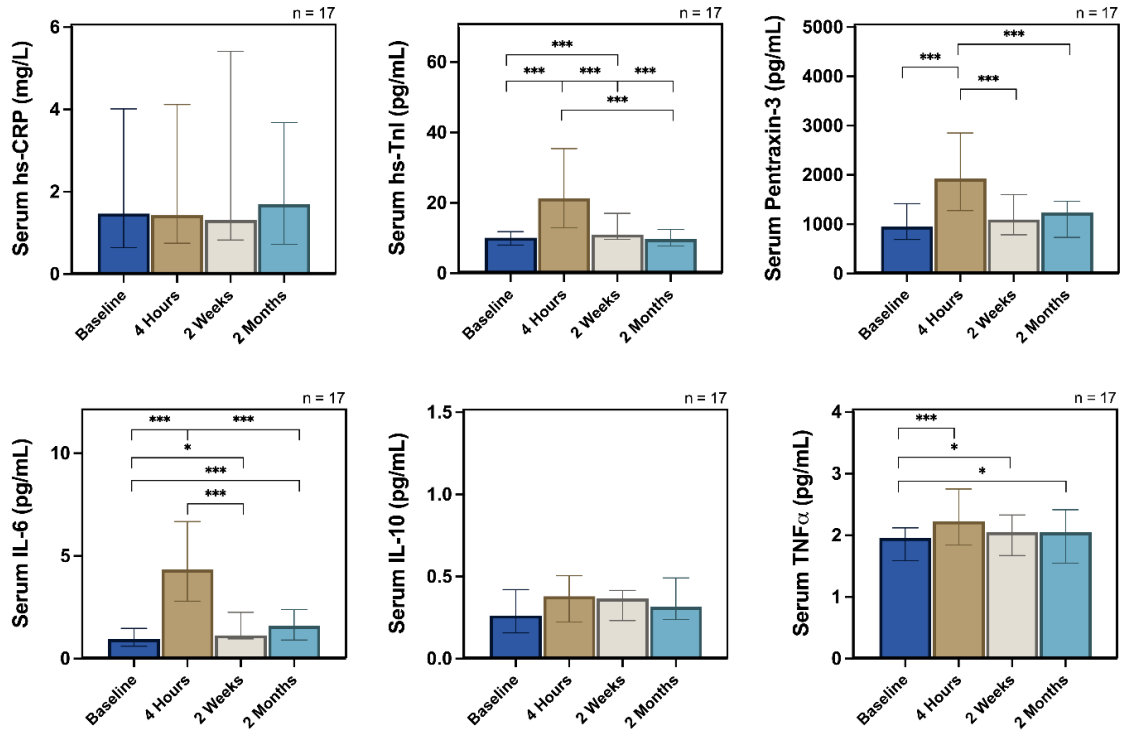
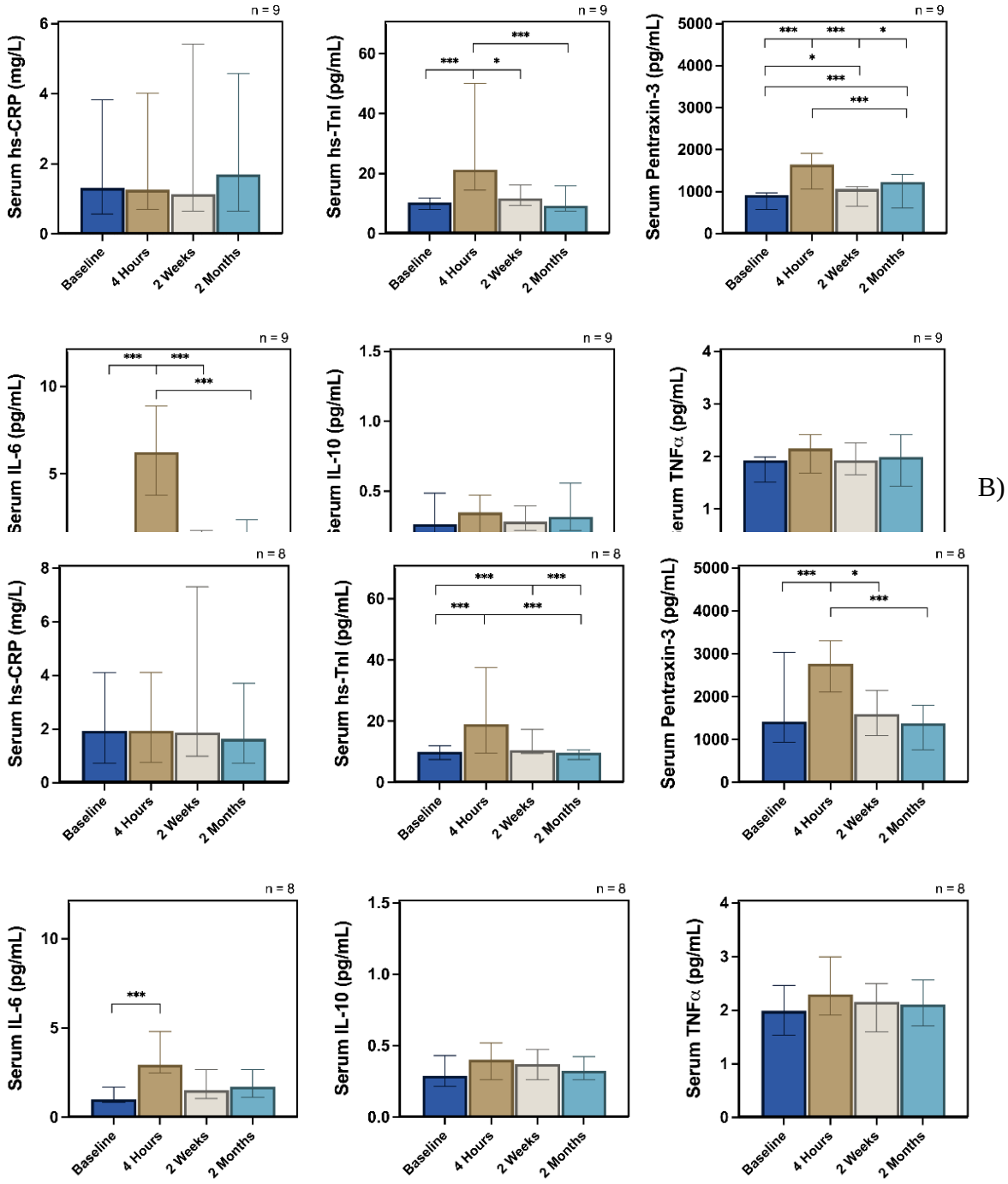


Fig 7.6: shows the inflammatory biomarker response following elective percutaneous coronary intervention. * p<0.05 ** p<0.01 *** p<0.001

Fig 7.7: Inflammatory biomarker response following angioplasty with drug coated balloon (A) or drug eluting stent (B).

A)



B)

Fig 7.7 shows the inflammatory response after elective angioplasty with drug coated balloon (A) or drug eluting stent (B). * $p<0.05$ ** $p<0.01$ *** $p<0.001$

7.4 Discussion

There are only limited data about the role of monocytes following PCI. Fakuda et al. were the first to link circulating monocytes with in-stent neointimal hyperplasia. They demonstrated that circulating monocytes increase after PCI with bare metal stent, peak at two days and the maximum monocyte level positively correlated with in-stent neointimal volume (81). Our study is novel as we demonstrated the monocyte subset response after elective PCI in the modern era. We have demonstrated that the population of intermediate monocytes decreased in the immediate post-PCI period (four hours), recovered at two weeks and then significantly increased further at two months. The classical monocytes appeared to follow the opposite pattern to intermediate monocytes while the non-classical monocytes did not change significantly, suggesting that there was a shift from classical to intermediate monocytes and vice versa. Subgroup analysis showed few differences in the monocyte response between DCB and DES groups, most notable being that in the DES group the intermediate monocytes are significantly increased at two months compared to baseline while in the DCB group they were not. The fact that intermediate monocytes, a highly pro-atherosclerotic monocyte subset, remained persistently elevated two months after elective, uncomplicated PCI is a concern and requires further validation and investigation. Our study included a small number of patients and should be regarded as hypothesis generating. However, a possible explanation could be that the stent as a metallic foreign material represents a persistent stimulus for the monocyte response. This hypothesis is consistent with previous studies which have shown that platelet and neutrophil activation is greater after stenting compared to balloon angioplasty only (243).

The current classification of monocytes, which introduced the intermediate subset, was established in 2010 (242). Since then, there has been an increasing interest about their role in cardiovascular diseases. Over the last ten years, studies have shown that intermediate

monocytes are an independent predictor of cardiovascular events in stable patients (79) (80). In a large prospective study of almost 1000 patients being referred for elective coronary angiography, intermediate monocytes were the only subset independently predictive of adverse cardiovascular events (79). In addition, stable angina patients with elevated levels of the highly proatherogenic lipoprotein (a) (Lp(a)) have significantly elevated levels of intermediate monocytes and oxidized phospholipids (OxPL) (244). The biomarker OxPL/apoB (oxidized phospholipids on apolipoprotein B-100) correlates with intermediate monocytes but not with the other monocyte subsets, suggesting that this is the link between the atherogenic Lp(a) and the more proinflammatory intermediate monocytes (244).

The predictive role of intermediate monocytes has also been demonstrated in patients with acute coronary syndrome. Intermediate and non-classical monocytes are significantly increased in patients with unstable angina when compared with stable patients (245). Furthermore, in unstable angina patients with intermediate-to-high cardiovascular risk (as determined by GRACE score) intermediate monocytes are increased independently of traditional risk factors (245). In patients with STEMI, intermediate monocytes increase significantly in the early stages and are independent predictors of cardiovascular events at two years (246). Beyond the context of coronary artery disease, intermediate monocytes significantly increase in advanced stages of peripheral vascular disease and are associated with risk of restenosis following peripheral vascular angioplasty (247) (248). In addition, they have been shown to be independently associated with and be linked to the pathogenesis of atrial fibrillation (249).

Furthermore, I have demonstrated changes in the gene expression of CD14+ leucocytes, indicating changes in the functional profile of leucocytes. IL18 showed decreased expression at two weeks, CXCR4 and IL1β decreased at two months, while pentraxin 3 increased at two

weeks and two months. The decrease of IL18 and IL1β was mainly driven by the DES group. The expression profile represents a pool of all CD14+ leucocytes rather than specific monocytes subsets. Future studies focusing on specific subsets could help gain insight of the gene alterations that take place at the monocyte subset level.

Monocytes play a central role in the crosstalk between T-lymphocytes, endothelial cells and smooth muscle cells mediated by cytokines (33). In this present study, we have demonstrated alterations in the gene expression of various inflammatory mediators following PCI. IL18, IL1β and CXCR4 have decreased expression while PTX3 has increased expression. Interestingly, IL1β, CXCR4 and PTX3 had sustained different levels of expression two months later, indicating that the change in gene expression is not a transient response even after uncomplicated PCI for elective patients. IL18 is a pleiotropic proinflammatory cytokine playing roles in neointimal formation, smooth muscle cell migration as well as plaque vulnerability. Higher levels of IL18 have been associated with increased risk of in-stent restenosis (112). Monocytes are one of the main producers of IL1β, a cytokine that is known to induce an inflammatory response in vessel wall and is closely related to atherosclerosis as shown by the recent CANTOS trial (250). Expression of CXCR4 receptor has recently been shown to be atheroprotective by a variety of mechanisms such as maintaining arterial integrity, preserving endothelial function and promoting a normal contractility of smooth muscle cells (251). PTX3 is produced locally at sites of inflammation by a number of cells such as monocytes, endothelial cells, smooth muscle cells, dendritic cells and fibroblasts (252). It increases after elective PCI and the post-PCI levels are predictive of major adverse cardiovascular events (253).

The humoral inflammatory response post-PCI has been studied extensively over the last few decades. Our study is the first to demonstrate that TNFα remains significantly elevated two months after elective uncomplicated PCI. TNFα is a key pro-inflammatory cytokine acting locally at sites of vascular injury such as PCI. It promotes the interaction between circulating leucocytes and endothelial cells (254). Clinical and pre-clinical data have shown that it is associated with restenosis (255). Our finding that IL6 peaks at four hours post-PCI and remains significantly elevated up to two months later is consistent with a large previous study that had showed that IL6 levels peak 24hours post-PCI and return to baseline by three months (256). IL6 is a multifunctional cytokine known to induce other acute phase proteins and play a central role in inflammation and tissue injury. It increases immediately post-PCI in the coronary sinus circulation and correlates positively with late loss index at six months (257).

Targeting the residual inflammation after PCI is one of the main avenues current cardiovascular research pursues in order to improve patient outcomes (108,240). In this study, we have demonstrated that intermediate monocytes, a highly proatherogenic monocyte subset, remain significantly elevated two months following elective, uncomplicated PCI. This might be a potential target of the immune system that could lead to improved patient outcomes. Furthermore, we have demonstrated differences in the elicited inflammatory response between two different, modern PCI strategies. It might be that the PCI strategy could be one of the ways to modulate the elicited inflammatory response post-PCI and improve patient outcomes.

Limitations

This study has a number of limitations. First, I recruited a small number of patients and few patients were lost to follow up. This makes it difficult to draw definite conclusions about any subgroup comparisons. Second, I was only able to follow up the patients up to two months post PCI. Longer follow up would provide additional information about the monocyte response and

strengthen the value of our findings. Third, I studied a limited number of genes and inflammatory mediators. A more comprehensive gene expression analysis would provide a greater understanding of the changes of various monocyte subsets.

7.5 Conclusion

In conclusion, our study explored the monocyte response following elective PCI. We have demonstrated that the intermediate monocytes significantly decreased acutely at four hours, recovered at two weeks and significantly increased at two months. Subgroup analysis demonstrated that intermediate monocytes were significantly elevated two months post PCI in the DES group but not in the DCB group. Analysis of pooled CD14+ leucocytes has demonstrated that the monocyte response was accompanied by changes in gene expression of important inflammatory mediators, which were maintained up to two months. Finally, we have described the inflammatory biomarker response after elective PCI and demonstrated that some important inflammatory mediators (TNFα, IL6) remained significantly elevated up to two months. Future, larger studies should focus on the differences between DCB and DES in terms of monocyte response, monocyte subset gene expression and also inflammatory biomarkers.

Chapter 8. Myocardial inflammation after elective percutaneous coronary intervention: A proof of concept study

8.1 Introduction

It is well established that inflammation is the underlying pathophysiological process leading to sequalae of percutaneous coronary intervention such as restenosis (34,258). Leucocyte infiltration of the vessel wall takes place at an early stage following vascular injury post angioplasty, while macrophages accumulate at a later stage (34,258). While the majority of the studies have focused on the inflammatory reaction in the vessel wall post-angioplasty, animal data have demonstrated that the main foci of inflammation extend many millimetres away from the injured vessel into the myocardial tissue (259). Limited human data have also shown that coronary stents induce an inflammatory reaction which involves the distal coronary artery as well as the surrounding myocardium (260).

Cardiovascular magnetic resonance (CMR) enhanced with ultrasmall superparamagnetic particles of iron oxide (USPIO) is a relatively recent technique that has been used successfully to image cellular, myocardial inflammation (251) (252) . USPIO, which are both phagocytosed by cardiac macrophages but also passively present in myocardial interstitium, cause local inhomogeneities in the magnetic field that can be detected by CMR (263). USPIO-enhanced CMR has been used successfully to image cellular myocardial inflammation in several conditions such as acute myocardial infarction, takotsubo cardiomyopathy and chronic ischaemic cardiomyopathy (261,263).

To date, USPIO-enhanced CMR has not been used to assess myocardial inflammation post elective angioplasty. The purpose of my proof-of-concept study was to investigate the myocardial inflammation elicited following elective, uncomplicated angioplasty, as assessed by USPIO-enhanced CMR.

8.2 Methods

The methods for this study have been described previously in chapters 2.1 – 2.3 (page 52). In brief, in this prospective, pilot, proof-of-concept study I recruited adult patients (>18 years old) undergoing elective PCI for de novo coronary artery disease, either with Drug Coated Balloon (DCB) or Drug Eluding Stent (DES) at the discretion of the operator. Patients with previous myocardial infarction, previous PCI, significant inflammatory conditions on immunosuppression, significant renal impairment (defined as an estimated glomerular filtration rate <30mL/min/1.73m2) as well as pregnant women were excluded. I also recruited healthy volunteers as a control group. All patients underwent USPIO-enhanced CMR two weeks after PCI as described previously.

Statistical analysis

A visual density-curve inspection and Shapiro-Wilk tests were used to determine normality. If normally distributed, continuous variables were expressed as mean ± standard deviation. Continuous variables that were not normally distributed were expressed as median (interquartile range). The Welch two sample t-test or paired samples t-test were used for group comparison as appropriate. 'PCI myocardium' from patients undergoing PCI was compared with both 'remote myocardium' and 'healthy myocardium'. 'Remote myocardium' was considered to be myocardium from the same patient in the lateral wall representing the circumflex territory and 'healthy myocardium' was considered from subjects not undergoing PCI. All statistical analysis was performed using R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

8.3 Results

Five patients undergoing elective PCI and three healthy volunteers were recruited in the study. All patients were male with mean age 68.4 (11.2) years old. Table 8.1 shows their baseline

clinical characteristics and table 8.2 shows the angiographic and procedural characteristics. All patients underwent PCI in LAD while one patient underwent PCI in LAD and RCA at the same procedure. Table 8.3 shows the baseline and follow up biomarkers. One patient had significantly elevated troponin I at baseline and follow up, even though the patient did not have unstable symptoms. As this was a proof-of-concept study, we have run the analysis with and without this patient.

Table 8.1: Baseline patient characteristics.

Variable	Patients (N=5)
Age (years)	68.4 (11.2)
Male	5 (100%)
Previous MI	0 (0%)
Previous PCI	0 (0%)
Diabetes	1 (20%)
Stroke	0 (0%)
Hypertension	2 (40%)
Peripheral vascular disease	0 (0%)
Atrial fibrillation	0 (0%)
Hypercholesterolaemia	3 (60%)
Mean estimated glomerular filtration rate	75.2 (12.4)
Rheumatoid arthritis	0 (0%)
Chronic obstructive pulmonary disease	1 (20%)
Family history of IHD	2 (40%)
Ever smoker	2 (40%)
Medications	
Aspirin	5 (100%)
Clopidogrel	5 (100%)
Statin	4 (80%)
Betablocker	5 (100%)
Nitrate	3 (60%)
ACE inhibitor / ARB	1 (20%)
Calcium channel blocker	1 (20%)

Table 8.1: Baseline clinical characteristics of patients undergoing elective PCI

Table 8.2: Angiographic and procedural characteristics.

Variable	Patients (N=5)
Radial access	5 (100%)
Vessel treated	
LAD treated	5 (100%)
LAD and RCA treated	1 (20%)
PCI strategy	
Drug coated balloon	3 (60%)
Drug eluting stent	2 (40%)
TIMI flow pre-PCI (2 or 3)	5 (100%)
TIMI flow post-PCI (3)	5 (100%)
Mean vessel diameter (mm)	3.05 (0.37)
Mean lesion length (mm)	35.2 (20.03)
Other coronary artery disease (<50% in diameter)	5 (100%)

Table 8.2: Angiographic and procedural characteristics of patients undergoing elective PCI

LAD: left anterior descending artery, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction

Table 8.3: change in R2* values as well as the biomarker values at baseline and follow up.

Patient	PCI territory	Treatment strategy	R2* change in LAD	R2* change in Cx	R2* change in liver	
1	LAD	DES	29.23	15.39	233.28	
2	LAD	DCB	10.17	7.47	151.81	
3	LAD	DES	25.17	18.87	278.37	
4	LAD/RCA	DCB	26.75	-0.33	182.13	
5	LAD	DCB	5.29	4.48	175.44	
Patient	Baseline hs CRP mg/L	Baseline hs Troponin I pg/mL	Baseline Pentraxin 3 pg/mL	Baseline IL6 pg/mL	Baseline IL 10 pg/mL	Baseline TNFa pg/mL
1	1.39	7.8	1031.27	0.94	<0.3	3.02
2	7.86	206.3	1528.18	1.75	<0.3	0.28
3	5.22	11.8	1051.30	<0.6	<0.3	0.34
4	1.31	6.8	309.00	<0.6	<0.3	<0.2
5	0.77	20.3	706.87	1.39	<0.3	<0.2
Patient	4h - hs CRP mg/L	4h - hs Troponin I pg/mL	4h - Pentraxin 3 pg/mL	4h - IL6 pg/mL	4h - IL 10 pg/mL	4h - TNFa pg/mL
1	1.55	16.5	1270.34	4.47	<0.3	2.87
2	9.48	245.5	2181.57	4.76	<0.3	<0.2
3	5.41	29.2	2201.11	3.26	<0.3	0.32
4	1.26	13.9	903.73	1.93	<0.3	<0.2
5	0.79	23.7	1221.76	8.32	<0.3	0.24
Patient	2 weeks - hs CRP mg/L	2 weeks - hs Troponin I pg/mL	2 weeks - Pentraxin 3 pg/mL	2 weeks - IL6 pg/mL	2 weeks - IL 10 pg/mL	2 weeks - TNFa pg/mL
1	1.22	1.73	8.1	610.37	0.60	<0.3
2	1.35	8.96	323.7	1190.86	1.44	<0.3
3	1.34	6.87	13.4	1193.70	0.78	<0.3

4	1.20	1.14	10.3	308.06	0.80	<0.3
5	2.30	0.78	16.8	773.23	1.09	<0.3

Table 8.3 showing the change in R2* values as well as the biomarker values at baseline and follow up.

Table 8.4: R2* change in patients and healthy volunteers.

Characteristic	Overall, N = 8[1]	Volunteer, N = 3[1]	PCI, N = 5[1]	p-value[2]
Change R2* - LAD	16.6 (9.2)	12.2 (4.0)	19.3 (10.8)	0.2
Change R2* - Cx	9.7 (7.6)	10.5 (8.7)	9.2 (7.9)	0.8
Change R2* - Liver	195.7 (46.6)	181.4 (43.8)	204.5 (51.0)	0.5

[1] Mean (SD)

[2] Welch Two Sample t-test

Table 8.4 shows the R2* change in PCI area, remote myocardium, healthy myocardium and liver in patients and healthy volunteers

As demonstrated in figures 8.1, 8.2 and table 8.4, the PCI area had a numerically larger change in R2* values when compared to healthy myocardium or remote myocardium, which did not reach statistical significance; PCI area (LAD) vs healthy myocardium (LAD) (19.3 ± 10.8 vs 12.2 ± 4.0, p = 0.2); PCI area (LAD) vs remote myocardium (Cx) (19.3 ± 10.8 vs 9.2±7.9, p =0.1). There was no difference in comparing remote myocardium (Cx) from patients with PCI with healthy Cx myocardium (9.2 ±7.9 vs 10.5 ± 8.7, p=0.8), or healthy (LAD) vs healthy (Cx) (12.2±4.0 vs 10.5±8.7, p=0.6). These results were the same independently of whether the patient with elevated baseline troponin I was included or not. The change in R2* in PCI area did not correlate with any of the biomarkers measured at the same time (two weeks post PCI). Figure 8.3 demonstrates the T2* mapping of a patient and a healthy volunteer pre- and post-USPIO.

Fig 8.1: Change in R2* values in patients following elective PCI and healthy volunteers.

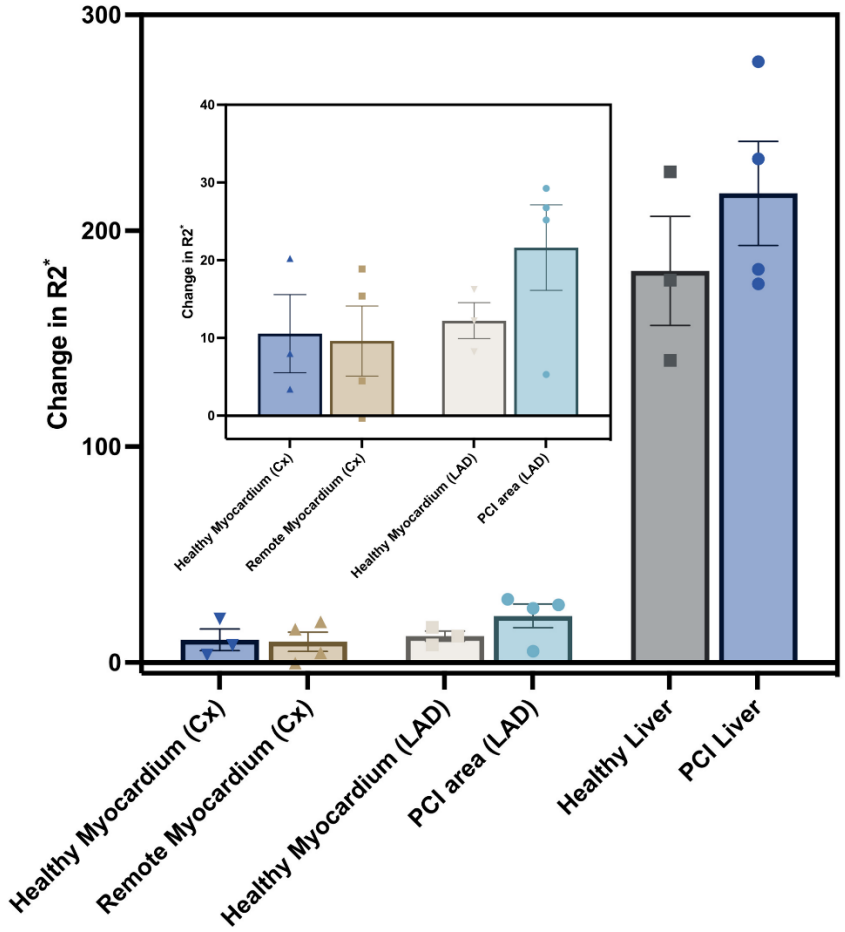


Fig 8.1 shows the USPIO-uptake (Change in R2* (s^{-1})) in PCI area (LAD) and remote myocardium (Cx) following elective PCI, and healthy volunteers. The patient with high baseline troponin is excluded.

USPIO: ultrasmall superparamagnetic particles of iron oxide, LAD: left anterior descending, Cx: circumflex, PCI: percutaneous coronary intervention

Fig 8.2: Change in R2* values in patients following elective PCI and healthy volunteers.

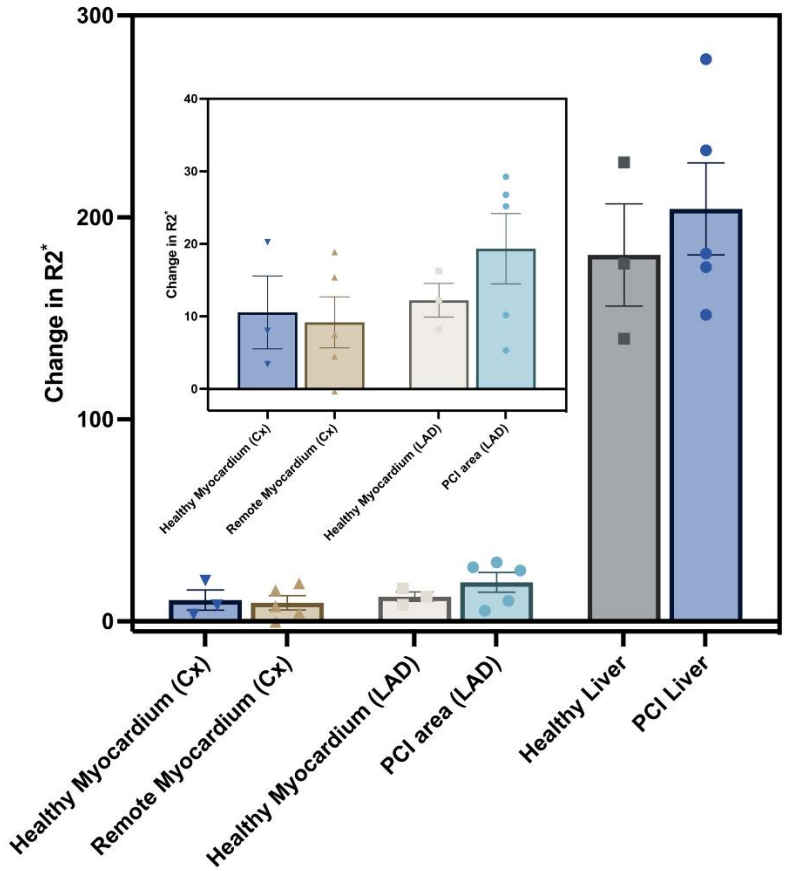


Fig 8.2 shows the USPIO-uptake (Change in R2* (s^{-1}) in PCI area (LAD) and remote myocardium (Cx) following elective PCI, and healthy volunteers. The patient with high baseline troponin is included.

USPIO: ultrasmall superparamagnetic particles of iron oxide, LAD: left anterior descending, Cx: circumflex, PCI: percutaneous coronary intervention

Fig 8.3: Example of USPIO-enhanced T2* map, pre and post USPIO.

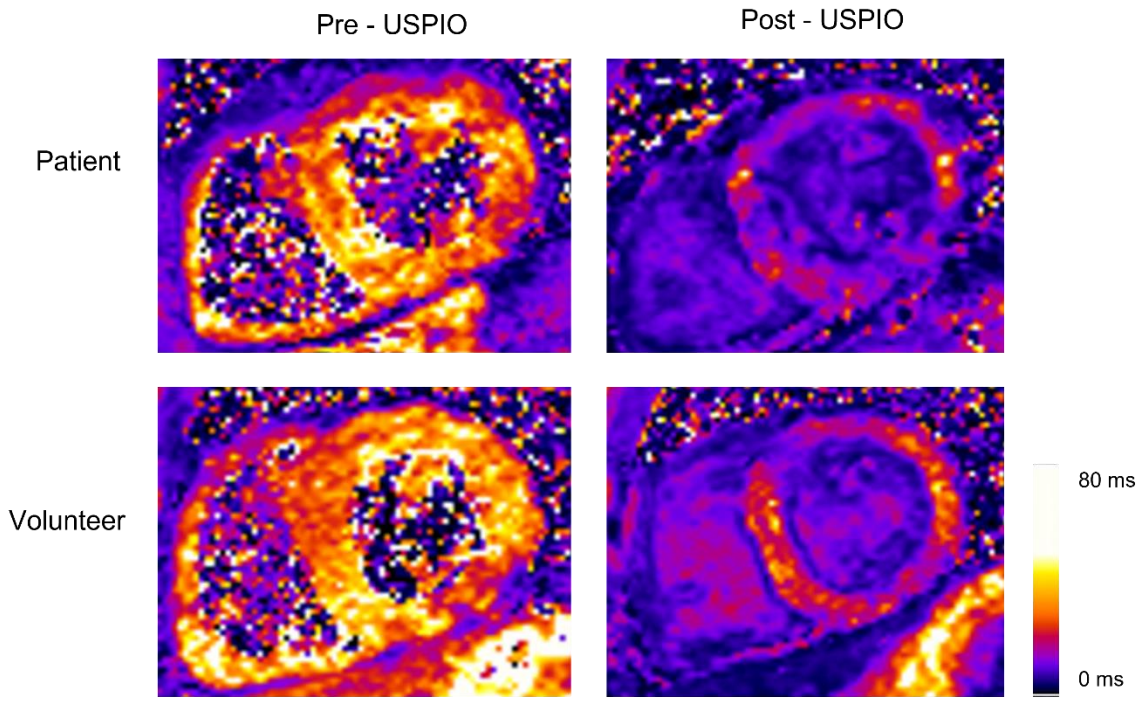


Fig 8.3 shows the T2* map of a patient and a healthy volunteer pre- and post-USPIO

8.4 Discussion

For the first time I have utilised USPIO-enhanced CMR to assess the myocardial inflammation elicited after uncomplicated, elective PCI. As a proof-of-concept study, I have demonstrated a small absolute, but statistically non-significant, increase in inflammation in the PCI area when compared to remote myocardium or healthy myocardium two weeks after PCI.

There is an abundance of data indicating that both pre- and post-inflammatory status, as assessed by blood inflammatory biomarkers, can predict patient outcomes including mortality, cardiovascular mortality as well as in-stent restenosis (36,264). Direct mechanical trauma is the main mechanism driving the inflammatory reaction post uncomplicated PCI. Vessel wall injury either from the balloon inflations or stent deployment causes coronary endothelial injury as well as plaque disruption, microembolisation and release of prothrombotic/proinflammatory material (36). The inflammatory reaction in the vessel wall, following angioplasty is an

important underlying mechanism for stent-failure processes such as in-stent restenosis, thrombosis and neo-atherosclerosis (97,258). Even though the majority of the studies have focused on the vessel wall as the site of inflammation, it has been demonstrated that the main foci of perivascular inflammation following balloon angioplasty of porcine coronary arteries are not limited to the adventitia but extend into the myocardium away from the injured vessel wall (259). The concept of, myocardial inflammation post angioplasty, is supported by sparse human data. Gomes et al. demonstrated that stents induce a chronic inflammatory reaction which extends into the distal coronary artery but also in the surrounding myocardium (260).

USPIO-enhanced CMR is a relatively new technique which has allowed imaging of myocardial cellular inflammation. It has been used successfully to image myocardial inflammation in patients with ST elevation myocardial infarction and demonstrate that myocardial inflammation is a key driver for chronic ischaemic cardiomyopathy as well as takotsubo cardiomyopathy (134,263,265). The elicited inflammation following elective, uncomplicated PCI is of smaller magnitude compared to the inflammation elicited following STEMI. Nevertheless, localising the elicited inflammation into the myocardium rather than just the vessel wall is important and might be related to patient outcomes. In our study, we were only able to demonstrate a small numerical increase R2* signal in PCI area which was not statistically significant. Microembolisation, which can occur during PCI, has a characteristic pattern of LGE on CMR. In particular it starts from the endocardium and extends distally towards the mid-myocardium and epicardium and has a patchy pattern. No LGE was seen in any of our patients, therefore microembolisation did not occur in these patients and did not influence the results.

Limitations

My study is not without limitations. Firstly, as a proof-of-concept study the number of patients was limited. Secondly, the cost of each USPIO vial was £1608 ($1985, EUR 1826) therefore

this cost make the method prohibitive for clinical implementation. Thirdly, more recently a R2*/R1 ratio at 75 hours post-USPIO infusion has been proposed for identifying active macrophage infiltration (263). However, this potential novel parameter was not known at the beginning of our study.

8.5 Conclusion

In conclusion, for the first time, I have utilised USPIO-enhanced CMR to assess myocardial inflammation post elective, uncomplicated PCI. I have demonstrated a small, numerical increase in inflammation which was not statistically significant. However, my results open the way for larger studies in this novel area.

Chapter 9. Discussion

There is increasing evidence from randomised clinical trials and registry studies about the safety and efficacy of DCB-only angioplasty compared to DES for de novo coronary artery disease. However, a lot of the studies are limited by long recruitment periods and relatively small number of patients in the DCB group compared to the DES group. Therefore, there is currently very limited 'real world' data about the safety of DCB-only angioplasty for de novo disease in routine clinical practice.

The first few chapters of this thesis have utilised a large database from Norfolk & Norwich University Hospital, the cardiac centre with one of the highest implantation of DCB in the UK, to study the safety and efficacy of DCB-only angioplasty for de novo disease compared to second generation DES, as part of routine clinical practice.

The role of inflammation in the pathogenesis of atherosclerosis and sequalae of PCI is well established (33). The monocytes have gained a lot of interest over the last few years, as various monocyte subsets (only recently classified) have been associated with adverse cardiovascular events, restenosis after angioplasty for peripheral vascular disease and linked to the pathogenesis of atrial fibrillation (79,247,249). However, there is no data about the monocyte subset response after elective, uncomplicated PCI. The last chapter of this thesis reports our prospective study on the monocyte response following elective, uncomplicated PCI with modern PCI techniques (DCB and DES).

9.1 Interpretation of findings and clinical implications

a) Same-day discharge of elective patients following DCB-only angioplasty.

In view of the concern about acute vessel closure following angioplasty without a stent, chapter three of this thesis investigated the safety of same-day discharge of elective patients following DCB-only angioplasty for de novo coronary artery disease. We concluded that the protocol

followed in Norfolk & Norwich University Hospital, to discharge patients on the same day if they are pain-free with no ECG changes and no more than B dissection is safe. Implementing this protocol in clinical practice will provide safe discharge of elective patients on the same day of their procedure without unnecessary hospital stay, whilst ensuring at the same time that cost is kept to a minimum.

b) Long-term safety of DCB-only angioplasty

In view of a meta-analysis which raised a concern about possible increased late mortality signal associated with paclitaxel DCB angioplasty for peripheral vascular disease, in chapter four I investigated the possibility of a late mortality signal with paclitaxel DCB angioplasty for coronary artery disease (200). I concluded that there is no evidence of increased late mortality associated with paclitaxel DCB-only angioplasty for de novo coronary artery disease, as compared with non-paclitaxel second generation DES. On the contrary, the propensity score matched analysis demonstrated that patients treated exclusively with DCB-only angioplasty in the index and subsequent procedures had significantly better long-term survival.

c) Safety of DCB-only angioplasty for de novo coronary artery disease in routine, elective clinical practice

In chapter five, I investigated the safety of DCB-only angioplasty for de novo coronary artery disease in routine clinical practice as compared with second generation DES. I concluded that DCB-only angioplasty for stable angina due to de novo disease is safe in terms of mortality and net cardiac events including unplanned TLR, which is the most interesting endpoint for elective patients. My study included mainly large vessels and patients were followed up for a median of >3.5 years indicating sustained results in the long term. Multivariable Cox regression

analysis showed that the only independent predictors of mortality were increasing age and frailty score.

d) Safety of DCB-only angioplasty for STEMI

In chapter six, I investigated the safety of DCB-only angioplasty in patients with STEMI due to de novo disease as compared with second generation DES. I concluded that DCB-only angioplasty is safe in terms of all-cause mortality and net cardiac events including unplanned TLR. My study included mainly large vessels and followed up the patients for a median of >3 years indicating sustained results in the long term. Multivariable Cox regression analysis showed history of heart failure, increasing frailty and increasing acuity score as independent predictors of worse mortality while family history of IHD was an independent predictor of better survival.

Together, the results of the previous studies provide reassurance to interventional cardiologists that DCB-only angioplasty for de novo disease is safe in the short-term but also in the long-term, in terms of all-cause mortality and net cardiac events including target lesion revascularisation. Even though DES remain the standard of care, the above studies have demonstrated that the necessity of stent implantation may be assessed per each case and provide an equipoise for further randomised studies. DCB-only angioplasty can be an alternative PCI strategy with demonstrated safety in de novo disease of all vessel sizes, in elective patients and patients with STEMI.

e) Inflammatory response following elective PCI

In chapter seven, I investigated the inflammatory response following elective PCI, focusing on the monocytes. I demonstrated that intermediate monocytes decrease significantly acutely (at

four hours), recover at two weeks and then increase significantly at two months. Subgroup analysis showed that compared to baseline, these changes were significant only in the DES group while in the DCB group they did not reach statistical significance. Gene expression analysis of CD14+ leucocytes showed IL18 had decreased expression at two weeks, CXCR4 and IL1β decreased at two months, while pentraxin 3 increased at two weeks and two months. In terms of humoral biomarkers, hsTnI remains elevated till two weeks post PCI while IL6 and TNFα remain elevated till two months post PCI.

The intermediate monocytes have been associated with adverse cardiovascular events, restenosis following angioplasty for peripheral vascular disease and linked to the pathogenesis of atrial fibrillation. The fact that they remain significantly increased two months following elective, uncomplicated PCI warrants further validation and investigation.

f) Myocardial inflammation after elective angioplasty

Finally, in chapter eight, my proof-of-concept study investigated the inflammation elicited after elective angioplasty utilising USPIO-enhanced CMR. I found out a numerical but not statistically significant increase in the change of R2* in the PCI area compared to the remote myocardium or healthy myocardium. This is the first study that has utilised USPIO-enhanced CMR to investigate in vivo the cellular inflammation post elective angioplasty and localise it to the myocardium. Given the small number of patients recruited in this proof-of-concept study, I did not expect to find a statistically significant difference. The finding that there is a numerical, even though not statistically significant, increase in cellular inflammation in the PCI area compared to remote myocardium or healthy myocardium, following elective, uncomplicated PCI is intriguing and warrants further investigation. Further, larger studies are needed to verify the results.

9.2 Future work

The work in this thesis has helped establish some important aspects of patient outcomes and physiology in relation to DES and DCB. However, it also opens avenues for future research. I will now discuss research projects that could be considered in the future.

a) In this thesis I have compared DCB vs DES for patients presenting with stable angina or STEMI. In the STEMI study we excluded patients with cardiogenic shock, cardiac arrest or intubation. It would be important to analyse various other groups such as:
    1) NSTEMI
    2) Bifurcation lesions. Bifurcation lesions account for approximately 20% of all PCIs and are associated with worse outcomes when compared to non-bifurcation lesions (266). Currently, provisional strategy is recommended with 2-stent strategies being reserved for complex bifurcation disease with high-risk characteristics (266). There are scarce data only comparing DCB-only vs DES-only for bifurcation lesions. Our database includes a large number of bifurcation lesions treated only with DCB. It is important to analyse our database and compare DCB-only vs DES-only strategies for bifurcation lesions.
    3) Multivessel PCI. Recent data have shown that a hybrid strategy (DCB and stent) compared with stent only in the context of multivessel PCI improves patient outcomes (267). In our database, there is a considerable number of patients with multivessel PCI treated with DCB-only, rather than hybrid strategy (DCB and DES). It would be important to analyse our database and identify if DCB-only angioplasty for multivessel disease improves patient outcomes compared to stent-only strategy.
    4) Stent thrombosis. There are limited data about the optimal management of patients with stent thrombosis, even though there is general agreement that avoidance of

further metal is desirable unless there is a specific anatomical reason (268). There are no data about the role of DCB in the treatment of stent thrombosis. It would be important to analyse our large database and investigate the outcomes of patients presenting with stent thrombosis and treated with DCB.

5) STEMI with cardiogenic shock, cardiac arrest or intubation. This is one of the highest-risk and most difficult to treat group of patients. Stent under-sizing due to extreme vasoconstriction and antiplatelet malabsorption during a prothrombotic state are some of the factors that increase the risk of stent failure in that context. DCB-only angioplasty has the potential to ameliorate that risk. It would be important to compare DCB vs DES for this complex group of patients.

b) In my thesis, I have not explored the reasons for DCB failure, for example inadequate lesion preparation, undersized DCB, failure to detect high grade dissection, geographical miss. It would be important for such an analysis to take place aiming to detect high-risk characteristics for DCB-failure and how to ameliorate these.

c) Intravascular imaging and physiology testing is strongly advised to guide angioplasty with stents (269). The role of intravascular imaging in angioplasty for de novo disease with DCB is less well defined. It would be important for future prospective studies to clarify the role of intravascular imaging in de novo disease treated with DCB, in terms of assessment of lesion preparation, detection of dissections, determining need for treatment in cases of diffuse disease. It will also be interesting to assess the results of physiology-guided DCB in patients especially if diffuse disease.

d) Recent work has shown that very-late stent-related events occurred between 1 and 5 years after PCI at a rate of about 2%/year with all stent types, with no plateau evident (270). Even though my studies had relatively long follow-up (median >3 years in STEMI cohort and >3.5 years in the elective cohort), it would be valuable to reassess

the outcomes of these patients after 5 years. It might be that DCB-only angioplasty is able to ameliorate very-late angioplasty-related events.

e) My studies have demonstrated that there is equipoise between DCB and DES in de novo disease in real world patients. A large, multicentre randomised controlled trial comparing DCB vs DES for de novo disease, in all vessel sizes and all clinical presentations, is needed to provide the highest level of evidence.

f) In my studies, I included patients after 2015 (Stable angina) and 2016 (STEMI), which represent the years with high and stable rate of DCB angioplasty compared to DES. It will be very interesting to analyse the previous years (before 2015) which represent the learning curve of DCB-angioplasty for our department.

g) In addition to safety and clinical effectiveness, a cost-effectiveness analysis of DCB vs DES is also necessary.

h) The small number of patients and relatively short follow up were two important limitations of my study on inflammatory response post PCI. It would be valuable if a future study could clarify:

   1) How long the circulating intermediate monocytes remain significantly elevated post -PCI?
   2) Is there a significant difference between DCB and DES in terms of the monocyte subset response?
   3) Is the monocyte subset response associated with restenosis?

   Such a study could also pave the way for selective blockade of the specific inflammatory pathway in patients who have undergone PCI in a randomised trial and assess patient outcomes.

9.3 Conclusion

In conclusion this thesis assessed the outcomes of patients treated with DCB-only angioplasty for de novo disease and explored the inflammatory response following elective PCI. I have demonstrated that same-day discharge is safe following elective DCB angioplasty as long as the patient is pain-free with no ECG changes and no more than type B dissection. I demonstrated that there is no evidence of late mortality signal associated with paclitaxel DCB angioplasty. DCB-only angioplasty for stable angina or STEMI due to de novo disease, as compared with second generation DES is safe in terms of all-cause mortality and net cardiac events including unplanned TLR. Finally, I have demonstrated that circulating intermediate monocytes, decrease significantly four hours after elective PCI, recover at two weeks and increase significantly after two months. TNFα and IL6 remain significantly elevated two months after elective PCI.

Overall, these findings suggest that DCB-only angioplasty is a safe alternative PCI strategy that could be considered on a case by case basis. However, given the limited randomised data to date, there should be adequate shared decision with patients as well as appropriate governance and local outcomes monitoring in place. The finding that circulating intermediate monocytes, a highly pro-atherosclerotic monocyte subset, remain significantly increased two months after elective, uncomplicated PCI requires further validation and investigation.

Appendix

Table 1: Biomarkers, monocyte subsets and gene expression at baseline, 4hours, 2 weeks and 2 months post PCI

Characteristic	**Overall**, N = 26[1]	**DCB**, N = 10	**DES**, N = 15	**p-value**[2]
E_hsCRP_Baseline, Median (IQR)	1.43 (0.57 – 3.83)	1.39 (0.67 – 3.48)	1.82 (0.60 – 4.02)	0.85
E_hsCRP_4 Hours, Median (IQR)	1.43 (0.71 – 4.12)	1.35 (0.79 – 4.00)	1.68 (0.67 – 3.98)	0.95
E_hsCRP_2 Weeks, Median (IQR)	1.32 (0.88 – 4.47)	1.16 (0.84 – 4.89)	1.34 (1.06 – 3.78)	0.51
E_hsCRP_2 Months, Median (IQR)	1.70 (0.81 – 3.02)	1.7 (0.8 – 2.4)	2.4 (0.9 – 3.6)	0.93
E_hsTropI_Baseline, Median (IQR)	9.35 (7.80 – 11.20)	9.90 (8.38 – 11.35)	9.30 (7.80 – 10.75)	0.58
E_hsTropI_4 Hours, Median (IQR)	21 (14 – 38)	20 (14 – 28)	26 (16 – 39)	0.63
E_hsTropI_2 Weeks, Median (IQR)	10.0 (8.8 – 15.4)	11.0 (9.2 – 15.7)	9.9 (8.7 – 12.2)	0.49
E_hsTropI_2 Months, Median (IQR)	9.2 (7.3 – 10.9)	9.2 (8.4 – 13.9)	9.7 (7.4 – 9.9)	0.60
E_Pent3_Baseline, Median (IQR)	975 (735 – 1,303)	936 (682 – 982)	1,051 (877 – 1,982)	0.071
E_Pent3_4 Hours, Median (IQR)	1,925 (1,329 – 2,620)	1,726 (1,249 – 1,886)	2,398 (1,686 – 2,969)	**0.031**
E_Pent3_2 Weeks, Median (IQR)	1,090 (845 – 1,559)	1,051 (779 – 1,092)	1,194 (981 – 1,872)	0.055
E_Pent3_2 Months, Median (IQR)	1,234 (753 – 1,460)	1,230 (737 – 1,380)	1,399 (852 – 1,788)	0.22
E_IL6_Baseline, Median (IQR)	0.93 (0.55 – 1.40)	0.88 (0.50 – 1.35)	0.94 (0.74 – 1.43)	0.59
E_IL6_4 Hours, Median (IQR)	4.47 (3.10 – 6.39)	5.63 (4.03 – 7.86)	4.09 (2.88 – 4.90)	0.14

Characteristic	**Overall**, N = 26[1]	**DCB**, N = 10	**DES**, N = 15	**p-value**[2]
E_IL6_2 Weeks, Median (IQR)	1.03 (0.80 – 1.75)	1.02 (0.84 – 1.24)	1.03 (0.80 – 2.01)	0.76
E_IL6_2 Months, Median (IQR)	1.50 (0.91 – 2.01)	1.36 (0.91 – 2.06)	1.61 (0.98 – 1.96)	0.60
E_IL10_Baseline, Median (IQR)	0.25 (0.13 – 0.39)	0.25 (0.13 – 0.37)	0.25 (0.21 – 0.42)	0.62
E_IL10_4 Hours, Median (IQR)	0.33 (0.10 – 0.44)	0.33 (0.14 – 0.42)	0.35 (0.13 – 0.48)	0.79
E_IL10_2 Weeks, Median (IQR)	0.34 (0.24 – 0.40)	0.28 (0.21 – 0.38)	0.37 (0.29 – 0.46)	0.26
E_IL10_2 Months, Median (IQR)	0.32 (0.26 – 0.42)	0.32 (0.22 – 0.55)	0.34 (0.26 – 0.41)	0.73
E_TNFa_Baseline, Median (IQR)	1.73 (1.27 – 2.00)	1.88 (1.41 – 1.97)	1.49 (1.24 – 2.12)	0.81
E_TNFa_4 Hours, Median (IQR)	1.86 (1.34 – 2.32)	2.04 (1.79 – 2.32)	1.74 (1.34 – 2.33)	0.47
E_TNFa_2 Weeks, Median (IQR)	1.91 (1.45 – 2.22)	1.92 (1.52 – 2.16)	1.68 (1.49 – 2.28)	0.85
E_TNFa_2 Months, Median (IQR)	2.05 (1.55 – 2.35)	1.99 (1.48 – 2.39)	2.16 (1.98 – 2.31)	0.73
Classical_Mo_Baseline, Median (IQR)	83 (76 – 89)	87 (79 – 90)	82 (74 – 88)	0.24
Classical_Mo_4 Hours, Median (IQR)	92 (81 – 97)	91 (81 – 96)	94 (84 – 99)	0.41
Classical_Mo_2 Weeks, Median (IQR)	83.2 (79.4 – 86.7)	83.0 (74.8 – 86.8)	85.0 (81.4 – 86.6)	0.43
Classical_Mo_2 Months, Median (IQR)	72 (67 – 82)	82 (69 – 91)	72 (67 – 78)	0.14
Intermediate_Mo_Baseline, Median (IQR)	9 (7 – 15)	8 (7 – 11)	11 (7 – 16)	0.50

Characteristic	**Overall**, N = 26[1]	**DCB**, N = 10	**DES**, N = 15	**p-value**[2]
Intermediate_Mo_4 Hours, Median (IQR)	5.1 (2.0 – 9.5)	7.4 (3.2 – 9.9)	3.3 (0.9 – 8.8)	0.31
Intermediate_Mo_2 Weeks, Median (IQR)	12.1 (8.4 – 15.1)	13.2 (10.1 – 15.8)	9.7 (7.5 – 13.6)	0.16
Intermediate_Mo_2 Months, Median (IQR)	21 (10 – 25)	16 (7 – 21)	25 (13 – 25)	0.093
Non_Classical_Mo_Baseline, Median (IQR)	6.7 (2.7 – 9.3)	3.9 (2.3 – 6.9)	7.2 (5.2 – 9.7)	0.16
Non_Classical_Mo_4 Hours, Median (IQR)	2.3 (0.0 – 8.7)	1.6 (0.4 – 6.5)	4.2 (0.0 – 8.6)	0.98
Non_Classical_Mo_2 Weeks, Median (IQR)	4.8 (2.5 – 7.5)	4.2 (2.3 – 6.3)	6.0 (2.7 – 7.8)	0.50
Non_Classical_Mo_2 Months, Median (IQR)	5.8 (2.2 – 7.9)	2.5 (1.3 – 8.6)	6.1 (5.8 – 7.8)	0.30
PCR_MMP9_Baseline	1	1	1	
PCR_MMP9_2 Weeks, Median (IQR)	0.77 (0.42 – 1.62)	0.79 (0.61 – 1.55)	0.72 (0.36 – 1.56)	0.51
PCR_MMP9_2 Months, Median (IQR)	0.95 (0.50 – 1.36)	1.02 (0.87 – 1.38)	0.87 (0.35 – 1.06)	0.28
PCR_IL10_Baseline	1	1	1	
PCR_IL10_2 Weeks, Median (IQR)	0.96 (0.57 – 1.66)	0.56 (0.41 – 1.14)	1.16 (0.76 – 2.46)	**0.031**
PCR_IL10_2 Months, Median (IQR)	0.75 (0.54 – 0.91)	0.57 (0.46 – 0.77)	1.62 (0.65 – 2.70)	**0.046**
PCR_CCL2_Baseline	1	1	1	
PCR_CCL2_2 Weeks, Median (IQR)	1.10 (0.67 – 1.94)	1.43 (0.82 – 2.43)	1.18 (0.49 – 1.90)	0.63
PCR_CCL2_2 Months, Median (IQR)	1.00 (0.63 – 2.37)	1.57 (1.00 – 3.17)	0.88 (0.57 – 1.52)	0.26
PCR_CXCR4_Baseline	1	1	1	

Characteristic	**Overall**, N = 26[1]	**DCB**, N = 10	**DES**, N = 15	**p-value**[2]
PCR_CXCR4_2 Weeks, Median (IQR)	0.83 (0.64 – 1.12)	0.71 (0.57 – 0.91)	0.98 (0.68 – 1.23)	0.17
PCR_CXCR4_2 Months, Median (IQR)	0.72 (0.52 – 0.86)	0.66 (0.51 – 0.82)	0.79 (0.72 – 0.87)	0.44
PCR_TNF_Baseline	1	1	1	
PCR_TNF_2 Weeks, Median (IQR)	0.78 (0.52 – 1.13)	0.89 (0.57 – 1.44)	0.79 (0.69 – 1.01)	0.80
PCR_TNF_2 Months, Median (IQR)	0.66 (0.36 – 1.10)	1.07 (0.58 – 1.53)	0.65 (0.35 – 0.96)	0.26
PCR_TREM1_Baseline	1	1	1	
PCR_TREM1_2 Weeks, Median (IQR)	0.89 (0.67 – 1.21)	0.96 (0.65 – 1.14)	0.92 (0.85 – 1.25)	0.40
PCR_TREM1_2 Months, Median (IQR)	0.90 (0.62 – 1.32)	0.90 (0.71 – 1.31)	1.08 (0.59 – 1.33)	0.73
PCR_PTX3_Baseline	1	1	1	
PCR_PTX3_2 Weeks, Median (IQR)	1.13 (0.94 – 1.36)	1.10 (0.95 – 1.59)	1.17 (0.96 – 1.34)	0.80
PCR_PTX3_2 Months, Median (IQR)	1.38 (1.08 – 1.98)	1.34 (1.11 – 1.50)	1.85 (1.05 – 2.20)	0.73
PCR_CD36_Baseline	1	1	1	
PCR_CD36_2 Weeks, Median (IQR)	0.90 (0.65 – 1.27)	1.01 (0.67 – 1.25)	0.96 (0.71 – 1.34)	>0.99
PCR_CD36_2 Months, Median (IQR)	0.91 (0.71 – 1.12)	0.91 (0.75 – 1.32)	0.99 (0.74 – 1.07)	0.80
PCR_IL18_Baseline	1	1	1	
PCR_IL18_2 Weeks, Median (IQR)	0.88 (0.74 – 0.97)	0.95 (0.74 – 1.15)	0.87 (0.82 – 0.91)	0.47
PCR_IL18_2 Months, Median (IQR)	0.95 (0.86 – 1.04)	0.99 (0.95 – 1.17)	0.86 (0.69 – 0.97)	0.14

Characteristic	**Overall**, N = 26[1]	**DCB**, N = 10	**DES**, N = 15	**p-value**[2]
PCR_IL1B_Baseline	1	1	1	
PCR_IL1B_2 Weeks, Median (IQR)	0.83 (0.49 – 0.98)	0.89 (0.52 – 1.08)	0.82 (0.58 – 0.93)	0.63
PCR_IL1B_2 Months, Median (IQR)	0.69 (0.51 – 0.88)	0.81 (0.67 – 1.16)	0.62 (0.42 – 0.80)	0.30

Abbreviations

ACE: angiotensin converting enzyme

ACS: acute coronary syndrome

AF: atrial fibrillation

ARB: angiotensin receptor blocker

PAC: admitted patient care

BA: balloon angioplasty

BMI: body mass index

BMS: bare metal stent

CABG: coronary artery bypass graft

cDNA: complimentary deoxyribonucleic acid

CAG: confidentiality advisory group

CCL2: C-C motif ligand 2

CD: cluster of differention

CI: confidence interval

CMR: cardiovascular magnetic resonance

COPD: chronic obstructive pulmonary disease

CRP: C-reactive protein

CXCR4: C-X-C motif chemokine receptor 4

DAPT: dual antiplatelet therapy

DES: drug eluting stent

DCB: drug coated balloon

DEX: dexamethasone eluting stent

ECG: electrocardiogram

ECM: extra cellular matrix

EDTA: ethylenediaminetetraacetic acid

ELISA: enzyme linked immunosorbent assay

eGFR: estimated glomerular filtration rate

FSC(A): forward scatter area

FSC(H): forward scatter height

HASTE: half-fourier acquisition single short turbo spin echo

HES: hospital episode statistics

HR: hazard ratio

ICD: injuries and causes of death

IHD: ischaemic heart disease

IL: interleukin

ION: iron oxide nanoparticles

ISR: in-stent restenosis

LAD: left anterior descending

LCx: left circumflex

LMS: left main stem

LGE: late gadolinium enhancement

Lp-PLA_2: lipoprotein-associated phospholipase A_2

MACE: major adverse cardiovascular events

MACS: magnetic activated cell sorting

MB: main branch

MCP-1: monocyte chemoattractant protein-1

MI: myocardial infarction

MMP: matrix metalloproteinases

MRI: magnetic resonance imaging

NHLBI: national heart, lung and blood institute

NSTEMI: non-ST elevation myocardial infarction

OCT: optical coherence tomography

OPCS: office of population censuses and surveys

OxPL/a[pB: oxidized phospholipids on apolipoprotein B

PBS: phosphate buffered saline

PC: percutaneous coronary intervention

PES: paclitaxel eluting stent

POBA: plain old balloon angioplasty

PTX-3: pentraxin-3

qPCR: quantitative polymerase chain reaction

RCA: right coronary artery

RIR: residual inflammatory risk

RNA: ribonucleic acid

ROI: region of interest

SB: side branch

SD: standard deviation

SES: sirolimus eluting stent

SMC: smooth muscle cell

SPSS: statistical package for the social sciences

SSFP: steady state free precession

SST: serum separator tube

STEMI: ST elevation myocardial infarction

TIA: transient ischaemic attack

TIMI: thrombolysis in myocardial infarction

TLR: target lesion revascularisation

TNFα: tumour necrosis factor α

TREM-1: triggering receptor expressed on myeloid cells

USPIO: ultrasmall superparamagnetic particles of iron oxide

WBC: white blood cell

References

1. Merinopoulos I, Gunawardena T, Wickramarachchi U, Ryding A, Eccleshall S, Vassiliou V. Percutaneous Coronary Intervention in the Elderly: Are Drug-coated Balloons the Future? Curr Cardiol Rev. 2018;14(1):45–52.
2. Merinopoulos I, Gunawardena T, Stirrat C, Cameron D, Eccleshall SC, Dweck MR, et al. Diagnostic Applications of Ultrasmall Superparamagnetic Particles of Iron Oxide for Imaging Myocardial and Vascular Inflammation. JACC Cardiovasc Imaging. 2021;14(6):1249–64.
3. Barton M, Gruntzig J, Husmann M, Rosch J. Balloon Angioplasty - The Legacy of Andreas Gruntzig, M.D. (1939-1985). Front Cardiovasc Med [Internet]. 2014;1(December):1–25. Available from: http://journal.frontiersin.org/article/10.3389/fcvm.2014.00015/abstract
4. Gruntzig A, Senning A, Siegenthaler W. Nonoperative dilatation of coronary-artery stenosis: percutaneous transluminal coronary angioplasty. N Engl J Med. 1979;301(2):61–8.
5. Gruntzig A, King S, Scblumpf M, Siegenthaler W. Long-term follow-up after percutaneous transluminal coronary angioplasty. N Engl J Med. 1987;316:1127–32.
6. Sigwart U, Puel J, Mirkvitch V, Joffre F, Kappenberger L. Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. N Engl J Med. 1987;316:701–6.
7. Serruys P, Jaegere P, Kiemeneij F, Al E. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. N Engl J Med. 1994;331:489–95.
8. Fischman D, Leon M, Baim D, Schatz R, Al E. A randomised comparison of coronary stent placement and balloon angioplasty in the treatment of coronary artery disease. N

Engl J Med. 1994;331:496–501.

9. Serruys PW, Kutryk MJB, Ong ATL. Coronary-Artery Stents. N Engl J Med [Internet]. 2006;354(5):483–95. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra051091

10. Stefanini GG, Holmes DR. Drug-Eluting Coronary-Artery Stents. N Engl J Med [Internet]. 2013;368(3):254–65. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra1210816

11. Li L, Geraghty OC, Mehta Z, Rothwell PM. Age-specific risks, severity, time course, and outcome of bleeding on long-term antiplatelet treatment after vascular events: a population-based cohort study. Lancet [Internet]. 2017;6736(17):1–10. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673617307705

12. Buccheri D, Caramanno G, Geraci S, Cortese B. Should we reconsider dual antiplatelet therapy duration following bioresorbable scaffold angioplasty? J Thorac Dis. 2017 Mar;9(3):417–8.

13. D'Souza S, Ferrante G, Tyczynski P, Mario C Di. Biodegradable Stents - A New Era? Eur Cardiol. 2008;4(2):82–4.

14. Ramadugu P, Latha Alikatte K. A Review on Biodegradable and Bioabsorbable Stents for Coronary Artery Disease. J Bioequiv Availab [Internet]. 2016;08(02):64–7. Available from: https://www.omicsonline.org/open-access/a-review-on-biodegradable-and-bioabsorbable-stents-for-coronary-arterydisease-jbb-1000269.php?aid=66484

15. Peng X, Qu W, Jia Y, Wang Y, Yu B, Tian J. Bioresorbable Scaffolds: Contemporary Status and Future Directions. Front Cardiovasc Med. 2020;7(November):1–14.

16. Yusuf S, Mehra S, Chrolavicius S et al. Effects of Clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med. 2001;345(7):494–502.

17. Mauri L, Kereiakes DJ, Yeh RW, Driscoll-Shempp P, Cutlip DE, Steg PG, et al. Twelve or 30 Months of Dual Antiplatelet Therapy after Drug-Eluting Stents. N Engl J Med [Internet]. 2014;371(23):2155–66. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1409312

18. Alfredsson J, Neely B, Neely ML, Bhatt DL, Goodman SG, Tricoci P, et al. Predicting the risk of bleeding during dual antiplatelet therapy after acute coronary syndromes. Heart. 2017 Aug;103(15):1168–76.

19. Song JW, Soh S, Shim JK. Dual antiplatelet therapy and non-cardiac surgery: Evolving issues and anesthetic implications. Korean J Anesthesiol. 2017;70(1):13–21.

20. Byrne R, Joner M, Alfonso F, Kastrati A. Drug-coated balloon therapy in coronary and peripheral artery disease. Nat Rev Cardiol. 2014;11(1):13–23.

21. Richelsen RKB, Overvad TF, Jensen SE. Drug-Eluting Balloons in the Treatment of Coronary De Novo Lesions: A Comprehensive Review. Cardiol Ther [Internet]. 2016;5(2):133–60. Available from: http://link.springer.com/10.1007/s40119-016-0064-4

22. Ng VG, Mena C, Pietras C, Lansky AJ. Local delivery of paclitaxel in the treatment of peripheral arterial disease. Eur J Clin Invest. 2015;45(3):333–45.

23. Sehgal SN. Sirolimus: Its discovery, biological properties, and mechanism of action. Transplant Proc. 2003;35(3 SUPPL.):S7–14.

24. Jeger R V., Eccleshall S, Wan Ahmad WA, Ge J, Poerner TC, Shin ES, et al. Drug-Coated Balloons for Coronary Artery Disease: Third Report of the International DCB Consensus Group. JACC Cardiovasc Interv. 2020;13(12):1391–402.

25. Yerasi C, Case BC, Forrestal BJ, Torguson R, Weintraub WS, Garcia-Garcia HM, et al. Drug-Coated Balloon for De Novo Coronary Artery Disease: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(9):1061–73.

26. Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, Byrne RA, et al. 2018 ESC/EACTS Guidelines on myocardial revascularization The Task Force on myocardial revascularization of the European Society of Cardiology (ESC) and European Association for Cardio-Thoracic Surgery (EACTS) Developed with the special contribution of th. Eur Heart J [Internet]. 2018;00:1–96. Available from: https://academic.oup.com/eurheartj/advance-article-abstract/doi/10.1093/eurheartj/ehy394/5079120

27. Kufner S, Joner M, Schneider S, Tölg R, Zrenner B, Repp J, et al. Neointimal Modification With Scoring Balloon and Efficacy of Drug-Coated Balloon Therapy in Patients With Restenosis in Drug-Eluting Coronary Stents: A Randomized Controlled Trial. JACC Cardiovasc Interv. 2017;10(13):1332–40.

28. Jeger R V., Farah A, Ohlow MA, Mangner N, Möbius-Winkler S, Weilenmann D, et al. Long-term efficacy and safety of drug-coated balloons versus drug-eluting stents for small coronary artery disease (BASKET-SMALL 2): 3-year follow-up of a randomised, non-inferiority trial. Lancet. 2020;396(10261):1504–10.

29. Jeger R V, Farah A, Ohlow M, Mangner N, Möbius-winkler S, Leibundgut G, et al. Drug-coated balloons for small coronary artery disease (BASKET-SMALL 2): an open-label non-inferiority trial. Lancet. 2018;6736(18):1–8.

30. Tang Y, Qiao S, Tian J, Gao R, Su X, Chen Y, et al. Drug-Coated Balloon Versus Drug-Eluting Stent for Small-Vessel Disease: The RESTORE SVD China Randomized Trial. JACC Cardiovasc Interv. 2018;11(23):2381–92.

31. Welt FGP, Rogers C. Inflammation and restenosis in the stent era. Arterioscler Thromb Vasc Biol. 2002;22(11):1769–76.

32. Libby P, Theroux P. 27. Pathophysiology of coronary artery disease. Circulation [Internet]. 2005;111(25):3481–8. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/15983262

33. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32(9):2045–51.

34. Toutouzas K, Colombo A, Stefanadis C. Inflammation and restenosis after percutaneous coronary interventions. Eur Heart J. 2004;25(19):1679–87.

35. Niccoli G, Montone RA, Ferrante G, Crea F. The evolving role of inflammatory biomarkers in risk assessment after stent implantation. J Am Coll Cardiol. 2010;56(22):1783–93.

36. Tucker B, Vaidya K, Cochran BJ, Patel S. Inflammation during percutaneous coronary intervention— prognostic value, mechanisms and therapeutic targets. Cells. 2021;10(6).

37. Cao DJ. Macrophages in Cardiovascular Homeostasis and Disease. Circulation. 2018;138(22):2452–5.

38. Liuzzo G, Buffon A, Biasucci LM, Gallimore JR, Caligiuri G, Vitelli A, et al. Enhanced inflammatory response to coronary angioplasty in patients with severe unstable angina. Circulation. 1998;98(22):2370–6.

39. Buffon A, Liuzzo G, Biasucci LM, Pasqualetti P, Ramazzotti V, Rebuzzi AG, et al. Preprocedural serum levels of C-reactive protein predict early complications and late restenosis after coronary angioplasty. J Am Coll Cardiol. 1999;34(5):1512–21.

40. Meuwissen M, Piek JJ, van der Wal AC, Chamuleau SAJ, Koch KT, Teeling P, et al. Recurrent unstable angina after directional coronary atherectomy is related to the extent of initial coronary plaque inflammation. J Am Coll Cardiol [Internet]. 2001;37(5):1271–6. Available from: http://dx.doi.org/10.1016/S0735-1097(01)01133-0

41. Moreno PR, Bernardi VH, López-Cuéllar J, Newell JB, McMellon C, Gold HK, et al.

Macrophage infiltration predicts restenosis after coronary intervention in patients with unstable angina. Vol. 94, Circulation. 1996. p. 3098–102.

42. Pietersma A, Kofflard M, De Wit LEA, Stijnen T, Koster JF, Serruys PW, et al. Late lumen loss after coronary angioplasty is associated with the activation status of circulating phagocytes before treatment. Vol. 91, Circulation. 1995. p. 1320–5.

43. Chan AW, Bhatt DL, Chew DP, Reginelli J, Schneider JP, Topol EJ, et al. Relation of inflammation and benefit of statins after percutaneous coronary interventions. Circulation. 2003;107(13):1750–6.

44. Hoshida S, Nishino M, Takeda T, Tanouchi J, Yamada Y, Hori M. A persistent increase in C-reactive protein is a risk factor for restenosis in patients with stable angina who are not receiving statins. Atherosclerosis. 2004;173(2):285–90.

45. Walter DH, Fichtlscherer S, Sellwig M, Auch-Schwelk W, Schächinger V, Zeiher AM. Preprocedural C-reactive protein levels and cardiovascular events after coronary stent implantation. J Am Coll Cardiol. 2001;37(3):839–46.

46. Ferrante G, Niccoli G, Biasucci LM, Liuzzo G, Burzotta F, Galiuto L, et al. Association between C-reactive protein and angiographic restenosis after bare metal stents: an updated and comprehensive meta-analysis of 2747 patients. Cardiovasc Revascularization Med. 2008;9(3):156–65.

47. Rasmussen LM, Andresen JL, Ledet T. Vascular Smooth Muscle Cells. Clin Appl Microcirc Res. 2019;467–80.

48. Rudolph T, Schaps KP, Steven D, Koester R, Rudolph V, Berger J, et al. Interleukin-3 is elevated in patients with coronary artery disease and predicts restenosis after percutaneous coronary intervention. Int J Cardiol [Internet]. 2009;132(3):392–7. Available from: http://dx.doi.org/10.1016/j.ijcard.2007.12.060

49. Arron H V, Cannon CP, Murphy SA, Braunwald E, Gibson CM. Association Between

White Blood Cell Count, Epicardial Blood Flow, Myocardial Perfusion, and Clinical Outcomes in the Setting of Acute Myocardial Infarction A. Circulation. 2000;102:2329–34.

50. Gurm HS, Bhatt DL, Gupta R, Ellis SG, Topol EJ, Lauer MS. Preprocedural white blood cell count and death after percutaneous coronary intervention. Am Heart J. 2003;146(4):692–8.

51. Park DW, Cheol WL, Yun SC, Kim YH, Hong MK, Kim JJ, et al. Prognostic impact of preprocedural C reactive protein levels on 6-month angiographic and 1-year clinical outcomes after drug-eluting stent implantation. Heart. 2007;93(9):1087–92.

52. Park DW, Yun SC, Lee JY, Kim WJ, Kang SJ, Lee SW, et al. C-reactive protein and the risk of stent thrombosis and cardiovascular events after drug-eluting stent implantation. Circulation. 2009;120(20):1987–95.

53. Delhaye C, Maluenda G, Wakabayashi K, Ben-Dor I, Lemesle G, Collins SD, et al. Long-Term Prognostic Value of Preprocedural C-Reactive Protein After Drug-Eluting Stent Implantation. Am J Cardiol. 2010;105(6):826–32.

54. Ishii H, Toriyama T, Aoyama T, Takahashi H, Amano T, Hayashi M, et al. Prognostic values of C-reactive protein levels on clinical outcome after mplantation of sirolimus-eluting stents in patients on hemodialysise. Circ Cardiovasc Interv. 2009;2(6):513–8.

55. Niccoli G, Conte M, Cosentino N, Todaro D, Brugaletta S, Montone R, et al. Baseline C-reactive protein serum levels and in-stent restenosis pattern after m-TOR inhibitors drug-eluting stent implantation. J Invasive. 2011;23:16–20.

56. Oemrawsingh RM, Cheng JM, Martijn Akkerhuis K, Kardys I, Degertekin M, Van Geuns RJ, et al. High-sensitivity C-reactive protein predicts 10-year cardiovascular outcome after percutaneous coronary intervention. EuroIntervention. 2016;12(3):345–51.

57. Montone RA, Niccoli G. Predictive value of C-reactive protein after drug-eluting stent implantation: An update view. Future Cardiol. 2018;14(5):355–8.

58. de Stefano A, Mannucci L, Tamburi F, Cardillo C, Schinzari F, Rovella V, et al. Lp-PLA 2 , a new biomarker of vascular disorders in metabolic diseases. Int J Immunopathol Pharmacol. 2019;33:0–3.

59. Khuseyinova N, Imhof A, Rothenbacher D, Trischler G, Kuelb S, Scharnagl H, et al. Association between Lp-PLA2 and coronary artery disease: Focus on its relationship with lipoproteins and markers of inflammation and hemostasis. Atherosclerosis. 2005;182(1):181–8.

60. Yin Y jie, Chen Y chun, Xu L, Zhao X hai, Song Yang. Relationship of lipoprotein-associated phospholipase A2(Lp-PLA2) and periprocedural myocardial injury in patients undergoing elective percutaneous coronary intervention. IJC Hear Vasc [Internet]. 2020;28:100541. Available from: https://doi.org/10.1016/j.ijcha.2020.100541

61. Shah B, Baber U, Pocock SJ, Krucoff MW, Ariti C, Gibson CM, et al. White blood cell count and major adverse cardiovascular events after percutaneous coronary intervention in the contemporary era insights from the Paris study (Patterns of non-adherence to anti-platelet regimens in stented patients registry). Circ Cardiovasc Interv. 2017;10(8):1–8.

62. Forrester JS, Fishbein M, Helfant R, Fagin J. A paradigm for restenosis based on cell biology: Clues for the development of new preventive therapies. J Am Coll Cardiol. 1991;17(3):758–69.

63. Nobuyoshi M, Kimura T, Ohishi H, Horiuchi H, Nosaka H, Hamasaki N, et al. Restenosis after percutaneous transluminal coronary angioplasty: Pathologic observations in 20 patients. J Am Coll Cardiol [Internet]. 1991;17(2):433–9. Available

from: http://dx.doi.org/10.1016/S0735-1097(10)80111-1

64. Hojo Y, Ikeda U, Katsuki TA, Mizuno O, Fukazawa H, Kurosaki K, et al. Interleukin 6 expression in coronary circulation after coronary angioplasty as a risk factor for restenosis. Heart. 2000;84(1):83–7.

65. Clausell N, De Lima VC, Molossi S, Liu P, Turley E, Gotlieb AI, et al. Expression of tumour necrosis factor a and accumulation of fibronectin in coronary artery restenotic lesions retrieved by atherectomy. Heart. 1995;73(6):534–9.

66. Hokimoto S, OGAWA H, Saito T, Oshima S, Noda K, Soejima H, et al. Increased plasma antigen levels of MCP1 in patients with restenosis after PTCA 2000.pdf.

67. Ikeda U, Shimada K, Cipollone F, Mezzetti A. Elevated circulating levels of monocyte chemoattractant protein-1 in patients with restenosis after coronary angioplasty. Arterioscler Thromb Vasc Biol. 2001;21(6):1090–1.

68. Hokimoto S, Oike Y, Saito T, Kitaoka M, Oshima S, Noda K, et al. Increased expression of monocyte chemoattractant protein-1 in atherectomy specimens from patients with restenosis after percutaneous transluminal coronary angioplasty. Circ J. 2002;66(1):114–6.

69. Ikeda H, Nakayama H, Oda T, Kuwano K, Yamaga A, Ueno T, et al. Neutrophil activation after percutaneous transluminal coronary angioplasty. 1989;126(6):1–5.

70. Neumann FJ, Ott I, Gawaz M, Puchner G, Schömig A. Neutrophil and platelet activation at balloon-injured coronary artery plaque in patients undergoing angioplasty. J Am Coll Cardiol. 1996;27(4):819–24.

71. Inoue T, Sakai Y, Morooka S, Hayashi T, Takayanagi K, Takabatake Y. Expression of polymorphonuclear leukocyte adhesion molecules and its clinical significance in patients treated with percutaneous transluminal coronary angioplasty. J Am Coll Cardiol. 1996;28(5):1127–33.

72. Inoue T, Sakai Y, Hoshi K, Yaguchi I, Fujito T, Morooka S. Lower expression of neutrophil adhesion molecule indicates less vessel wall injury and might explain lower restenosis rate after cutting balloon angioplasty. Circulation. 1998;97(25):2511–8.

73. Iqbal J, Gunn J, Serruys PW. Coronary stents: Historical development, current status and future directions. Br Med Bull. 2013;106(1):193–211.

74. Grewe PH, Deneke T, Machraoui A, Barmeyer J, Müller KM. Acute and chronic tissue response to coronary stent implantation: Pathologic findings in human specimen. J Am Coll Cardiol. 2000;35(1):157–63.

75. Chung IM, Gold HK, Schwartz SM, Ikari Y, Reidy MA, Wight TN. Enhanced extracellular matrix accumulation in restenosis of coronary arteries after stent deployment. J Am Coll Cardiol. 2002;40(12):2072–81.

76. Farb A, Weber DK, Kolodgie FD, Burke AP, Virmani R. Morphological predictors of restenosis after coronary stenting in humans. Circulation. 2002;105(25):2974–80.

77. Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz RS, et al. Pathology of acute and chronic coronary stenting in humans. Circulation. 1999;99(1):44–52.

78. Ghattas A, Griffiths HR, Devitt A, Lip GYH, Shantsila E. Monocytes in coronary artery disease and atherosclerosis: Where are we now? J Am Coll Cardiol. 2013;62(17):1541–51.

79. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, et al. CD14++CD16+ monocytes independently predict cardiovascular events: A cohort study of 951 patients referred for elective coronary angiography. J Am Coll Cardiol. 2012;60(16):1512–20.

80. Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, et al. CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic

kidney disease. Eur Heart J. 2011;32(1):84–92.

81. Fukuda D, Shimada K, Tanaka A, Kawarabayashi T, Yoshiyama M, Yoshikawa J. Circulating Monocytes and In-Stent Neointima after Coronary Stent Implantation. J Am Coll Cardiol. 2004;43(1):18–23.

82. Colombo A, Sangiorgi G. The Monocyte: The Key in the Lock to Reduce Stent Hyperplasia? J Am Coll Cardiol. 2004;43(1):24–6.

83. Liu Y, Imanishi T, Ikejima H, Tsujioka H, Ozaki Y, Kuroi A, et al. Association between circulating monocyte subsets and in-stent restenosis after coronary stent implantation in patients with ST-elevation myocardial infarction. Circ J. 2010;74(12):2585–91.

84. Gaspardone A, Crea F, Versaci F, Tomai F, Pellegrino A, Chiariello L, et al. Predictive value of C-reactive protein after successful coronary-artery stenting in patients with stable angina. Am J Cardiol. 1998;82(4):515–8.

85. Gottsauner-Wolf M, Zasmeta G, Hornykewycz S, Nikfardjam M, Stepan E, Wexberg P, et al. Plasma levels of C-reactive protein after coronary stent implantation. Eur Heart J. 2000;21(14):1152–8.

86. Gach O, Legrand V, Biessaux Y, Chapelle JP, Vanbelle S, Pierard LA. Long-Term Prognostic Significance of High-Sensitivity C-Reactive Protein Before and After Coronary Angioplasty in Patients With Stable Angina Pectoris. Am J Cardiol. 2007;99(1):31–5.

87. Saleh N, Svane B, Hansson LO, Jensen J, Nilsson T, Danielsson O, et al. Response of serum C-reactive protein to percutaneous coronary intervention has prognostic value. Clin Chem. 2005;51(11):2124–30.

88. Inoue T, Kato T, Uchida T, Sakuma M, Nakajima A, Shibazaki M, et al. Local release of C-reactive protein from vulnerable plaque or coronary arterial wall injured by

stenting. J Am Coll Cardiol. 2005;46(2):239–45.

89. Inoue T, Uchida T, Yaguchi I, Sakai Y, Takayanagi K, Morooka S. Stent-induced expression and activation of the leukocyte integrin Mac-1 is associated with neointimal thickening and restenosis. Circulation. 2003;107(13):1757–63.

90. Demyanets S, Tentzeris I, Jarai R, Katsaros KM, Farhan S, Wonnerth A, et al. An increase of interleukin-33 serum levels after coronary stent implantation is associated with coronary in-stent restenosis. Cytokine. 2014;67(2):65–70.

91. Jones GT, Kay IP, Chu JWS, Wilkins GT, Phillips L V., McCormick M, et al. Elevated plasma active matrix metalloproteinase-9 level is associated with coronary artery in-stent restenosis. Arterioscler Thromb Vasc Biol. 2006;26(7):121–5.

92. Ge J, Shen C, Liang C, Chen L, Qian J, Chen H. Elevated matrix metalloproteinase expression after stent implantation is associated with restenosis. Int J Cardiol. 2006;112(1):85–90.

93. Nakano M, Otsuka F, Yahagi K, Sakakura K, Kutys R, Ladich ER, et al. Human autopsy study of drug-eluting stents restenosis: Histomorphological predictors and neointimal characteristics. Eur Heart J. 2013;34(42):3304–13.

94. Zhang MC, Cresswell N, Tavora F, Mont E, Zhao Z, Burke A. In-stent restenosis is associated with neointimal angiogenesis and macrophage infiltrates. Pathol Res Pract [Internet]. 2014;210(12):1026–30. Available from: http://dx.doi.org/10.1016/j.prp.2014.04.004

95. Yoneda S, Abe S, Kanaya T, Oda K, Nishino S, Kageyama M, et al. Late-phase inflammatory response as a feature of in-stent restenosis after drug-eluting stent implantation. Coron Artery Dis. 2013;24(5):368–73.

96. Nakazawa G, Otsuka F, Nakano M, Vorpahl M, Yazdani SK, Ladich E, et al. The pathology of neoatherosclerosis in human coronary implants: Bare-metal and drug-

eluting stents. J Am Coll Cardiol. 2011;57(11):1314–22.

97. Park SJ, Kang SJ, Virmani R, Nakano M, Ueda Y. In-stent neoatherosclerosis: A final common pathway of late stent failure. J Am Coll Cardiol. 2012;59(23):2051–7.

98. Otsuka F, Vorpahl M, Nakano M, Foerst J, Newell JB, Sakakura K, et al. Pathology of second-generation everolimus-eluting stents versus first-generation sirolimus-and paclitaxel-eluting stents in humans. Circulation. 2014;129(2):211–23.

99. Otsuka F, Byrne RA, Yahagi K, Mori H, Ladich E, Fowler DR, et al. Neoatherosclerosis: Overview of histopathologic findings and implications for intravascular imaging assessment. Eur Heart J. 2015;36(32):2147–59.

100. Dibra A, Ndrepepa G, Mehilli J, Dirschinger J, Pache J, Schühlen H, et al. Comparison of C-reactive protein levels before and after coronary stenting and restenosis among patients treated with sirolimus-eluting versus bare metal stents. Am J Cardiol. 2005;95(10):1238–40.

101. Gaspardone A, Versaci F, Tomai F, Citone C, Proietti I, Gioffrè G, et al. C-Reactive Protein, Clinical Outcome, and Restenosis Rates After Implantation of Different Drug-Eluting Stents. Am J Cardiol. 2006;97(9):1311–6.

102. Kim JY, Ko YG, Chi YS, Park S, Hwang KC, Choi D, et al. Comparison of effects of drug-eluting stents versus bare metal stents on plasma C-reactive protein levels. Am J Cardiol. 2005;96(10):1384–8.

103. Kang WC, Ahn TH, Moon CI, Han SH, Shin EK, Kim JS, et al. Comparison of inflammatory markers and angiographic outcomes after implantation of sirolimus and paclitaxel-eluting stents. Heart. 2009;95(12):970–5.

104. Kang WC, Moon C Il, Lee K, Han SH, Suh SY, Moon J, et al. Comparison of inflammatory markers for the prediction of neointimal hyperplasia after drug-eluting stent implantation. Coron Artery Dis. 2011;22(8):526–32.

105. Hsieh IC, Chen CC, Hsieh MJ, Yang CH, Chen DY, Chang SH, et al. Prognostic impact of 9-month high-sensitivity C-reactive protein levels on long-term clinical outcomes and in-stent restenosis in patients at 9 months after drug-eluting stent implantation. PLoS One. 2015;10(9):1–12.

106. Shiba M, Itaya H, Iijima R, Nakamura M. Influence of Late Vascular Inflammation on Long-Term Outcomes Among Patients Undergoing Implantation of Drug Eluting Stents: Role of C-Reactive Protein. J Am Heart Assoc. 2016;5(9):1–10.

107. Kalkman DN, Aquino M, Claessen BE, Baber U, Guedeney P, Sorrentino S, et al. Residual inflammatory risk and the impact on clinical outcomes in patients after percutaneous coronary interventions. Eur Heart J. 2018;39(46):4101–8.

108. Guedeney P, Claessen BE, Kalkman DN, Aquino M, Sorrentino S, Giustino G, et al. Residual Inflammatory Risk in Patients With Low LDL Cholesterol Levels Undergoing Percutaneous Coronary Intervention. J Am Coll Cardiol. 2019;73(19):2401–9.

109. Hudzik B, Szkodzinski J, Pietka-Rzycka A, Danikiewicz A, Wojnar R, Lekston A, et al. Plasma pentraxin 3 may be a more sensitive marker of inflammatory response than high-sensitivity C-reactive protein after bare-metal stent compared to drug-eluting stent implantation. J Interf Cytokine Res. 2013;33(5):280–4.

110. Liu H, Guo X, Wang C, Jie Y, Chen G, Zhang L, et al. Prognostic value of plasma pentraxin-3 levels in patients with stable coronary artery disease after drug-eluting stent implantation. Mediators Inflamm. 2014;2014.

111. Kimura S, Sugiyama T, Hishikari K, Nakagama S, Nakamura S, Misawa T, et al. Relationship of systemic pentraxin-3 values with coronary plaque components on optical coherence tomography and post-percutaneous coronary intervention outcomes in patients with stable angina pectoris. Atherosclerosis [Internet]. 2020;292(November

2019):127–35. Available from: https://doi.org/10.1016/j.atherosclerosis.2019.11.022

112. Liu W, Liu Y, Jiang H, Ding X, Zhu R, Li B, et al. Plasma levels of interleukin 18, interleukin 10, and matrix metalloproteinase-9 and -137G/C polymorphism of interleukin 18 are associated with incidence of in-stent restenosis after percutaneous coronary intervention. Inflammation. 2013;36(5):1129–35.

113. Monraats PS, Kurreeman FAS, Pons D, Sewgobind VDKD, de Vries FR, Zwinderman AH, et al. Interleukin 10: A new risk marker for the development of restenosis after percutaneous coronary intervention. Genes Immun. 2007;8(1):44–50.

114. Liu X, Zhang R, Hou J, Wu J, Zhang M, Fang S, et al. Interleukin-35 promotes early endothelialization after stent implantation by regulating macrophage activation. Clin Sci. 2019;133(7):869–84.

115. Katsaros KM, Kastl SP, Zorn G, Maurer G, Wojta J, Huber K, et al. Increased Restenosis Rate After Implantation of Drug-Eluting Stents in Patients With Elevated Serum Activity of Matrix Metalloproteinase-2 and -9. JACC Cardiovasc Interv. 2010;3(1):90–7.

116. Du JB, Zhang W, Li N, Jiang H, Liu Y, Gao J, et al. Association study of matrix metalloproteinase 3 5A/6A polymorphism with in-stent restenosis after percutaneous coronary interventions in a Han Chinese population. J Int Med Res. 2020;48(1).

117. Koppara T, Cheng Q, Yahagi K, Mori H, Sanchez OD, Feygin J, et al. Thrombogenicity and early vascular healing response in metallic biodegradable polymer-based and fully bioabsorbable drug-eluting stents. Circ Cardiovasc Interv. 2015;8(6):1–9.

118. Otsuka F, Pacheco E, Perkins LEL, Lane JP, Wang Q, Kamberi M, et al. Long-term safety of an everolimus-eluting bioresorbable vascular scaffold and the cobalt-chromium XIENCE v stent in a porcine coronary artery model. Circ Cardiovasc Interv.

2014;7(3):330–42.

119. Rampat R, Williams T, Mayo T, Mengozzi M, Ghezzi P, Hildick-Smith D, et al. Association between inflammatory biomarkers and neointimal response following elective implantation of the ABSORB bioresorbable vascular scaffold. Coron Artery Dis. 2019;30(3):183–7.

120. Chovatiya R, Medzhitov R. Stress, inflammation, and defense of homeostasis. Mol Cell [Internet]. 2014;54(2):281–8. Available from: http://dx.doi.org/10.1016/j.molcel.2014.03.030

121. Bandaru S, Ala C, Salimi R, Akula M, Ekstrand M, Devarakonda S, et al. Targeting Filamin A Reduces Macrophage Activity and Atherosclerosis. Circulation. 2019;140(1):67–9.

122. Ma Y, Mouton AJ, Lindsey ML. Cardiac macrophage biology in the steady-state heart, the aging heart, and following myocardial infarction. Transl Res [Internet]. 2018;191:15–28. Available from: https://doi.org/10.1016/j.trsl.2017.10.001

123. Bjørnerud A, Johansson L. The utility of superparamagnetic contrast agents in MRI: Theoretical consideration and applications in the cardiovascular system. NMR Biomed. 2004;17(7):465–77.

124. Dulińska-Litewka J, Łazarczyk A, Hałubiec P, Szafrański O, Karnas K, Karewicz A. Superparamagnetic Iron Oxide Nanoparticles—Current and Prospective Medical Applications. Materials (Basel) [Internet]. 2019;12(4):617. Available from: http://www.mdpi.com/1996-1944/12/4/617

125. Bietenbeck M, Florian A, Sechtem U, Yilmaz A. The diagnostic value of iron oxide nanoparticles for imaging of myocardial inflammation - Quo vadis? J Cardiovasc Magn Reson [Internet]. 2015;17(1):1–12. Available from: http://dx.doi.org/10.1186/s12968-015-0165-6

126. Cortajarena AL, Ortega D, Ocampo SM, Gonzalez-García A, Couleaud P, Miranda R, et al. Engineering Iron Oxide Nanoparticles for Clinical Settings. Nanobiomedicine. 2014;1(1):2.

127. Stirrat C, Vesey A, McBride O, Alam S, Wallace W, Semple S, et al. Ultra-small superparamagnetic particles of iron oxide in magnetic resonance imaging of cardiovascular disease. J Vasc Diagnostics. 2014;2:99–112.

128. Wang YXJ, Hussain SM, Krestin GP. Superparamagnetic iron oxide contrast agents: Physicochemical characteristics and applications in MR imaging. Eur Radiol. 2001;11(11):2319–31.

129. Bean CP, Livingston JD. Superparamagnetism. J Appl Phys. 1959;30(4):S120–9.

130. Weissleder R, Nahrendorf M, Pittet MJ. Imaging macrophages with nanoparticles. Nat Mater [Internet]. 2014;13(2):125–38. Available from: http://dx.doi.org/10.1038/nmat3780

131. Neuwelt EA, Papisov M, Weissleder R, Bogdanov A. Long-circulating iron oxides for MR imaging. Adv Drug Deliv Rev. 1995;16(617):321–34.

132. Weissleder R, Elizondo G, Wittenberg J, Rabito C, Bengele H, Josephson L. Ultrasmall Agents for Superparamagnetic of a New Class Iron Oxide : of Contrast Agents for MR Imaging. Radiology. 1990;175:489–93.

133. Alam SR, Stirrat C, Spath N, Zamvar V, Pessotto R, Dweck MR, et al. Myocardial inflammation, injury and infarction during on-pump coronary artery bypass graft surgery. J Cardiothorac Surg. 2017;12(1):1–10.

134. Stirrat CG, Alam SR, MacGillivray TJ, Gray CD, Dweck MR, Raftis J, et al. Ferumoxytol-enhanced magnetic resonance imaging assessing inflammation after myocardial infarction. Heart. 2017;103(19):1528–35.

135. Stirrat CG, Alam SR, MacGillivray TJ, Gray CD, Forsythe R, Dweck MR, et al.

Ferumoxytol-enhanced magnetic resonance imaging methodology and normal values at 1.5 and 3T. J Cardiovasc Magn Reson [Internet]. 2016;18(1):1–9. Available from: http://dx.doi.org/10.1186/s12968-016-0261-2

136. Yilmaz A, Rösch S, Klingel K, Kandolf R, Helluy X, Hiller KH, et al. Magnetic resonance imaging (MRI) of inflamed myocardium using iron oxide nanoparticles in patients with acute myocardial infarction - Preliminary results. Int J Cardiol [Internet]. 2013;163(2):175–82. Available from: http://dx.doi.org/10.1016/j.ijcard.2011.06.004

137. Toth GB, Varallyay CG, Horvath A, Bashir MR, Choyke PL, Daldrup-Link HE, et al. Current and potential imaging applications of ferumoxytol for magnetic resonance imaging. Kidney Int. 2017;92(1):47–66.

138. Knobloch G, Colgan T, Wiens C, Wang X, Schubert T, Hernando D, et al. Relaxivity of ferumoxytol at 1.5T and 3.0T. Invest Radiol. 2018;53(5):257–63.

139. Metz S, Bonaterra G, Rudelius M, Settles M, Rummeny EJ, Daldrup-Link HE. Capacity of human monocytes to phagocytose approved iron oxide MR contrast agents in vitro. Eur Radiol. 2004;14(10):1851–8.

140. Neuwelt EA, Hamilton BE, Varallyay CG, Rooney WR, Edelman RD, Jacobs PM, et al. Ultrasmall superparamagnetic iron oxides (USPIOs): A future alternative magnetic resonance (MR) contrast agent for patients at risk for nephrogenic systemic fibrosis (NSF)? Kidney Int. 2009;75(5):465–74.

141. Simon GH, Bauer J, Saborovski O, Fu Y, Corot C, Wendland MF, et al. T1 and T2 relaxivity of intracellular and extracellular USPIO at 1.5T and 3T clinical MR scanning. Eur Radiol. 2006;16(3):738–45.

142. Chovatiya R, Medzhitov R. Stress, inflammation, and defense of homeostasis. Mol Cell [Internet]. 2014;54(2):281–8. Available from: http://dx.doi.org/10.1016/j.molcel.2014.03.030

143. Yilmaz A, Dengler MA, Van Der Kuip H, Yildiz H, Rösch S, Klumpp S, et al. Imaging of myocardial infarction using ultrasmall superparamagnetic iron oxide nanoparticles: A human study using a multi-parametric cardiovascular magnetic resonance imaging approach. Eur Heart J. 2013;34(6):462–75.

144. Sosnovik DE, Nahrendorf M, Deliolanis N, Novikov M, Aikawa E, Josephson L, et al. Fluorescence tomography and magnetic resonance imaging of myocardial macrophage infiltration in infarcted myocardium in vivo. Circulation. 2007;115(11):1384–91.

145. Montet-Abou K, Daire JL, Hyacinthe JN, Jorge-Costa M, Grosdemange K, MacH F, et al. In vivo labelling of resting monocytes in the reticuloendothelial system with fluorescent iron oxide nanoparticles prior to injury reveals that they are mobilized to infarcted myocardium. Eur Heart J. 2010;31(11):1410–20.

146. Yang Y, Yang Y, Yanasak N, Schumacher A, Hu TCC. Temporal and noninvasive monitoring of inflammatory-cell infiltration to myocardial infarction sites using micrometer-sized iron oxide particles. Magn Reson Med. 2010;63(1):33–40.

147. Protti A, Dong X, Andia ME, Yu B, Dokukina K, Chaubey S, et al. Assessment of inflammation with a very small iron-oxide particle in a murine model of reperfused myocardial infarction. J Magn Reson Imaging. 2014;39(3):598–608.

148. Saleh A, Schroeter M, Jonkmanns C, Hartung HP, Mödder U, Jander S. In vivo MRI of brain inflammation in human ischaemic stroke. Brain. 2004;127(7):1670–7.

149. Saleh A, Schroeter M, Ringelstein A, Hartung HP, Siebler M, Mödder U, et al. Iron oxide particle-enhanced MRI suggests variability of brain inflammation at early stages after ischemic stroke. Stroke. 2007;38(10):2733–7.

150. Wang G, Serkova NJ, Groman E V., Scheinman RI, Simberg D. Feraheme (Ferumoxytol) Is Recognized by Proinflammatory and Anti-inflammatory Macrophages via Scavenger Receptor Type AI/II. Mol Pharm. 2019;16(10):4274–81.

151. Messroghli DR, Moon JC, Ferreira VM, Grosse-Wortmann L, He T, Kellman P, et al. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2 and extracellular volume: A consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imagin. J Cardiovasc Magn Reson. 2017;19(1):1–24.

152. Smith GC, Carpenter JP, He T, Alam MH, Firmin DN, Pennell DJ. Value of black blood T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2011;13(1):1–6.

153. Stirrat CG, Alam SR, MacGillivray TJ, Gray CD, Forsythe R, Dweck MR, et al. Ferumoxytol-enhanced magnetic resonance imaging methodology and normal values at 1.5 and 3T. J Cardiovasc Magn Reson [Internet]. 2016;18(1):1–9. Available from: http://dx.doi.org/10.1186/s12968-016-0261-2

154. Finn JP, Nguyen KL, Han F, Zhou Z, Salusky I, Ayad I, et al. Cardiovascular MRI with ferumoxytol. Clin Radiol. 2016;71(8):796–806.

155. Taktak S, Sosnovik D, Cima MJ, Weissleder R, Josephson L. Multiparameter magnetic relaxation switch assays. Anal Chem. 2007;79(23):8863–9.

156. Harisinghani M, Dixon T, Saksena M, Brachtel E, Blezek D, Dhawale P, et al. phy : Imaging Strategies to Optimize the Imag- ing of Lymph Nodes with Ferumoxtran-10 1. Imaging Ther Technol. 2004;24(3):867–78.

157. Alam SR, Shah ASV, Richards J, Lang NN, Barnes G, Joshi N, et al. Ultrasmall superparamagnetic particles of iron oxide in patients with acute myocardial infarction early clinical experience. Circ Cardiovasc Imaging. 2012;5(5):559–65.

158. Lagan J, Naish J, Clark D, Foden P, Caldwell J, Trafford A, et al. In vivo evidence of chronic myocardial inflammation in ischaemic cardiomyopathy using USPIO enhanced cardiovascular magnetic resonance. Eur Heart J. 2017;Abstract 3:693–4.

159. Ferreira VM, Schulz-Menger J, Holmvang G, Kramer CM, Carbone I, Sechtem U, et al. Cardiovascular Magnetic Resonance in Nonischemic Myocardial Inflammation: Expert Recommendations. J Am Coll Cardiol. 2018;72(24):3158–76.

160. Moon H, Park HE, Kang J, Lee H, Cheong C, Lim YT, et al. Noninvasive assessment of myocardial inflammation by cardiovascular magnetic resonance in a rat model of experimental autoimmune myocarditis. Circulation. 2012;125(21):2603–12.

161. Stirrat CG, Alam SR, MacGillivray TJ, Gray CD, Dweck MR, Dibb K, et al. Ferumoxytol-enhanced magnetic resonance imaging in acute myocarditis. Heart. 2018;104(4):300–5.

162. Mahrholdt H, Goedecke C, Wagner A, Meinhardt G, Athanasiadis A, Vogelsberg H, et al. Cardiovascular Magnetic Resonance Assessment of Human Myocarditis. Circulation. 2004;109(10):1250–8.

163. Scally C, Abbas H, Ahearn T, Srinivasan J, Mezincescu A, Rudd A, et al. Myocardial and Systemic Inflammation in Acute Stress-Induced (Takotsubo) Cardiomyopathy. Circulation [Internet]. 2019;139(13):1581–92. Available from: https://clinicaltrials.gov

164. Abdel-Aty H, Cocker M, Friedrich MG. Myocardial edema is a feature of Tako-Tsubo cardiomyopathy and is related to the severity of systolic dysfunction: Insights from T2-weighted cardiovascular magnetic resonance. Int J Cardiol. 2009;132(2):291–3.

165. Eitel I, Lücke C, Grothoff M, Sareban M, Schuler G, Thiele H, et al. Inflammation in takotsubo cardiomyopathy: Insights from cardiovascular magnetic resonance imaging. Eur Radiol. 2010;20(2):422–31.

166. Sagris M, Antonopoulos AS, Simantiris S, Oikonomou E, Siasos G, Tsioufis K, et al. Pericoronary fat attenuation index—a new imaging biomarker and its diagnostic and prognostic utility: a systematic review and meta-analysis. Eur Heart J Cardiovasc Imaging [Internet]. 2022;23(12):E526–36. Available from:

https://doi.org/10.1093/ehjci/jeac174

167. Antoniades C, Kotanidis CP, Berman DS. State-of-the-art review article. Atherosclerosis affecting fat: What can we learn by imaging perivascular adipose tissue? J Cardiovasc Comput Tomogr [Internet]. 2019;13(5):288–96. Available from: https://doi.org/10.1016/j.jcct.2019.03.006

168. Klüner L V., Oikonomou EK, Antoniades C. Assessing cardiovascular risk by using the fat attenuation index in coronary ct angiography. Radiol Cardiothorac Imaging. 2021;3(1).

169. Oikonomou EK, Marwan M, Desai MY, Mancio J, Alashi A, Hutt Centeno E, et al. Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. Lancet [Internet]. 2018;392(10151):929–39. Available from: http://dx.doi.org/10.1016/S0140-6736(18)31114-0

170. Marimuthu R, Francis H, Dervish S, Li SCH, Medbury H, Williams H. Characterization of human monocyte subsets by whole blood flow cytometry analysis. J Vis Exp. 2018;2018(140):1–10.

171. Garcia-Garcia HM, McFadden EP, Farb A, Mehran R, Stone GW, Spertus J, et al. Standardized end point definitions for coronary intervention trials: The academic research consortium-2 consensus document. Circulation. 2018;137(24):2635–50.

172. Rahman Z, Ullah M, Choudhury AK. Coronary Artery Dissection and Perforation Complicating Percutaneous Coronary Intervention – A Review. Cardiovasc J. 2011;3(2):239–47.

173. Park TK, Park YH, Song Y Bin, Oh JH, Chun WJ, Kang GH, et al. Long-term clinical outcomes of true and non-true bifurcation lesions according to medina classification - Results from the COBIS (COronary BIfurcation stent) II registry. Circ J.

2015;79(9):1954–62.

174. Gilbert T, Neuburger J, Kraindler J, Keeble E, Smith P, Ariti C, et al. Development and validation of a Hospital Frailty Risk Score focusing on older people in acute care settings using electronic hospital records: an observational study. Lancet [Internet]. 2018;391(10132):1775–82. Available from: http://dx.doi.org/10.1016/S0140-6736(18)30668-8

175. Merinopoulos I, Wickramarachchi U, Wardley J, Khanna V, Gunawardena T, Maart C, et al. Day case discharge of patients treated with drug coated balloon only angioplasty for de novo coronary artery disease : A single center experience. Catheter Cardiovasc Interv. 2020;95(1):105–8.

176. Neumann F-J, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, et al. ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J [Internet]. 2018;1–96. Available from: https://academic.oup.com/eurheartj/advance-article/doi/10.1093/eurheartj/ehy394/5079120

177. Jeger R V, Farah A, Ohlow M, Mangner N, Möbius-winkler S, Leibundgut G, et al. Drug-coated balloons for small coronary artery disease non-inferiority trial. 2018;6736(18):1–8.

178. Wickramarachchi U, Eccleshall S. Drug-coated Balloon-only Angioplasty for Native Coronary Disease Instead of Stents. Interv Cardiol Rev. 2016;11(2):110–5.

179. Kumar S, Anantharaman R, Das P, Hobbs J, Densem C, Ansell J, et al. Radial approach to day case intervention in coronary artery lesions (RADICAL): A single centre safety and feasibility study. Heart. 2004;90(11):1340–1.

180. Kiemeneij F, Laarman GJ, Slagboom T, Van Der Wieken R. Outpatient coronary stent implantation. J Am Coll Cardiol [Internet]. 1997;29(2):323–7. Available from: http://dx.doi.org/10.1016/S0735-1097(96)00486-X

181. Cortese B, Silva Orrego P, Agostoni P, Buccheri D, Piraino D, Andolina G, et al. Effect of Drug-Coated Balloons in Native Coronary Artery Disease Left with a Dissection. JACC Cardiovasc Interv. 2015;8(15):2003–9.

182. Black AJ, Namay DL, Niederman AL, Lembo NJ, Roubin GS, Douglas JS, et al. Tear or dissection after coronary angioplasty. Morphologic correlates of an ischemic complication. Circulation [Internet]. 1989;79(5):1035–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2523763

183. Ludman PF, Gavalova L. National Audit of Percutaneous Coronary Interventions Annual Report [Internet]. 2015. Available from: http://www.ucl.ac.uk/nicor/audits/adultpercutaneous/reports

184. Theuerle J, Yudi M, Farouque O, Andrianopoulos N, Scott P, Ajani A, et al. Utility of the ACC/AHA lesion classification as a predictor of procedural, 30-day and 12-month outcomes in the contemporary percutaneous coronary intervention era. Catheter Cardiovasc Interv. 2018;92(3):227–34.

185. Albertal M, Langenhove G Van, Regar E, Kay IP, Foley D, Sianos G, et al. Uncomplicated moderate coronary artery dissections after balloon angioplasty : good outcome without stenting. Heart. 2001;86:193–8.

186. K J, A B. Unit Costs of Health and Social Care 2021. 2021;

187. Amin AP, Pinto D, House JA, Rao S V., Spertus JA, Cohen MG, et al. Association of Same-Day Discharge After Elective Percutaneous Coronary Intervention in the United States With Costs and Outcomes. JAMA Cardiol [Internet]. 2018;63110:1–9. Available from: http://cardiology.jamanetwork.com/article.aspx?doi=10.1001/jamacardio.2018.3029

188. Rinfret S, Kennedy WA, Lachaine J, Lemay A, Rods-Cabau J, Cohen DJ, et al. Economic impact of same-day home discharge after uncomplicated transradial

percutaneous coronary intervention and bolus-only abciximab regimen. JACC Cardiovasc Interv [Internet]. 2010;3(10):1011–9. Available from: http://dx.doi.org/10.1016/j.jcin.2010.07.011

189. Merinopoulos I, Gunawardena T, Wickramarachchi U, Richardson P, Maart C, Sreekumar S, et al. Long-term safety of paclitaxel drug-coated balloon-only angioplasty for de novo coronary artery disease: the SPARTAN DCB study. Clin Res Cardiol [Internet]. 2020; Available from: https://doi.org/10.1007/s00392-020-01734-6

190. Rigatelli G, Zuin M, Nikolov P, Mileva N, Vassilev D. One- and 3-year outcomes of percutaneous bifurcation left main revascularization with modern drug-eluting stents: a systematic review and meta-analysis. Clin Res Cardiol [Internet]. 2020;(0123456789). Available from: https://doi.org/10.1007/s00392-020-01679-w

191. Neumann JT, Goßling A, Sörensen NA, Blankenberg S, Magnussen C, Westermann D. Temporal trends in incidence and outcome of acute coronary syndrome. Clin Res Cardiol [Internet]. 2020;(Mi). Available from: https://doi.org/10.1007/s00392-020-01612-1

192. Colleran R, Kastrati A. Percutaneous coronary intervention: balloons, stents and scaffolds. Clin Res Cardiol [Internet]. 2018;107(0):55–63. Available from: http://dx.doi.org/10.1007/s00392-018-1328-x

193. Jeger R V, Farah A, Ohlow M, Mangner N, Möbius-winkler S, Leibundgut G, et al. Drug-coated balloons for small coronary artery disease (BASKET-SMALL 2): an open-label randomised non-inferiority trial. Lancet. 2018;6736(18):1–8.

194. Rissanen TT, Uskela S, Eränen J, Mäntylä P, Olli A, Romppanen H, et al. Drug-coated balloon for treatment of de-novo coronary artery lesions in patients with high bleeding risk (DEBUT): a single-blind, randomised, non-inferiority trial. Lancet [Internet]. 2019;6736(19):1–10. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S0140673619311262

195. Vos NS, Fagel ND, Amoroso G, Herrman J-PR, Patterson MS, Piers LH, et al. Paclitaxel-Coated Balloon Angioplasty Versus Drug-Eluting Stent in Acute Myocardial Infarction. JACC Cardiovasc Interv [Internet]. 2019;9(12(17)):1691–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1936879819309318

196. Fahrni G, Scheller B, Coslovsky M, Gilgen N, Farah A, Ohlow MA, et al. Drug-coated balloon versus drug-eluting stent in small coronary artery lesions: angiographic analysis from the BASKET-SMALL 2 trial. Clin Res Cardiol [Internet]. 2020;(0123456789). Available from: https://doi.org/10.1007/s00392-020-01603-2

197. Yu X, Xu F, Zhang W, Wang X, Lu D, Yang C, et al. Treatment of large de novo coronary lesions with paclitaxel-coated balloon only: results from a Chinese institute. Clin Res Cardiol [Internet]. 2019;108(3):234–43. Available from: http://dx.doi.org/10.1007/s00392-018-1346-8

198. Ali RM, Abdul Kader MASK, Wan Ahmad WA, Ong TK, Liew HB, Omar AF, et al. Treatment of Coronary Drug-Eluting Stent Restenosis by a Sirolimus- or Paclitaxel-Coated Balloon. JACC Cardiovasc Interv. 2019;12(6):558–66.

199. Cortese B. Sirolimus-Coated Balloons: A Ferrari for Our Coronary Patients or Another Elegant Town Car? JACC Cardiovasc Interv [Internet]. 2019;12(6):567–8. Available from: https://doi.org/10.1016/j.jcin.2018.12.034

200. Katsanos K, Kitrou P, Karnabatidis D, Spiliopoulos S, Krokidis M. Risk of death following application of paclitaxel-coated balloons and stents in the femoropopliteal artery of the leg: A systematic review and meta-analysis of randomized controlled trials. J Am Heart Assoc. 2018;7(24).

201. Rocha-Singh KJ, Duval S, Jaff MR, Schneider PA, Ansel GM, Lyden SP, et al. Mortality and Paclitaxel-Coated Devices: An Individual Patient Data Meta-Analysis.

Circulation. 2020;141(23):1859–69.

202. Schneider PA, Laird JR, Doros G, Gao Q, Ansel G, Brodmann M, et al. Mortality Not Correlated With Paclitaxel Exposure: An Independent Patient-Level Meta-Analysis of a Drug-Coated Balloon. J Am Coll Cardiol. 2019;73(20):2550–63.

203. Albrecht T, Schnorr B, Kutschera M, Waliszewski MW. Two-Year Mortality After Angioplasty of the Femoro-Popliteal Artery with Uncoated Balloons and Paclitaxel-Coated Balloons—A Pooled Analysis of Four Randomized Controlled Multicenter Trials. Cardiovasc Intervent Radiol [Internet]. 2019;42(7):949–55. Available from: https://doi.org/10.1007/s00270-019-02194-w

204. FDA. UPDATE: Treatment of Peripheral Arterial Disease with Paclitaxel-Coated Balloons and Paclitaxel-Eluting Stents Potentially Associated with Increased Mortality [Internet]. 2019 [cited 2019 Oct 28]. Available from: https://www.fda.gov/medical-devices/letters-health-care-providers/august-7-2019-update-treatment-peripheral-arterial-disease-paclitaxel-coated-balloons-and-paclitaxel

205. Waksman R, Pakala R. Drug-eluting balloon: The comeback kid? Circ Cardiovasc Interv. 2009;2(4):352–8.

206. Rissanen TT, Uskela S, Eränen J, Mäntylä P, Olli A, Romppanen H, et al. Drug-coated balloon for treatment of de-novo coronary artery lesions in patients with high bleeding risk (DEBUT): a single-blind, randomised, non-inferiority trial. Lancet. 2019;394(10194):230–9.

207. Giacoppo D, Alfonso F, Xu B, Bimmer EP, Pe J, Kang D, et al. Paclitaxel-coated balloon angioplasty vs . drug-eluting stenting for the treatment of coronary in-stent restenosis : a comprehensive , collaborative , individual patient data meta-analysis of 10 randomized clinical trials (DAEDALUS study). Eur Heart J. 2019;1–14.

208. Scheller B, Vukadinovic D, Jeger R, Rissanen TT, Scholz SS, Byrne R, et al. Survival

After Coronary Revascularization With Paclitaxel-Coated Balloons. J Am Coll Cardiol. 2020;75(9):1017–28.

209. Beckman JA, White CJ. Paclitaxel-Coated Balloons and Eluting Stents: Is There a Mortality Risk in Patients with Peripheral Artery Disease? Circulation. 2019;140(16):1342–51.

210. Mills JL, Conte MS, Murad MH. Critical review and evidence implications of paclitaxel drug-eluting balloons and stents in peripheral artery disease. J Vasc Surg [Internet]. 2019;70(1):3–7. Available from: https://doi.org/10.1016/j.jvs.2019.05.002

211. Saratzis A, Mani K, Zayed H. Paclitaxel Eluting Endovascular Technology and Long-Term Mortality: Safety Concern or a Reminder of an Obvious Literature Gap? Eur J Vasc Endovasc Surg [Internet]. 2019;57(3):327–8. Available from: https://doi.org/10.1016/j.ejvs.2019.01.030

212. Farb A, Malone M, Maisel W. Drug-coated devices for peripheral arterial disease. N Engl J Med. 2021;384(2):97–9.

213. Ludman PF. BCIS Audit returns adult interventional procedures [Internet]. 2019 [cited 2019 Oct 28]. Available from: http://www.bcis.org.uk/wp-content/uploads/2019/02/BCIS-Audit-2017-18-data-for-web-ALL-excl-TAVI-as-27-02-2019.pdf

214. Merinopoulos I, Gunawardena T, Corballis N, Bhalraam U, Gilbert T, Maart C, et al. Paclitaxel drug-coated balloon-only angioplasty for de novo coronary artery disease in elective clinical practice. Clin Res Cardiol [Internet]. 2022; Available from: https://doi.org/10.1007/s00392-022-02106-y

215. Neumann FJ, Sechtem U, Banning AP, Bonaros N, Bueno H, Bugiardini R, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J. 2020;41(3):407–77.

216. Jeger R V., Eccleshall S, Wan Ahmad WA, Ge J, Poerner TC, Shin ES, et al. Drug-Coated Balloons for Coronary Artery Disease: Third Report of the International DCB Consensus Group. JACC Cardiovasc Interv. 2020;13(12):1391–402.

217. Fahrni G, Scheller B, Coslovsky M, Gilgen N, Farah A, Ohlow M-A, et al. Drug-coated balloon versus drug-eluting stent in small coronary artery lesions: angiographic analysis from the BASKET-SMALL 2 trial. Clin Res Cardiol. 2020;109:1114–24.

218. Jeger R V, Farah A, Ohlow M-A, Mangner N, Möbius-Winkler S, Weilenmann D, et al. Long-term efficacy and safety of drug-coated balloons versus drug-eluting stents for small coronary artery disease (BASKET-SMALL 2): 3-year follow-up of a randomised, non-inferiority trial. Lancet. 2020;6736(20):1–7.

219. Jeger R V., Kaiser C, Mangner N, Kleber FX, Scheller B. Causes of death after treatment of small coronary artery disease with paclitaxel-coated balloons. Clin Res Cardiol [Internet]. 2021;110(2):307–11. Available from: https://doi.org/10.1007/s00392-020-01674-1

220. Merinopoulos I, Gunawardena T, Wickramarachchi U, Richardson P, Maart C, Sreekumar S, et al. Long-term safety of paclitaxel drug-coated balloon-only angioplasty for de novo coronary artery disease: the SPARTAN DCB study. Clin Res Cardiol [Internet]. 2021;110(2):220–7. Available from: https://doi.org/10.1007/s00392-020-01734-6

221. Rahman Z, Ullah M, Choudhury A. Coronary Artery Dissection and Perforation Complicating Percutaneous Coronary Intervention – A Review. Cardiovasc J. 2011;3(2):239–47.

222. Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, et al. 2018 ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J. 2019;40(2):87–165.

223. Venetsanos D, Lawesson SS, Panayi G, Tödt T, Berglund U, Swahn E, et al. Long-term efficacy of drug coated balloons compared with new generation drug-eluting stents for the treatment of de novo coronary artery lesions. Catheter Cardiovasc Interv. 2018;92(5):E317–26.

224. Rosenberg M, Waliszewski M, Krackhardt F, Chin K, Wan Ahmad WA, Caramanno G, et al. Drug Coated Balloon-Only Strategy in de Novo Lesions of Large Coronary Vessels. J Interv Cardiol. 2019;2019.

225. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J. 2018;39(2):119–77.

226. Palmerini T, Biondi-Zoccai G, Della Riva D, Mariani A, Sabaté M, Valgimigli M, et al. Clinical outcomes with drug-eluting and bare-metal stents in patients with ST-segment elevation myocardial infarction: Evidence from a comprehensive network meta-analysis. J Am Coll Cardiol [Internet]. 2013;62(6):496–504. Available from: http://dx.doi.org/10.1016/j.jacc.2013.05.022

227. Rissanen TT, Uskela S, Eränen J, Mäntylä P, Olli A, Romppanen H, et al. Drug-coated balloon for treatment of de-novo coronary artery lesions in patients with high bleeding risk (DEBUT): a single-blind, randomised, non-inferiority trial. Lancet. 2019;394(10194):230–9.

228. Ho HH. Preliminary experience with drug-coated balloon angioplasty in primary percutaneous coronary intervention. World J Cardiol. 2015;7(6):311.

229. Teacher L, Yellow D, King A, Zhang JC, Liu LH, Road Y. Study on the safety and effectiveness of drug-coated balloons in patients with acute myocardial infarction. J Cardiothorac Surg. 2021;16(1):1–7.

230. Nijhoff F, Agostoni P, Belkacemi A, Nathoe HM, Voskuil M, Samim M, et al. Primary

percutaneous coronary intervention by drug-eluting balloon angioplasty: The nonrandomized fourth arm of the DEB-AMI (drug-eluting balloon in ST-segment elevation myocardial infarction) trial. Catheter Cardiovasc Interv. 2015;86:S34–44.

231. Mehran R, Pocock SJ, Nikolsky E, Clayton T, Dangas GD, Kirtane AJ, et al. A Risk Score to Predict Bleeding in Patients With Acute Coronary Syndromes. J Am Coll Cardiol [Internet]. 2010;55(23):2556–66. Available from: http://dx.doi.org/10.1016/j.jacc.2009.09.076

232. Grines C, Browse K, Marco J, Rothbaum D, Stone G, O'Keefe J, et al. A comparison of immediate angioplasty with thrombolytic therapy for acute myocardial infarction. N Engl J Med. 1993;328(10):673–9.

233. Zijlstra F. Long-term benefit of primary angioplasty compared to thrombolytic therapy for acute myocardial infarction. Eur Heart J. 2000;21(18):1487–9.

234. Bangalore S, Amoroso N, Fusaro M, Kumar S, Feit F. Outcomes with various drug-eluting or bare metal stents in patients with st-segment-elevation myocardial infarction: A mixed treatment comparison analysis of trial level data from 34 068 patient-years of follow-up from randomized trials. Circ Cardiovasc Interv. 2013;6(4):378–90.

235. Chichareon P, Modolo R, Collet C, Tenekecioglu E, Vink MA, Oh PC, et al. Efficacy and Safety of Stents in ST-Segment Elevation Myocardial Infarction. J Am Coll Cardiol. 2019;74(21):2572–84.

236. Vos NS, Fagel ND, Amoroso G, Herrman JPR, Patterson MS, Piers LH, et al. Paclitaxel-Coated Balloon Angioplasty Versus Drug-Eluting Stent in Acute Myocardial Infarction: The REVELATION Randomized Trial. JACC Cardiovasc Interv. 2019;12(17):1691–9.

237. Gobić D, Tomulić V, Lulić D, Židan D, Brusich S, Jakljević T, et al. Drug-Coated

Balloon Versus Drug-Eluting Stent in Primary Percutaneous Coronary Intervention: A Feasibility Study. Am J Med Sci [Internet]. 2017;354(6):553–60. Available from: http://dx.doi.org/10.1016/j.amjms.2017.07.005

238. Urban P, Mehran R, Colleran R, Angiolillo DJ, Byrne RA, Capodanno D, et al. Defining high bleeding risk in patients undergoing percutaneous coronary intervention: a consensus document from the Academic Research Consortium for High Bleeding Risk. Eur Heart J. 2019;40(31):2632–53.

239. Merinopoulos I, Gunawardena T, Corballis NH, Tsampaisan V, Eccleshall S, SMITH J, et al. The role of inflammation in percutaneous coronary intervention, from balloon angioplasty to drug eluting stents. Minerva cardiol Angiol. 2022;

240. Zhao TX, Mallat Z. Targeting the Immune System in Atherosclerosis: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;73(13):1691–706.

241. Nidorf SM, Fiolet ATL, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, et al. Colchicine in Patients with Chronic Coronary Disease. N Engl J Med. 2020;383(19):1838–47.

242. Wong KL, Tai JJY, Wong WC, Han H, Sem X, Yeap WH, et al. Gene expression profiling reveals the defining features of the classical, intermediate,and nonclassical human monocyte subsets. Blood [Internet]. 2011;118(5):e16–31. Available from: http://dx.doi.org/10.1182/blood-2010-12-326355

243. Inoue T, Sohma R, Miyazaki T, Iwasaki Y, Yaguchi I, Morooka S. Comparison of activation process of platelets and neutrophils after coronary stent implantation versus balloon angioplasty for stable angina pectoris. Am J Cardiol. 2000;86(10):1057–62.

244. Krychtiuk KA, Kastl SP, Hofbauer SL, Wonnerth A, Goliasch G, Ozsvar-Kozma M, et al. Monocyte subset distribution in patients with stable atherosclerosis and elevated levels of lipoprotein(a). J Clin Lipidol [Internet]. 2015;9(4):533–41. Available from:

http://dx.doi.org/10.1016/j.jacl.2015.04.005

245. Zeng S, Zhou X, Ge L, Ji WJ, Shi R, Lu RY, et al. Monocyte subsets and monocyte-platelet aggregates in patients with unstable angina. J Thromb Thrombolysis. 2014;38(4):439–46.

246. Zhou X, Liu XL, Ji WJ, Liu JX, Guo ZZ, Ren D, et al. The kinetics of circulating monocyte subsets and monocyte-platelet aggregates in the acute phase of st-elevation myocardial infarction associations with 2-year cardiovascular events. Med (United States). 2016;95(18):e3466.

247. Wildgruber M, Aschenbrenner T, Wendorff H, Czubba M, Glinzer A, Haller B, et al. The "intermediate" CD14++ CD16+ monocyte subset increases in severe peripheral artery disease in humans. Sci Rep. 2016;6(July):1–8.

248. Wildgruber M, Czubba M, Aschenbrenner T, Wendorff H, Hapfelmeier A, Glinzer A, et al. Increased intermediate CD14++CD16++ monocyte subset levels associate with restenosis after peripheral percutaneous transluminal angioplasty. Atherosclerosis [Internet]. 2016;253:128–34. Available from: http://dx.doi.org/10.1016/j.atherosclerosis.2016.09.002

249. Suzuki A, Fukuzawa K, Yamashita T, Yoshida A, Sasaki N, Emoto T, et al. Circulating intermediate CD1411CD161 monocytes are increased in patients with atrial fibrillation and reflect the functional remodelling of the left atrium. Europace. 2017;19(1):40–7.

250. Mai W, Liao Y. Targeting IL-1β in the Treatment of Atherosclerosis. Front Immunol. 2020;11(December):1–8.

251. Döring Y, Noels H, Van Der Vorst EPC, Neideck C, Egea V, Drechsler M, et al. Vascular CXCR4 limits atherosclerosis by maintaining arterial integrity: Evidence from mouse and human studies. Circulation. 2017;136(4):388–403.

252. Ristagno G, Fumagalli F, Bottazzi B, Mantovani A, Olivari D, Novelli D, et al. Pentraxin 3 in cardiovascular disease. Front Immunol. 2019;10(April):1–11.

253. Liu H, Guo X, Wang C, Jie Y, Chen G, Zhang L, et al. Prognostic value of plasma pentraxin-3 levels in patients with stable coronary artery disease after drug-eluting stent implantation. Mediators Inflamm. 2014;2014.

254. Tousoulis Di, Oikonomou E, Economou EK, Crea F, Kaski JC. Inflammatory cytokines in atherosclerosis: Current therapeutic approaches. Eur Heart J. 2016;37(22):1723–35.

255. Monraats PS, Pires NMM, Schepers A, Agema WRP, Boesten LSM, Vries MR, et al. Tumor necrosis factor-α plays an important role in restenosis development. FASEB J. 2005;19(14):1998–2004.

256. Leibundgut G, Lee J-H, Segev A, Strauss B, Tsimikas S. Acute and Long-Term Effect of Percutaneous Coronary Intervention on Serially-Measured Oxidative, Inflammatory, and Coagulation Biomarkers in Patients with Stable Angina. J Thromb Thrombolysis. 2016;41(4):569–80.

257. Hojo Y, Ikeda U, Katsuki TA, Mizuno O, Fukazawa H, Fujikawa H, et al. Chemokine expression in coronary circulation after coronary angioplasty as a prognostic factor for restenosis. Atherosclerosis. 2001;156(1):165–70.

258. Costa MA, Simon DI. Molecular basis of restenosis and drug-eluting stents. Circulation. 2005;111(17):2257–73.

259. Okamoto E, Couse T, Leon H De, Vinten-johansen J, Goodman RB, Scott NA, et al. Pericasvular inflammation after balloon angioplasty of porcine coronary arteries. Circulation. 2001;2228–35.

260. Gomes WJ, Giannotti-Filho O, Paez RP, Hossne NA, Catani R, Buffalo E. Coronary Artery and Myocardial Inflammatory Reaction Induced by Intracoronary Stent. Ann

Thorac Surg. 2003;76(5):1528–32.

261. Merinopoulos I, Gunawardena T, Stirrat C, Cameron D, Eccleshall SC, Dweck MD, et al. Diagnostic Applications of Ultrasmall Superparamagnetic Particles of Iron Oxide for Imaging Myocardial and Vascular Inflammation. JACC Cardiovasc Imaging. 2020;585–610.

262. Tsampasian V, Swift AJ, Assadi H, Chowdhary A, Swoboda P, Sammut E, et al. Myocardial inflammation and energetics by cardiac MRI: a review of emerging techniques. BMC Med Imaging [Internet]. 2021;21(1). Available from: https://doi.org/10.1186/s12880-021-00695-0

263. Lagan J, Naish JH, Simpson K, Zi M, Cartwright EJ, Foden P, et al. Substrate for the Myocardial Inflammation–Heart Failure Hypothesis Identified Using Novel USPIO Methodology. JACC Cardiovasc Imaging. 2021;14(2):365–76.

264. Merinopoulos I, Gunawardena T, Corballis N, Tsampasian V, Eccleshall S, Smith J, et al. The role of inflammation in percutaneous coronary intervention, from balloon angioplasty to drug eluting stents. Minerva cardiol Angiol. 2022;

265. Scally C, Abbas H, Ahearn T, Srinivasan J, Mezincescu A, Rudd A, et al. Myocardial and systemic inflammation in acute stress-induced (Takotsubo) cardiomyopathy. Circulation. 2019;139(13):1581–92.

266. Raphael CE, O'Kane PD. Contemporary approaches to bifurcation stenting. JRSM Cardiovasc Dis [Internet]. 2021;10:204800402199219. Available from: https://doi.org/10.1177/2048004021992190

267. Shin E-S, Jun EJ, Kim S, Kim B, Kim T-H, Sohn C-B, et al. Clinical Impact of Drug-Coated Balloon–Based Percutaneous Coronary Intervention in Patients With Multivessel Coronary Artery Disease. JACC Cardiovasc Interv. 2023;

268. Stefanini GG, Alfonso F, Barbato E, Byrne R, Capodanno D, Colleran R, et al.

Management of Myocardial Revascularization Failure: An Expert Consensus Document of the EAPCI. EuroIntervention. 2020;

269. Truesdell AG, Alasnag MA, Kaul P, Rab ST, Riley RF, Young MN, et al. Intravascular Imaging During Percutaneous Coronary Intervention: JACC State-of-the-Art Review. Vol. 81, Journal of the American College of Cardiology. 2023. p. 590–605.

270. Madhavan M V., Kirtane AJ, Redfors B, Généreux P, Ben-Yehuda O, Palmerini T, et al. Stent-Related Adverse Events >1 Year After Percutaneous Coronary Intervention. J Am Coll Cardiol. 2020;75(6):590–604.