Chronic Total Coronary Occlusions, Distal Collateral Supply and Implications of Recanalisation

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Abstract

The optimal treatment for patients with a chronic total coronary occlusion (CTO) is controversial, both in terms of the decision whether to revascularise and with respect to the physiological effect of recanalisation on revascularisation strategy.

Physiological lesion assessment in the form of fractional flow reserve (FFR) is now well established for the purpose of guiding multi-vessel revascularisation. CTOs are frequently associated with multi-vessel disease and the collateral dependent myocardium distal to the occlusion is often supplied by a collateral supply from another epicardial coronary. The haemodynamic effect of collateral donation upon collateral donor vessel flow may have important implications for the vessel's FFR; rendering it unreliable at predicting ischaemia should the CTO be revascularised. These haemodynamic changes along with the changes in the target vessel after recanalisation of the occlusion are not well described.

The ability to form a functional collateral supply varies considerably between individuals. As arteriogenesis is an endothelium dependent process we hypothesised that biomarkers of endothelial health might be associated with the degree of functional collateralisation measured invasively in the coronary vessel segment distal to a CTO.

In this series of studies, I first compare long-term outcomes between patients with a chronic total coronary occlusion treated electively by medical therapy or CTO PCI. I go on to study the haemodynamic changes associated with CTO PCI. Firstly, the associated changes in physiology in the non-CTO vessels and how these changes might influence best revascularisation strategy. Secondly, the haemodynamics in the recently recanalised CTO vessel and the effect that might have on physiological optimization of the PCI result. Finally, I compare coronary haemodynamics taken at the time of CTO PCI with biomarkers of endothelial health to investigate a link between their levels and degree of functional collateralisation. The over-arching goal of this thesis is to add to our

understanding of how best to manage patients with a chronic total coronary occlusion.

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List of abbreviations

APV	Average peak velocity
BMR	Basal microvascular resistance
CABG	Coronary artery bypass graft
CAG	Confidentiality advisory group
CC grade	Collateral connection grade
CCS class	Canadian Cardiovascular Society (angina) class
CFR	Coronary flow velocity reserve
CFVR	Collateral flow velocity reserve
CI	Confidence interval
CKD	Chronic kidney disease
CMR	Cardiac magnetic resonance
CTO	Chronic total coronary occlusion
DFV-PGR	Diastolic flow velocity-pressure gradient relation
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
FFR	Fractional flow reserve
FFR_{coll}	Fractional collateral flow reserve
GTN	Glyceryl trinitrate
HMRv	Hyperaemic microvascular resistance
HRA	Health Research Authority
HSR	Hyperaemic stenosis resistance
ICAM-1	Intercellular adhesion molecule-1
iFR	Instantaneous wave-free ratio
IPV	Instantaneous peak velocity
LAD	Left anterior descending artery
LCx	Left circumflex artery
LV	Left ventricular
MI	Myocardial infarction
MPS	Myocardial perfusion scintigraphy
NYHA class	New York Heart Association (heart failure) class

OMT	Optimal medical therapy
Pa	Aortic pressure
Pd	Distal coronary pressure (measured at the tip of the Combowire)
PCI	Percutaneous coronary intervention
PET	Positron emission tomography
QCA	Quantitative coronary angiography
RAP	Right atrial pressure
RCA	Right coronary artery
RPP	Rate pressure product
SD	Standard deviation
SPECT	Single photon emission computed tomography
STEMI	ST elevation myocardial infarction
TF	Tissue factor
TIMI	Thrombolysis in myocardial infarction
UA	Unstable angina
WIA	Wave intensity analysis
BEW	*Backwards expansion wave*
eBCW	*Early backwards compression wave*
lBCW	*Late backwards compression wave*
eFCW	*Early forwards compression wave*
FEW	*Forwards expansion wave*
lFCW	*Late forwards compression wave*

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Declaration

I confirm that this work is original and that if any passages or diagrams have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources. I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised.

1.0 Introduction

1.1 Chronic total coronary occlusions

1.1.1 Definiton and pathology

A chronic total coronary occlusion (CTO) is defined by the consensus document produced on behalf of the Euro CTO club as complete occlusion of a coronary artery of ≥3 months duration with complete interruption of antegrade flow as assessed by invasive coronary angiography (Thrombolysis in myocardial infarction (TIMI) grade 0 flow)[1]. The American consensus document also includes occlusions with minimal contrast penetration through the lesion without distal opacification (TIMI grade I flow)[2]. CTO is a common finding at the time of diagnostic angiography, and is found in between one fifth and half of patients with significant (any stenosis ≥70%) coronary disease[3,4].

The natural history of most CTOs is occlusion at the point of plaque rupture[5], rather than gradual luminal obliteration by atheromatous progression. In CTOs of a shorter duration, the occlusion will be made up of organised thrombus and necrotic core; over time, proteoglycans and dense collagen will replace this material followed by progressive calcification and negative remodelling[6].

1.1.2 The influence of a CTO upon treatment strategy

The presence of a CTO is strongly predictive of treatment strategy; patients with an identified CTO are more frequently treated either medically or with coronary artery bypass surgery (CABG) in preference over percutaneous intervention (PCI)[3,7]. This may in part be due to the perceived increased technical difficulty of recanalising a CTO relative to a non-occlusive lesion, which is borne out by historical success rates for PCI of CTOs in the range of 65-77% relative to rates of 96-97% for non-occlusive lesions[8–14]. More recently, in experienced hands, success rate of >85% have been reported[15,16]. However contemporary registries which have not selected high volume, expert operators continue to report success rates in the range of 60-70%[17,18].

In the presence of favourable patient and lesion characteristics, and in the hands of an experienced operator, success rates of ≥90% can be expected[1,19]. In the right hands PCI of CTOs is practicable, but a strategy of PCI to CTOs, indeed revascularisation of a CTO by any means is still not consistently adopted[3,7,19], perhaps contributed to by doubt as to its benefit.

1.1.3 Relief of symptoms

The primary indication and benefit of PCI is the relief of symptoms of angina. This is also the case for PCI of CTOs; it stands to reason and is widely accepted that if angina is caused by a CTO, opening the occlusion should improve symptoms. Indeed, observational data have consistently shown a significant improvement in symptoms of angina and reduced need for subsequent CABG in those undergoing successful PCI over those undergoing an unsuccessful procedure[9,10,20]. In their 2014 guidelines on revascularisation, the Task Force on Myocardial Revascularisation of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) recommend PCI of CTOs for symptomatic relief as class IIa indication, level of evidence B[21].

1.1.4 Chronic total occlusions and prognosis

1.1.4.1 Overview

There is now a wealth of observational data showing an association between successful PCI to a CTO and improved survival[9–14,18,22]. The comparator with successful PCI to CTOs in the literature has almost exclusively been those patients who have undergone a failed procedure[22]. A true comparison of medically treated CTOs versus those treated with PCI is lacking. This is the most pertinent comparison to inform the clinician who needs to make a decision with respect to revascularisation when confronted with an individual found to have a CTO on angiography.

The differences in outcome between successful versus failed CTO PCI procedures in the published observational studies appear to be due to fewer events at long-term follow up rather than an increase in directly related

peri-procedural adverse events, which one might imagine would be more likely if a procedure has failed[9–14,18,22]. However, the data is observational and has largely been carried out using the same approach. If there are unmeasured variables associated with CTO PCI failure, which are also associated with poor prognosis, then this might confound the findings of these studies. It is also conceivable that undergoing an unsuccessful attempt at PCI to a CTO in itself could be potentially harmful, in that important collateral branches subtending the myocardium distal to the occlusion could be disrupted.

Whilst there are no published randomized controlled trials reporting clinical outcomes between CTO PCI and medical therapy, a number of trials have reported clinical outcomes comparing PCI with medical therapy in non-occlusive coronary disease[23–27]. These trials have not demonstrated an outcome benefit of PCI over medical therapy[23–27]. Sceptics might reasonably question why there would be a prognostic benefit of CTO PCI when none seems to exist for PCI of non-occlusive coronary disease? If it does exist, the possible explanations for this disparity are: the improved contractility of what is often hibernating myocardium and therefore improvement in LV systolic function after CTO recanalisation; the particularly poor prognosis associated with the presence of a concomitant CTO in patients with STEMI; and the almost universal presence of inducible ischaemia providing the CTO segment is viable, which has not been universally present in patients included in trials of PCI versus medical therapy for non-occlusive disease[27]. Each will be dealt with in turn.

1.1.4.2 Improvement in Left Ventricular function

Many patients with CTOs have regional left ventricular (LV) impairment. Provided the effected territory is viable, revascularisation by PCI might be expected to result in improvement in regional ventricular function. Improvement in regional and as a consequence, overall LV function after PCI of CTOs has been demonstrated in several observational studies[12,28–30]. A consistent finding in these studies was an overall

improvement in wall motion in the CTO territory, whether measured by left ventriculography[28], echocardiography[12], or cardiac magnetic resonance imaging (CMR)[29,30]. This was often translated into an improvement in global LV systolic function[12,28,30].

Not all myocardial segments improve however. Kirschbaum and colleagues assessed 21 patients with CMR at baseline, 5 months and 3 years post CTO PCI[30]. They found overall significant improvements in ejection fraction and reductions in systolic volumes post PCI, which continued out to 3 years. Using a 16 segment model, of 109 myocardial segments in the perfusion territory of the treated CTO, 49 segments had abnormal systolic wall thickening, of which 55% improved post PCI.

More recently, the hypothesis that PCI of a CTO will improve LV systolic function has been tested in a randomized trial[31,32]. The EXPLORE trial randomized 304 patients after successful primary PCI (PPCI) for ST-segment myocardial infarction (STEMI) with a concurrent CTO in a non-infarct related artery to either elective PCI of the CTO within 7 days or standard medical treatment. The primary endpoints of left ventricular ejection fraction and left ventricular end-diastolic volume were assessed by CMR at four months. Perhaps surprisingly, there was no difference in left ventricular function at four months between PCI and medical therapy arms[32]. A potential criticism of the trial is that it included patients without confirmed myocardial viability and also those with normal contractility in the CTO territory. The smaller proportion of patients with impaired, but viable myocardium in the CTO segment are those in whom we would expect to see an improvement in myocardial contractility post CTO PCI, and the trial may have been underpowered to detect an improvement in this sub-group.

The degree of LV dysfunction is a potent predictor of prognosis in patients with coronary artery disease, with increased annual mortality in those with LV dysfunction and declining survival rates in proportion to severity[33,34]. The results of the STICH trial[35] have thrown some doubt upon whether revascularisation (in the case of the STICH trial, surgical

revascularisation) of viable myocardium in the context of an impaired left ventricle is prognostically beneficial[36] as the trial's primary outcome of a reduction in all cause mortality of CABG over medical therapy in patients with LV dysfunction was not met. However, a reduction in cardiac death and admissions to hospital was demonstrated[35]. It might be postulated that the improvement in LV function conferred by CTO PCI could translate into a survival benefit.

1.1.4.3 Adverse Prognosis in STEMI

The presence of a CTO in a non-infarct related artery in patients who present with STEMI has been shown to be associated with adverse outcomes in observational studies[37,38]. The presence of a CTO has been shown to be associated with early and late mortality[37]. Although patients with CTOs tend to have more co-morbid conditions and cardiovascular risk factors, a concomitant CTO appears to independently predict adverse outcomes in STEMI[37,38]. When compared with single vessel disease and multi-vessel disease without CTO, a concurrent CTO was also predictive of more severe LV dysfunction at baseline and a further reduction over the course of the first year post infarct[37].

The likely explanation for this association is twofold. Firstly, it is likely that in the presence of a CTO there would be less collateral support to the infarcting territory, resulting in more profound ischaemia. Secondly, the myocardium originally subtended by the CTO may depend upon the infarct related vessel for collateral supply, resulting in ischaemia to the collateral dependent bed and a greater area of infarct.

1.1.4.4 The importance of inducible ischaemia

It is well established that a greater magnitude of inducible ischaemia is associated with adverse outcomes[39–43]. Severity of abnormality on myocardial perfusion scanning by single photon emission computed tomography (SPECT) has been shown to be incrementally associated with reduced survival and myocardial infarction[39,40]. An observational study

which included 10,627 patients undergoing SPECT MPS, found that in the 1307 patients with >10% ischaemic myocardium, revascularisation (performed in 510 patients) was associated with a 50% risk-adjusted reduction in cardiac death when compared with those treated with medical therapy alone[42]. In a small randomized trial, patients with demonstrable ischaemia (by stress or ambulatory ECG), had a significant reduction in of death or MI at 2 year follow-up if treated with revascularisation by PCI or CABG compared with medical therapy alone[41]. The COURAGE trial compared an initial management strategy of optimal medical therapy (OMT) with OMT + PCI and found that the addition of PCI did not reduce the risk of death, myocardial infarction or other major cardiovascular events out to a median follow-up of 4.6 years[27]. Of the 2287 COURAGE patients, 314 were enrolled into a nuclear sub-study[43]. These patients underwent a baseline myocardial perfusion scintigraphy (MPS) by SPECT, which was repeated at 6-18 months. The primary endpoint of the study was achievement of ≥5% reduction in ischaemic burden, assessed by a change in reversible ischaemia assessed by summed difference score, this was achieved in 33% of the OMT + PCI group and 19% of the OMT group (p=0.0004). The addition of PCI to OMT was more likely to reduce the overall burden of ischaemia and if moderate to severe ischaemia was present at baseline (≥10%) it was more likely that a ≥5% reduction would be achieved. The sub-study was not powered to detect differences in clinical outcomes; however the outcome data was interesting. The magnitude of residual ischemia on follow-up MPS was found to be associated with an incremental risk for death or MI. Perhaps most interesting was the finding that a ≥5% reduction in ischaemic burden was associated with a reduction in death or myocardial infarction, this was most marked if baseline ischaemia was moderate to severe (≥10%). The task force on myocardial revascularisation of the European Society of Cardiology (ESC), European Association for Cardio-Thoracic Surgery (EACTS) and the European Association for Percutaneous Cardiovascular Interventions (EAPCI) suggest that in the presence of ≥10% demonstrable ischaemia, revascularisation

should be considered on the grounds of prognosis as a class I indication, level of evidence B[21].

If reduction of ischaemic burden does contribute to any prognostic benefit that might be gained by PCI to a CTO, the available evidence would suggest that any benefit would be related to the initial magnitude of ischaemia, and the degree to which it is reduced. This is supported by observational evidence in patients undergoing single vessel PCI to a CTO in which treating the left anterior descending artery (LAD) was associated with improved survival, but not the circumflex or right coronary artery (RCA)[44]. As one would expect, CTOs of the LAD are associated with a greater inducible perfusion defect than occlusions of the circumflex artery or RCA[45].

A frequent argument for not intervening upon a chronically occluded vessel is that the territory distal to the occlusion is well collateralised. It is well established that the presence of a collateral circulation can maintain normal resting myocardial blood flow; however this is seldom sufficient to prevent ischaemia with myocardial stress[45,46]. In 1978, Flameng et al. measured post-stenotic/post-occlusion coronary pressure and graft flow hyperaemia in 65 patients undergoing coronary bypass surgery[46]. They found that in well collateralised, chronically occluded vessels, distal pressure measurements and hyperaemic response to grafting were equivalent to that of vessels with a ≥90% non-occlusive epicardial stenosis. More recently, He et al reported the perfusion defect associated with CTOs in 71 consecutive patients with single vessel disease found on diagnostic angiography with no prior MI[45]. All patients with the exception of two had evidence of a perfusion defect, 86% of which were reversible. The two patients without perfusion defects had distal coronary occlusions, one an occlusion of the posterior descending artery arising from the RCA, and the other the atrio-ventricular branch of the circumflex artery. Collateral circulation was assessed angiographically, simply as 'present' (n=49) or 'absent' (n=22). There was no evidence that angiographically visible collaterals protected against stress hypoperfusion. It is likely there was a

referral bias as 49/71 patients underwent MPS prior to angiography, such that patients with small or absent perfusion defects may not have been referred for angiography. Nevertheless, the perfusion defects demonstrated were sizeable, with a mean defect size of 19±12%. In a recent published registry of 301 patients undergoing successful PCI to a CTO which examined the change in ischaemic burden measured by SPECT or positron emission tomography (PET) at baseline and after PCI, mean inducible ischaemic defect size was also sizeable at 13.1+/-11.9%[47]. The mean reduction in that ischaemic defect was 6.2+/-6.0% and 54% of patients experienced a $\geq$5% reduction in ischaemic burden. As in the COURAGE nuclear sub-study[43], It was more likely that a ≥5% reduction in ischaemic burden would be achieved post PCI if the defect at baseline was greater. Major adverse cardiovascular events (MACE) were higher at one year in those with a <5%, relative to a ≥5% reduction in ischaemic burden (28.6 vs 18.6%, p=0.042), this was largely driven by increased target vessel revascularisation (25 vs 14.3%, p=0.019). There was no difference in survival at 1 year, although there was at 6 years (78 vs 87%, p=0.018), there were far fewer patients followed up for this period than the initial study population however. This was a retrospective analysis and the follow-up MPS were performed on clinical grounds, it may be that the patients without a significant reduction in ischaemic burden were those with restenosis or sub-optimal PCI results. The suggestion however, is that the benefit of the absolute reduction of ischaemic burden on outcome which has been described[39–43,48], may also apply to the specific lesion subset of CTOs, treated with PCI.

1.1.5 Summary

Ultimately, whether recanalisation of a CTO is prognostically beneficial or not can only be answered by a randomized trial. Pending a trial sufficiently large and robust enough to answer the question; relying on the currently available observational data, even if it synthesised by meta-analysis[22], is potentially flawed. Management between clinicians with

respect to revascularisation strategy (or the absence of one) is sufficiently variable that an alternative approach to answering the question should be possible. Pre-treatment characteristics between patients treated by CTO PCI and those electively treated medically should overlap sufficiently for reasonable comparison. If such a comparison showed PCI of CTOs to be associated with improved clinical outcomes relative to elective medical therapy, it would add weight to the suggestion that recanalisation of CTOs is of prognostic benefit.

1.2 The influence of a CTO on modern management of multi-vessel disease

1.2.1 Introduction

The human coronary circulation in both health and in disease consists of a complex pre-existing anastomotic network rather than functional end-arteries. If an epicardial vessel is occluded, there is an associated gradual increase in diameter of these anastomotic collateral branch connections over time[49,50], through which the distal segment of the occluded vessel is filled**(figure 1.1).**

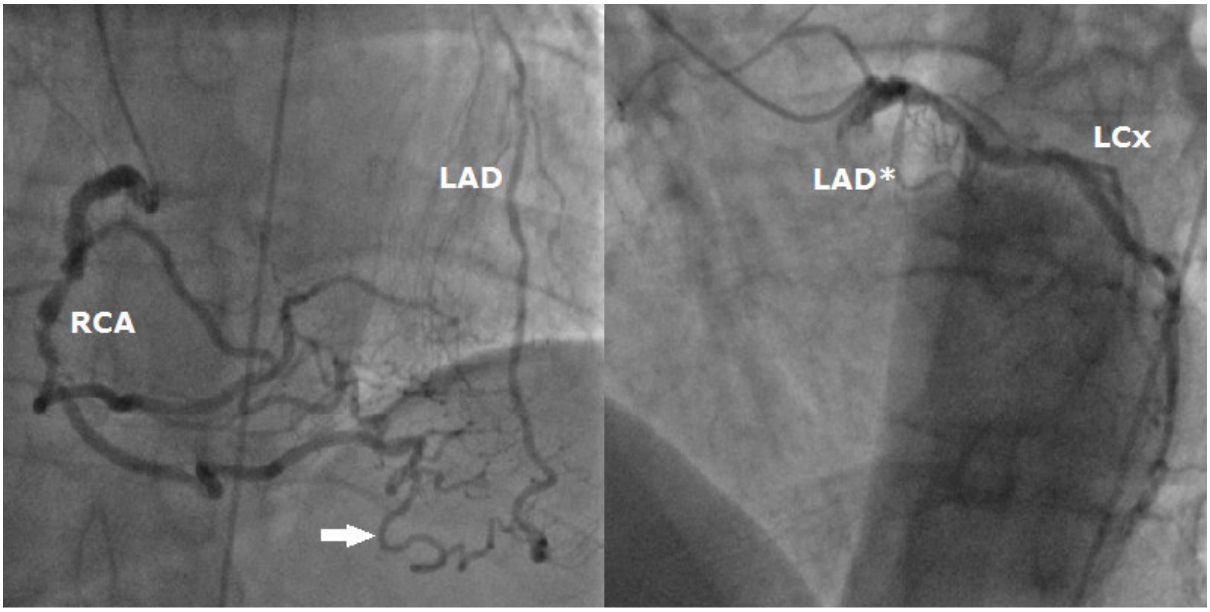

Figure 1.1. Left: Coronary angiogram demonstrating filling of the left anterior descending artery (LAD) by retrograde collateral branches arising from the right coronary artery (RCA), which have developed as a result of chronic occlusion of the left anterior descending artery. The arrow highlights the largest of these. Right: Coronary angiogram of the left coronary artery in the same projection as that for the right coronary artery (left). The LAD is completely occluded to antegrade flow at the point of the asterisk. The left circumflex artery (LCx) is also shown)

This coronary collateral supply can be sufficient to preserve resting left ventricular systolic function in spite of complete coronary occlusion, and in animal models has been shown to be sufficient to prevent ischaemia under stress[51–54]. In the presence of a chronic total coronary occlusion (CTO), if the collateral dependent myocardium is perfused by retrograde collateral branches, as is frequently the case, coronary physiology in the collateral donor vessel could be altered. If this effect is sufficiently large, the haemodynamic importance of a donor vessel coronary stenosis could change. The presence of a CTO may therefore result in flow limitation and myocardial ischaemia in coronary arterial territories remote from the occluded vessel. If so, the additional ischaemia generated by a CTO, relieved on recanalisation might even help to explain (in addition to the apparent detrimental effect of a concomitant CTO in the event of STEMI[37,38]) the frequent finding in published cohort studies of a survival benefit associated with successful CTO recanalisation[22], which is absent in clinical trials of PCI of non-occlusive lesions versus medical therapy[27]. If this phenomenon results in important changes to physiological stenosis

measurement indices such as the Fractional Flow Reserve (FFR), then as the evidence base for their use to guide revascularisation grows (as seems inevitable), the need to understand it will become increasingly important. In the following section, I will review what we know about coronary physiology related to this phenomenon, its importance, and highlight where further studies might inform our practice.

1.2.2 FFR and the effect of a change in donor vessel flow

The FFR is the ratio of the maximum myocardial blood flow in the presence of a coronary stenosis to the maximum myocardial blood flow in the absence of that stenosis[55]. Maximal myocardial blood flow is achieved by pharmacological vasodilatation, most commonly using adenosine given either intravenously or intra-coronary. It is dependent upon multiple morphologic determinants of resistance related to the stenosis, the extent of perfusion territory, and the presence of collateral myocardial blood flow.

The FAME study showed a clinical benefit in the use of physiological lesion assessment in the form of FFR to guide PCI in multi-vessel disease[56]. Patients randomised to treatment by FFR guided PCI (in which a lesion was treated if the FFR was ≤0.8) had a significant reduction in the composite primary endpoint of death, non-fatal myocardial infarction and repeat revascularisation at 1 year compared with those treated by angiographically guided PCI. At 2 year follow up, there was a significant difference in a composite of the harder end-points of death or myocardial infarction, favouring the FFR guided group[57]. The FAME II study showed a large increase in urgent revascularisation if treatment by PCI of lesions with an FFR ≤0.8 was deferred[58].

Although FFR is reported to be independent of changing haemodynamics[59], it is intimately related to total coronary flow through a stenosis[60,61]. Whilst PCI of a lesion remote from a vessel in which there is a stenosis will have no effect upon the characteristics of that stenosis, it would be expected to change the extent of collateral myocardial blood flow donated by the remote vessel. If the remote vessel makes a significant

collateral contribution to the treated vessel, the mass of myocardium the vessel perfuses may reduce, along with flow through the stenosis and as a consequence we might expect the FFR to increase. This is particularly relevant when considering the effect of a CTO on a remote vessel as the myocardium distal to a CTO is by definition entirely collateral dependent. This is illustrated schematically in **figure 1.2**. In addition, the angiographic degree of collateralisation is such that one might expect the influence on the remote/donor vessel(s) flow and FFR if a CTO is recanalised to be large. It has been suggested that the large increase in coronary flow through collateral donor vessels as a result of the additional flow through the collateral bed could be enough for minor atherosclerotic irregularities to generate enough resistance to become flow limiting. In support of this, Werner et al measured donor artery FFR prior to recanalisation of a CTO in assessing determinants of coronary steal; 18 of 45 patients in whom they reported an FFR measurement had an FFR of <0.8, only 8 of those had a visible lesion in that donor vessel[62].

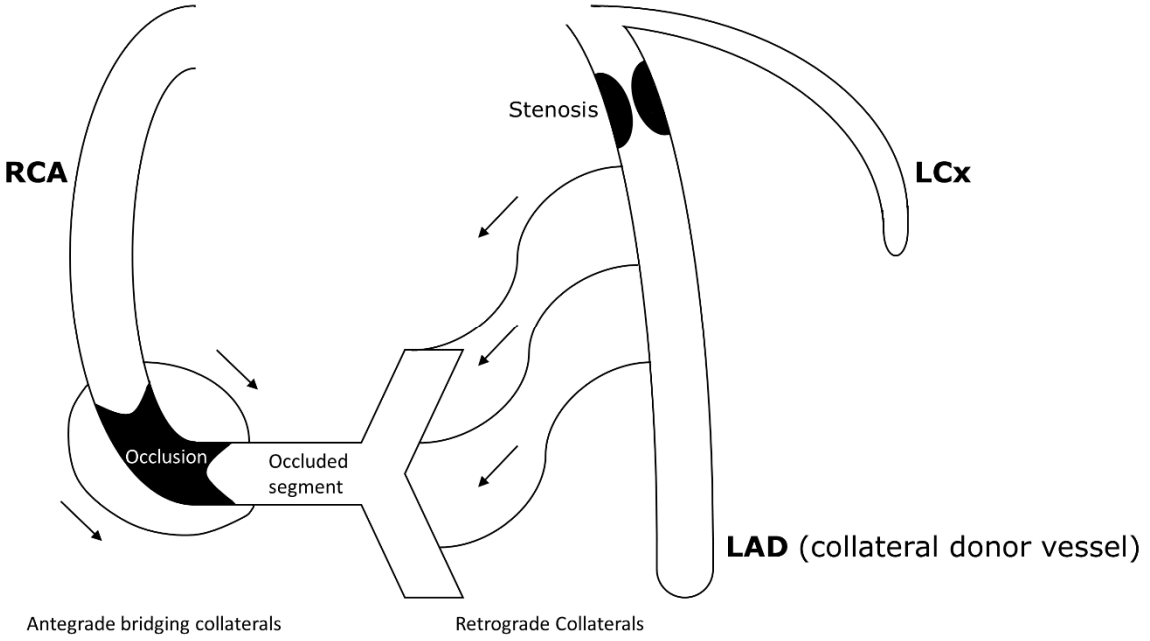

Figure 1.2 Schematic example of the effect of a CTO on the collateral donor vessel. In this example, the occluded segment of the right coronary artery (RCA) is in receipt of a blood supply from antegrade bridging collaterals arising proximally in the RCA and retrograde collaterals arising from the left anterior descending artery (LAD). The collaterals arising from the LAD originate distal to a stenosis, theoretically increasing blood flow through it relative to if the CTO were absent, which in turn should result in a greater pressure gradient across the stenosis and a lower (more haemodynamically significant) FFR. LCx represents left circumflex artery.

If the additional flow as a result of donating a collateral supply to collateral dependent myocardium is sufficient to significantly alter donor artery haemodynamics, we would expect an early reversal of the effect once the myocardium is rendered no longer collateral dependent. After CTO angioplasty it has been shown that both flow and pressure-derived recruitable collateral function in the target vessel diminishes rapidly[63,64] **(figure 1.3)**, and is not significantly different at 24 hours post PCI from the value taken at a mean time of just 48 minutes[63].

Several cases have been reported in which marked changes in non-target vessel FFR have occurred after PCI of a CTO[65–69]. Each case reported pre and post-PCI measurement of donor vessel FFR and involved PCI of either 1 or 2 CTOs. An impressive increase in FFR of 0.12 in 4 cases and

0.09 in another were reported, crossing the treatment threshold from <0.80 to ≥0.80 in 3 of the 5 cases. Although the change in FFR in these cases seems remarkably consistent, it is important to remember that these are individual published cases. The only attempt thus far to investigate this phenomenon systematically found a much more variable change in FFR[70]. In 14 cases, two of which involved PCI of 2 CTOs, mean change in FFR was an increase of 0.04 with a wide 95% confidence interval of the difference of 0.001–0.079, the standard deviation of the difference was 0.062[71]. The wide confidence interval would suggest that a larger study would be useful to give a more precise estimate of the change. The standard deviation however, implies that there is considerable variability to the change of FFR post CTO PCI, and that we cannot assume there will be a large increase in donor vessel FFR. It also highlights the utility of further studies which might identify features of donor vessel anatomy or haemodynamics which might predict a larger change in FFR.

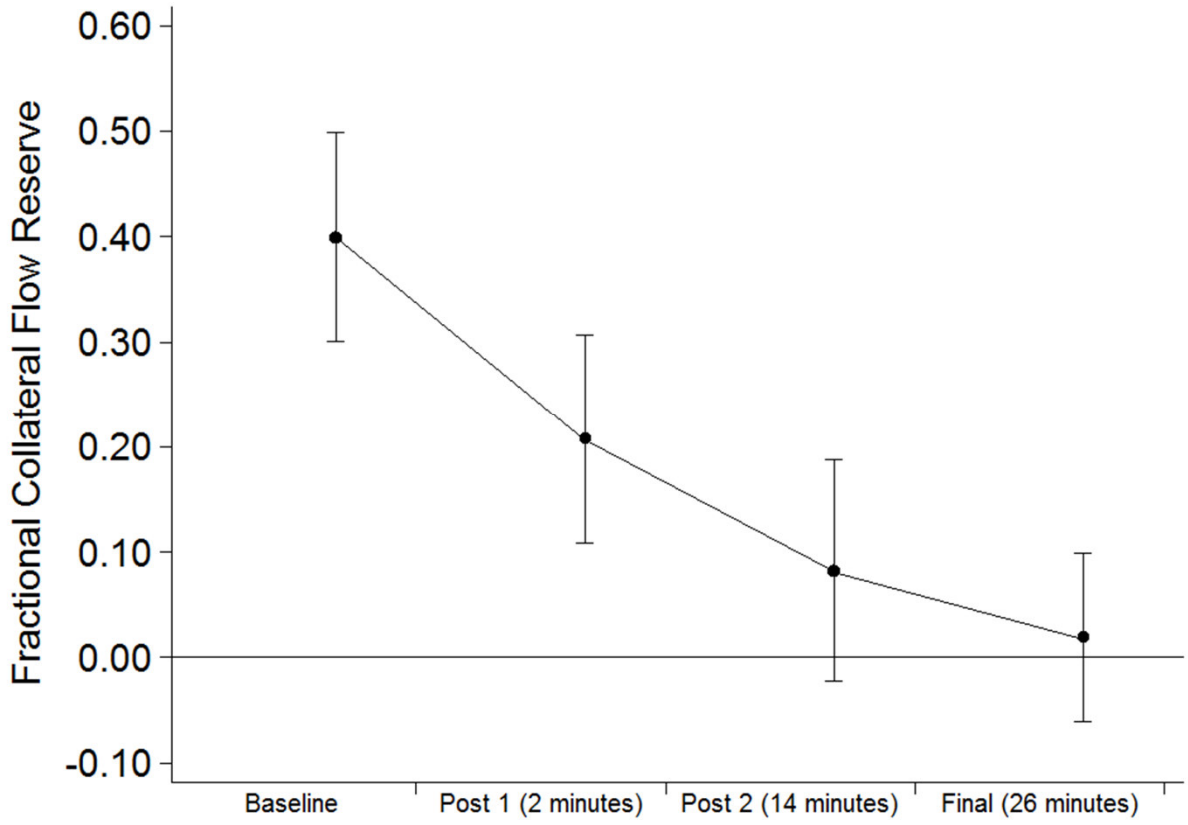

Figure 1.3. Data taken from Zimerano et al[64]. Changes in target vessel fractional collateral flow reserve (or received collateral supply) after PCI of chronic total occlusions. Fractional collateral flow reserve (FFR_{coll}) was measured at intervals in what was the occluded segment distal to a CTO at intervals after recanalisation, and diminished significantly at final measurement, measured by protocol approximately 26 minutes after restoration of antegrade flow in the CTO vessel. After CTO recanalisation FFR_{coll} was calculated incorporating pressure measured distal to a balloon re-occluding the vessel (wedge pressure): FFR_{coll} = myocardial FFR – coronary FFR. Coronary FFR = (distal pressure-wedge pressure)/(aortic pressure-wedge pressure), myocardial FFR = (distal pressure-central venous pressure)/aortic pressure-central venous pressure). Error bars represent 1 standard deviation.

The haemodynamic changes I predict and describe make the assumption of successful CTO recanalisation without additional haemodynamic effects. In recent years CTO PCI has developed considerably with greater success rates[15], this is in part the development of alternative approaches to CTO PCI, including dissection re-entry and retrograde approaches. The effects in the short and longer term of dissection re-entry techniques, which tend to involve longer stented segments, greater disruption of the vascular architecture, and a greater tendency for side branch occlusion upon haemodynamics and microvascular function are not well described. Any effect on the microvasculature is likely to be transient[72], however if the recanalisation technique results in side

branch occlusion, it may be that a proportion of myocardium perfused by the lost branch remains collateral dependent. This could result in a smaller effect on the collateral donor vessel. Although a retrograde approach often results in a damaged collateral vessel, if we expect them to regress after CTO recanalisation anyway[63,64], it seems unlikely that the use of a retrograde approach would have a long-term effect upon haemodynamics.

1.2.3 The need to understand the influence of a CTO on non-target vessel flow and FFR

It is now generally accepted that when presented with multi-vessel disease, we should aim for complete rather than incomplete revascularisation[73]. There is some evidence which supports the suggestion that complete revascularisation is associated with prognostic benefit[7,12]. In a large registry using New York State's Percutaneous Coronary Intervention Reporting System, 11294 patients with multi-vessel disease, treated by PCI were followed up for 18 months[7]. Incomplete revascularisation, performed in 69% of patients, was associated with increased mortality and those with two unattempted vessels including a CTO were at highest risk.

There is good evidence of a clinical benefit if FFR is used to guide multi-vessel angioplasty with clear thresholds to determine treatment. However, there is real doubt as to the effect of recanalisation of a CTO on non-target vessel haemodynamics. If the intention of treatment is that of complete revascularisation, at present we cannot be certain if a lesion with an FFR of ≤0.8 in a non-target vessel would still have an FFR of ≤0.8 once the occluded vessel is recanalised. The concern, is that should CTO revascularisation render a vessel's FFR above the treatment threshold of 0.8, the results of the FAME trial[56] would suggest that angioplasty of that vessel would be associated with adverse clinical outcomes. If the intention is to treat by PCI, the simple solution is to open the CTO and then re-assess the FFR of the other vessels. However we do not always have that luxury, in patients with three-vessel coronary disease coronary artery bypass graft surgery (CABG) has been shown to have superior long-term outcomes

compared with PCI, an effect which appears to be greater with increasing angiographic complexity[74] and with concomitant diabetes[75,76]. A vessel with a haemodynamically ambiguous lesion, the FFR of which is ≤0.8, but might move above that treatment threshold once myocardium receiving collaterals from it is revascularised, could be the difference between CABG and PCI being the most appropriate treatment. A haemodynamically ambiguous lesion would not necessarily be of low angiographic complexity, and the need to treat it might alter the long-term outcomes which can be achieved with angioplasty. What is becoming increasingly clear is that our ability to identify flow limiting lesions by angiography alone is limited[77,78] and knowledge of the FFR frequently changes management strategy[78–80].

There is less evidence for a clinical benefit for the use of FFR to guide coronary artery bypass graft placement than for angioplasty, but the occlusion rate is higher for grafts placed on haemodynamically non-significant lesions[81]**(figure 1.4)**. In a large retrospective cohort study, FFR guided graft placement was associated with a lower number of grafts, lower rate of angina and also a lower rate of graft failure than angiography guided graft placement, there was however, no difference in the primary clinical composite end-point of death, myocardial infarction or target vessel revascularisation[82].

A CTO is present in between one fifth and half of patients with significant coronary disease on coronary angiography[3,4]. If we consider assessment by FFR to guide revascularisation best practice, then the presence of a CTO, which may or may not alter the physiological significance of stenoses in the accompanying vessels is prevalent and there is therefore an uncertainty about the reliability of the FFR in a sizeable subset of patients.

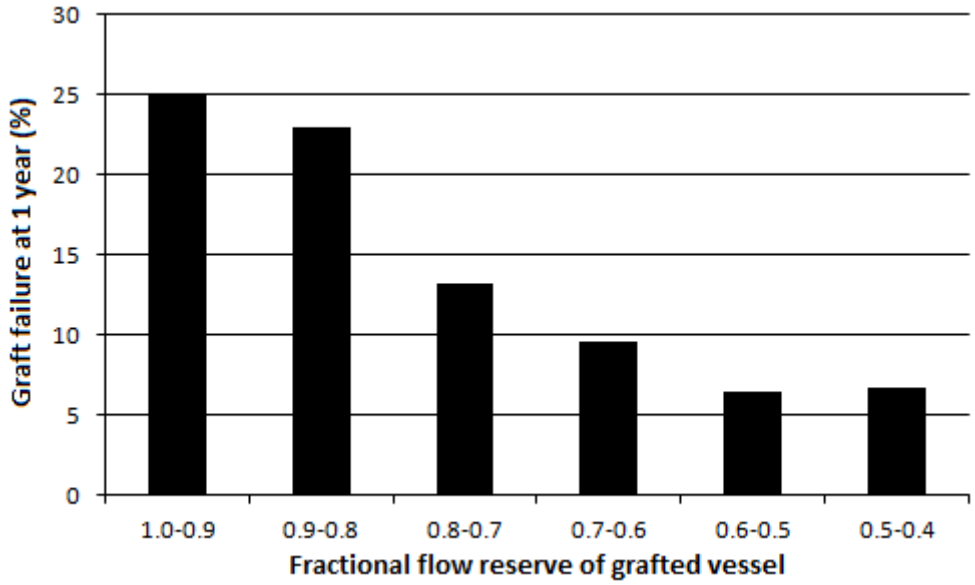

Figure 1.4. Data taken from Botman et al[81]. Percentage graft occlusion at 12 months post CABG by pre-operative FFR.

1.2.4 Relationship between pressure and flow

A particular coronary stenosis or vessel segment will have a characteristic relationship between coronary flow velocity and the associated pressure gradient. That relationship is described by the equation $\Delta P = FV + SV^2$ where ΔP is the pressure drop in mmHg, V is the coronary flow velocity in $cm \cdot sec^{-1}$, F is the coefficient of pressure loss due to viscous friction and S is the coefficient of pressure loss due to flow separation or localized turbulence downstream from the stenosis[83,84]. The equation describes the pressure gradient as a result of overall lesion severity, encompassing lesion length, diameter stenosis and induced turbulence as coronary flow velocity changes **(figure 1.5)**.

A vessel with a mild stenosis can accommodate a much larger increase in flow velocity before there is a given pressure gradient than a more severe stenosis. The FFR is dependent upon this flow velocity/pressure gradient relationship. Excluding other factors, it seems likely that the change in FFR of a vessel donating blood to collateral dependent myocardium on recanalisation of an accompanying CTO might be dependent on the vessel's flow velocity/pressure gradient curve and the degree to which there is a change in flow.

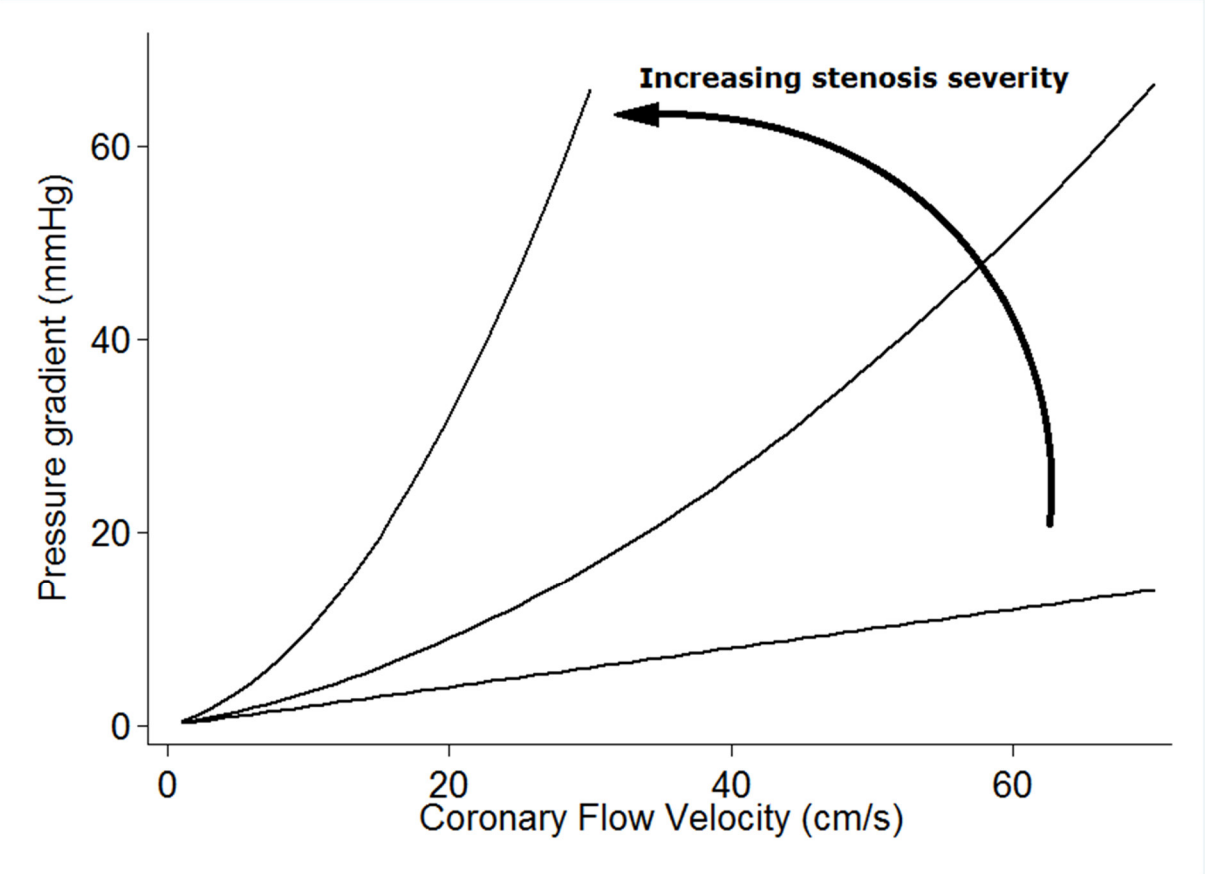

Figure 1.5. Predicted pressure gradient by stenosis severity described by the equation $\Delta P = FV + SV^2$. As the curves get steeper, stenosis severity increases.

Using pressure sensor tipped wires in combination with Doppler tipped wires it is possible to plot instantaneous flow velocity against pressure gradient as described in animal models by Gould, in clinical practice[85–87]. This was initially performed with separate Doppler and pressure tipped wires, but can now be performed with a combined wire (Volcano ComboWire, Volcano Corporation, San Diego, California)[87]. As in Gould's original work, coronary pressure gradient (defined as aortic pressure, measured from the guiding catheter minus distal coronary pressure, measured from the pressure wire) is plotted against coronary flow in mid-diastole, excluding the diastolic upstroke in coronary flow. During mid-diastole, compressive forces of the ventricle are minimal so coronary flow is only related to the severity of the lesion and to the driving pressure, theoretically minimizing any contribution other than resistance to flow across the stenosis to the flow-pressure gradient relationship. The technique takes measurements from a resting state, intermediate

hyperaemia and maximal hyperaemia and produces curves very similar to those produced by Gould **(figure 1.6)**.

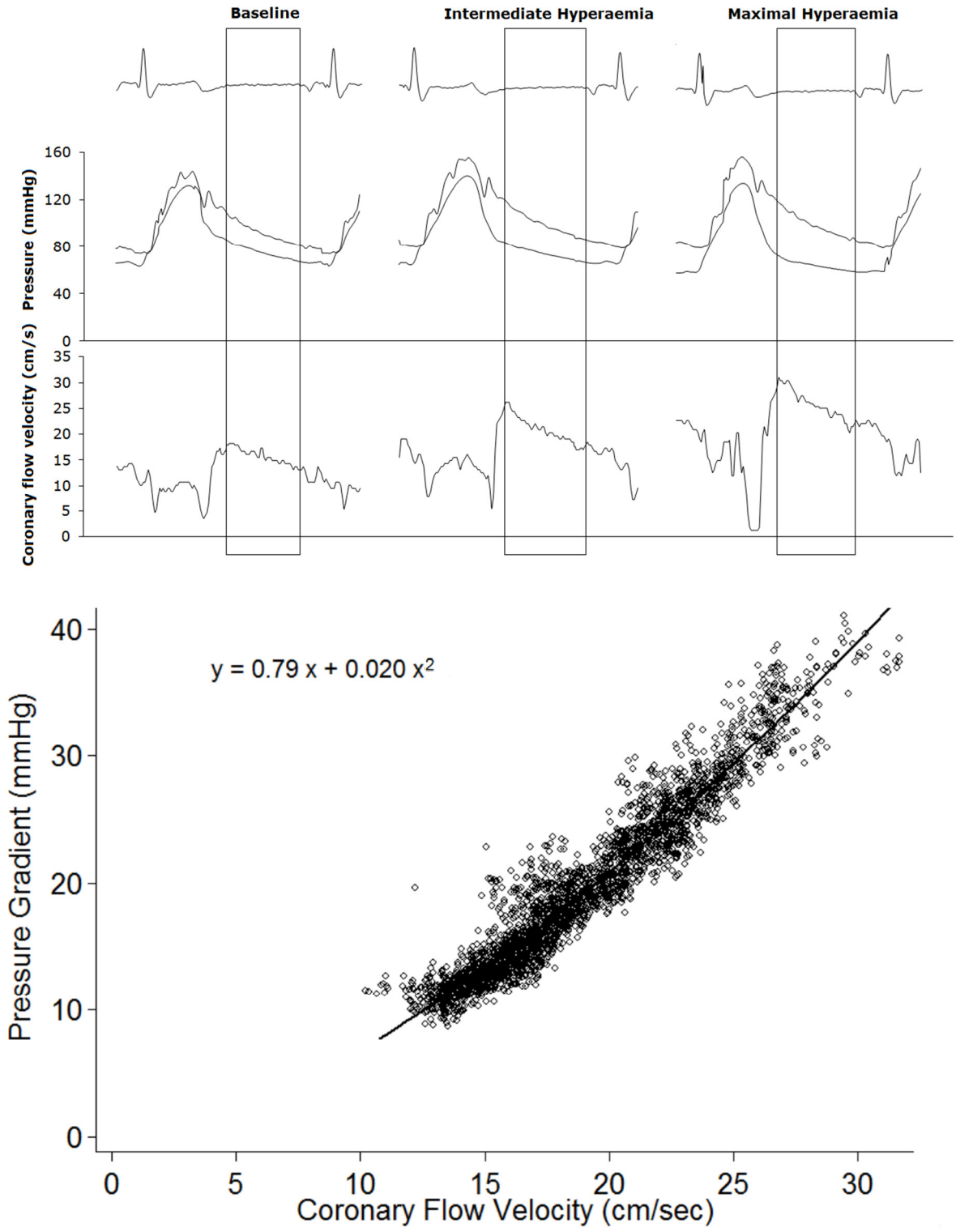

Figure 1.6. Top panel: example of simultaneous pressure and flow measurement for calculation of diastolic flow-pressure gradient, for each beat measurements are taken during the boxed diastolic periods. Bottom: calculation of the diastolic flow pressure-gradient slope using the formula $\Delta P = FV + SV^2$, 30 beats are used from baseline through to maximal hyperaemia, in this case F=0.79 and S=0.020.

Calculating diastolic flow-pressure gradient curves is relatively cumbersome when in FFR we have a validated means of identifying ischaemia which is simpler, more reproducible[86] and is associated with benefits in clinical outcome. What they add however, is a means of identifying the relationship between pressure gradient and flow in an individual vessel segment and allow us to predict the change in pressure gradient for a given change in flow velocity. It could even be that the characteristics of the slope could provide a means of identifying lesions which are likely to alter in haemodynamic importance after a change in subtended myocardium, such as PCI of a concomitant CTO.

Another possible explanation for a difference between patients with differing donor vessel lesion severity and how the change in FFR with concomitant CTO PCI might vary is that the contribution of collateral flow to distal pressure appears to be greater with more severe stenoses[88]. It is not known whether recruitable collateral function is improved in the non-target vessel after PCI of a CTO. If it is, the vessels (with more severe stenoses) which depend on collateral flow to a greater extent might see a larger change in distal perfusion and therefore FFR.

1.2.5 Flow to the collateral dependent myocardium

The change in remote, non-target vessel FFR post CTO PCI will be dependent upon the size of the change in flow, and therefore flow velocity across any stenosis. The magnitude of that change has not been studied, but the behaviour of the collateral dependent myocardium at rest and hyperaemia has been studied extensively.

The extent of collateralisation to an occluded segment can be readily assessed angiographically by the Rentrop grade[89], a measure of retrograde filling of the occluded vessel where: grade 0=no collaterals; grade 1=side branch filling of the recipient artery without filling of the main epicardial artery; grade 2=partial filling of the main epicardial recipient artery; and grade 3=complete filling of the main epicardial recipient artery. It might be expected that a higher Rentrop collateral filling grade would reflect greater

collateral perfusion, however it has not been shown to be related to invasive functional measures of collateral perfusion[90]. An alternative angiographic collateral grading system exists in the collateral connection (CC) grade[90] where: grade 0=no continuous connection between donor and recipient vessels, CC1=threadlike continuous connection and CC2=side branch–like connection. Pressure and flow derived measures of collateral function have been shown to be greater in those with CC2 collaterals. It might be that CTOs perfused via CC2 grade collaterals would have a greater predicted change in collateral donor vessel flow and FFR on recanalisation of a CTO.

Studies of collateral function under stress in man have demonstrated a consistent finding of a distal collateral supply seldom sufficient to prevent myocardial ischaemia under stress[45,46,91,92]. It should be borne in mind that all studies in man are confounded in that the study participants had sufficient symptoms to present to a cardiologist and undergo angiography, and in most cases revascularisation. Nevertheless, it seems unlikely that there is a population of patients with chronically occluded coronary arteries which behave differently to those studied, in any case, the study participants represent the very patients that present to us in clinical practice and in whom we must translate these results into best treatment.

With respect to the influence on donor vessel haemodynamics, the importance of the almost universal presence of inducible ischaemia in the collateral dependent myocardium is that the additional flow in collateral donor vessels as a result of a CTO is less than we would expect the flow through the CTO vessel to be should it be patent. Indeed, recanalisation of a CTO by PCI has been shown to result in an approximate 50% increase in absolute regional hyperaemic myocardial blood flow at 24 hours measured by cardiovascular magnetic resonance imaging, which was unchanged at 6 months[29]. Hyperaemic flow to the collateral dependent myocardium is therefore only approximately two thirds of expected, and this additional flow is often shared between two collateral donor coronary arteries and also antegrade collateral branches originating from the occluded vessel.

The response of the flow to the collateral dependent myocardium during Adenosine stress is also unpredictable. Early clinical work involving patients undergoing coronary artery bypass graft surgery showed that augmentation of coronary flow beyond an angiographically well collateralized occlusion is no better than that beyond an 80-90% coronary stenosis[46]. In a positron emission tomography study, Uren et al showed that vessels with 80-90% stenoses tend to have a coronary vasodilatory reserve of approximately 1, the microvasculature being already maximally dilated to maintain resting perfusion and therefore unable to dilate further in response to vasodilators[93]. The behaviour of chronically occluded vessels appears to be similar[46,94,95], however perfusion to the collateral dependent myocardium frequently diminishes with vasodilator stress, a phenomenon known as coronary steal. The mechanism of coronary steal is a fall in perfusion pressure at the origin of collateral vessels due to increased resistance to flow during hyperaemia, as there is a proportionally greater increase in conductance (or reduction in resistance) of the microvascular bed of the donor vessel myocardium relative to the low and fixed conductance of the collateral dependent myocardium, flow and perfusion actually falls during hyperaemia, rather than increases as we would usually expect[96].

1.2.6 Coronary steal

The phenomenon of coronary steal has been reported to occur in a very high proportion of well collateralised myocardial beds using positron emission tomography[94]. The conditions considered to be necessary for coronary steal to occur are: 1) there is sufficient resistance in the donor vessel to cause a pressure drop during hyperaemia; 2) resistance of the collateral vessel is not negligible; and 3) The microvascular resistance of the collateral dependent myocardium is fixed and lacks vasodilatory reserve[62,96]. The phenomenon has been investigated by Werner and colleagues, using the definition of coronary steal as a fall in coronary flow velocity over and above what would be expected by measurement variation

measured by intra-coronary Doppler wire during Adenosine infusion[62,95]. Using this definition, approximately one third of patients with a CTO exhibit steal, one third have no significant change and one third have an increase in flow during adenosine infusion. Werner showed that either a significant fall in pressure in the donor artery during Adenosine infusion (defined as an FFR of <0.8) or a lack of vasodilatory reserve in the collateral dependent myocardium, in addition to well-developed collateral vessels, was necessary for coronary steal to occur. From the same studies it was also concluded that coronary steal could not occur in patients with large (≥0.5mm) collateral vessels, however this was based on the absence of steal in only 3 patients. For coronary steal to occur, there has to be an alternative myocardial bed for flow to be redirected to, it should not occur to collateral flow through bridging collaterals. It is possible therefore, that the relative contribution of antegrade collateral flow and retrograde collateral flow changes during adenosine infusion, with a larger antegrade and diminished retrograde contribution.

From the point of view of considering the effect of a CTO on the physiology of the collateral donor vessel, the association between a lower donor vessel FFR and coronary steal may have important consequences. Although donor vessels with a lower FFR may be more sensitive to a change in flow, their tendency for coronary steal, or a lower flow reserve in the collateral dependent myocardium would suggest that the relative increase in hyperaemic donor artery flow as a consequence of the presence of a CTO would also be reduced, compared with a donor vessel with a higher FFR. Accordingly, one might expect a smaller change in donor vessel flow if the starting donor vessel FFR is lower and possibly therefore a smaller expected increase in FFR after CTO recanalisation. On the other hand, the presence of diffuse disease in the collateral donor vessel would be likely to be associated with a reduced coronary flow reserve[97]. The relative proportion of donor vessel flow attributable to the collateral dependent myocardium might therefore be increased and the effect on FFR greater.

1.2.7 Collateral dependent myocardial mass

The major driver of the large upstroke in coronary flow during early diastole, and therefore the predominant driver of coronary flow, is the negative pressure (or suction) generated by the relief of myocardial microcirculatory compression in early diastole[98]. This suction effect can be quantified by means of wave intensity analysis. Wave intensity represents the rate of energy per unit area transported by travelling waves in arteries and is derived from phasic changes in local pressure and flow velocity. A predominant pattern of 6 coronary waves measured by wave intensity analysis has since been described[98], and the effect of myocardial microcirculatory compression in systole and relaxation in early diastole can be estimated by the magnitude of the early backward compression wave(eBCW) and the backward expansion wave (BEW) respectively**(figure 1.7)**. The size of the eBCW has been shown to be greater with increasing myocardial contractility[99]. Accordingly, one would expect that with increasing downstream contracting myocardial mass the eBCW would also increase in size. The size of the BEW and consequently the size of the diastolic upstroke in coronary flow, being driven by the reverse of the mechanism of the eBCW, is therefore also likely to be related to the mass of myocardium relaxing in early diastole. The increased flow generated by a greater contracting myocardial mass provides an explanation why stenoses subtending a greater myocardial mass but with similar angiographic severities have been shown to have a significantly lower FFR[100]. Similarly, an inverse relationship between FFR and left ventricular ejection fraction has been demonstrated[101].

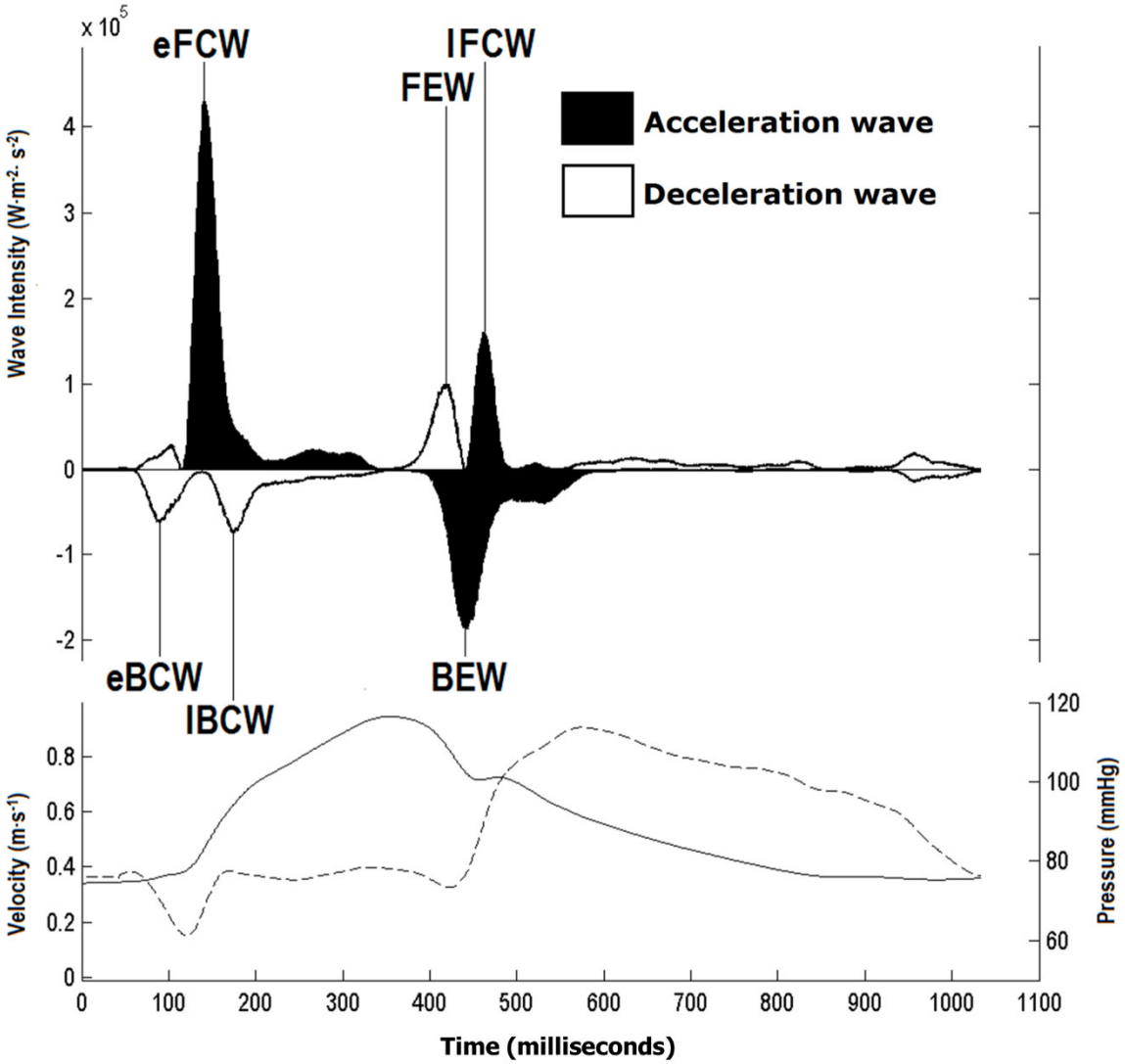

Figure 1.7. Wave intensity analysis, ensemble averaged coronary pressure (solid line) and flow velocity (dashed line) for a single cardiac cycle, measured in a non-dominant left circumflex artery in man. The onset of diastole can be identified by the dicrotic notch in the pressure trace at approximately 450 milliseconds, note the diastolic predominance of coronary flow. eBCW= early backward compression wave, BEW=backward expansion wave. Other waves: eFCW=early forwards compression wave, caused by left ventricular ejection; lBCW=late backwards compression wave, caused by the reflection of the eFCW against the microvasculature, bifurcations and coronary stenoses; FEW=forwards expansion wave, caused by the suction of left ventricular relaxation prior to closure of the aortic valve; lFCW= late forwards compression wave, caused by closure of the aortic valve.

A corollary of the inverse relationship between perfused, contracting myocardial mass and FFR, is that the quantitative change in perfused myocardial mass in a collateral donor vessel as a result of CTO PCI is likely to be related to the change in donor vessel FFR. This would apply to the absolute myocardial mass and also the collateral dependent mass relative to the myocardial mass in the ordinary perfusion territory of the collateral

donor vessel. One might expect the largest changes to occur in large collateral dependent coronary territories (such as that distal to a proximally occluded LAD), predominantly collateralised by a small vessel such as a non-dominant circumflex artery.

Vessel diameter is related to perfused myocardial mass[102], in the absence of ectasia in the donor vessel, the larger the donor vessel diameter, the smaller any change in FFR might be. Given the limited visualisation of the occluded vessel segment distal to a CTO and the likely reduction in diameter as a result of a chronic reduction in flow, the use of vessel diameter in the occluded segment is unlikely to be useful.

1.2.8 Viability of the collateral dependent myocardium

We would expect the increase in flow in a donor vessel associated with the additional supply of collateral dependent myocardium to be related to the collateral dependent myocardium's mass. We would therefore also expect there to be a similar relationship with the influence upon the FFR. If myocardium is infarcted or non-viable, then flow is very low indeed[103], however viable myocardium in the same territory has preserved microvascular function[104], it is likely that the territory can therefore be considered smaller and the change in flow related to the mass of viable myocardium. It is not clear whether coronary steal would be more likely in this situation.

1.2.9 Inherent variability of FFR measurement

As with all measurements, there is inherent variability to the measurement of FFR. The often quoted coefficient of variation, based upon 15 repeated measurements under baseline conditions is 4.8%[59], which is far superior to coronary flow reserve(10.5%). Put into context, this equates to a standard deviation of the difference between repeated measurements of 0.045 and, assuming the difference is unrelated to initial FFR, a coefficient of repeatability of 0.088**(figure 1.8)**. The largest study of FFR repeatability comes from the DEFER trial[105]. In patients enrolled in the trial,

FFR measurements were taken twice within a 10 minute interval. The mean absolute difference was reported as 0.03, with a standard deviation of 0.02. There was also no apparent association between the value of the FFR and the measurement variability.

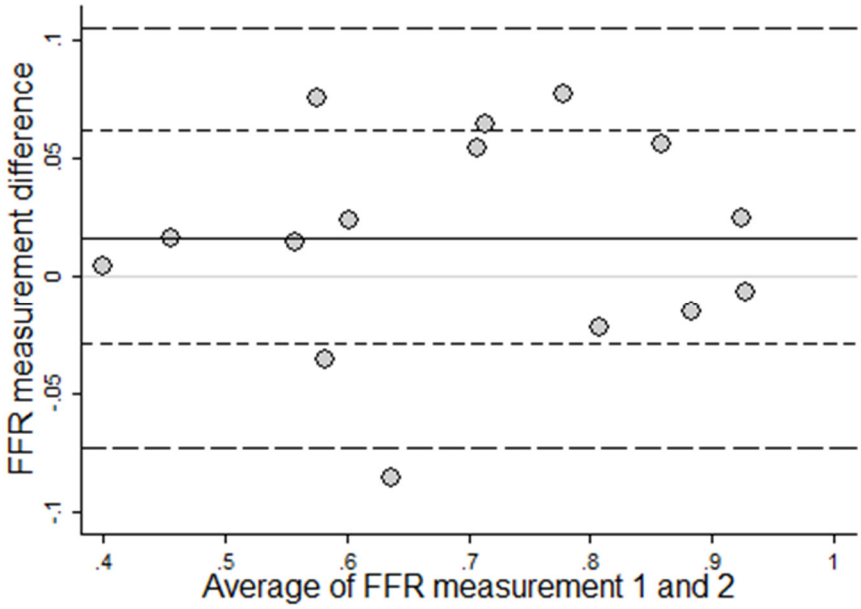

Figure 1.8. Bland-Altman plot of data taken from De Bruyne et al[59], the black solid horizontal line represents the mean difference between measurements (non-significant), short dashed lines represent one SDD either side of the mean difference and long dashed lines represent limits of agreement.

FFR measurement is highly reproducible, but we have grown to practice with that assumption in mind, such that clinical decisions are sometimes made based upon margins of as little as 0.01. Based upon the reported absolute difference from DEFER, we can estimate a standard deviation of the difference of 0.032, and a coefficient of repeatability of 0.063, which means 95% of repeat measurements will be within 0.063 of the initial measurement. It is therefore not particularly unusual for repeat FFR measurements taken within 10 minutes of one another, to differ by as much as 0.06 despite no action taken in the interim. If FFR is measured and re-measured after a longer interval, with a CTO angioplasty in the intervening period, even if the angioplasty has no direct effect itself, it would be reasonable to assume that the standard deviation of the difference might be larger than 0.032. The problem of publication bias in case reports is well recognised[106], and if a large change in FFR is encountered in the

expected direction (even if a large proportion of the change is due to simple measurement variability), it is more likely to be published than one in the unexpected direction, which when repeated has regressed towards the mean[107,108].

1.2.10 Summary

At present, when confronted with a chronic total coronary occlusion in the setting of multi-vessel disease, there is real uncertainty as to how large the influence of collateral donation is upon physiological lesion assessment indices on the collateral donor vessel. The existing literature we can base our decision making on includes case reports (which are likely to be subject to publication bias), one small study which reported a change with a very wide confidence interval and also inferences from our knowledge of coronary physiology. An understanding of the magnitude of the change in the index after PCI of the CTO, the mechanism of any change and the factors which influence that change would inform our revascularisation strategy in a sizeable subset of patients with multi-vessel disease. This can only realistically be achieved by further study of the phenomenon in the clinical setting.

1.3 The microvasculature distal to a CTO

1.3.1 The importance of the microvasculature

The modulation of tone within the coronary microcirculation is a major determinant of variations in coronary blood flow[109,110]. A dysfunctional microcirculation, lacking the capacity to vasodilate in response to increasing metabolic demands can be associated with myocardial ischaemia, even if no flow-limiting stenosis is present in the epicardial portion of the coronary artery[111]. Microcirculatory dysfunction, with or without flow-limiting epicardial coronary disease is associated with an adverse long-term prognosis[112,113]. In addition, given that the vasodilatory capacity of a microvascular bed has such a large influence over coronary flow both at rest and under hyperaemic conditions, microvascular

vasodilatory capacity has a major influence upon the now widely used pressure-wire based indices of physiological stenosis severity[114]. Lesions of the same anatomical severity can have large differences in FFR or instantaneous wave-free ratio (iFR) measurement dependent upon microvascular tone and the capacity of the microvasculature to dilate.

1.3.2 Make-up of the coronary circulation

The coronary circulation can be split broadly into three compartments: the large epicardial vessels between 0.5mm and 5mm in diameter; the small coronary arteries/pre-arterioles (100–500μm); and intra-mural arterioles (<100μm), which drain into the intra-myocardial capillaries and on into the coronary venous system[115] **(figure 1.9)**.

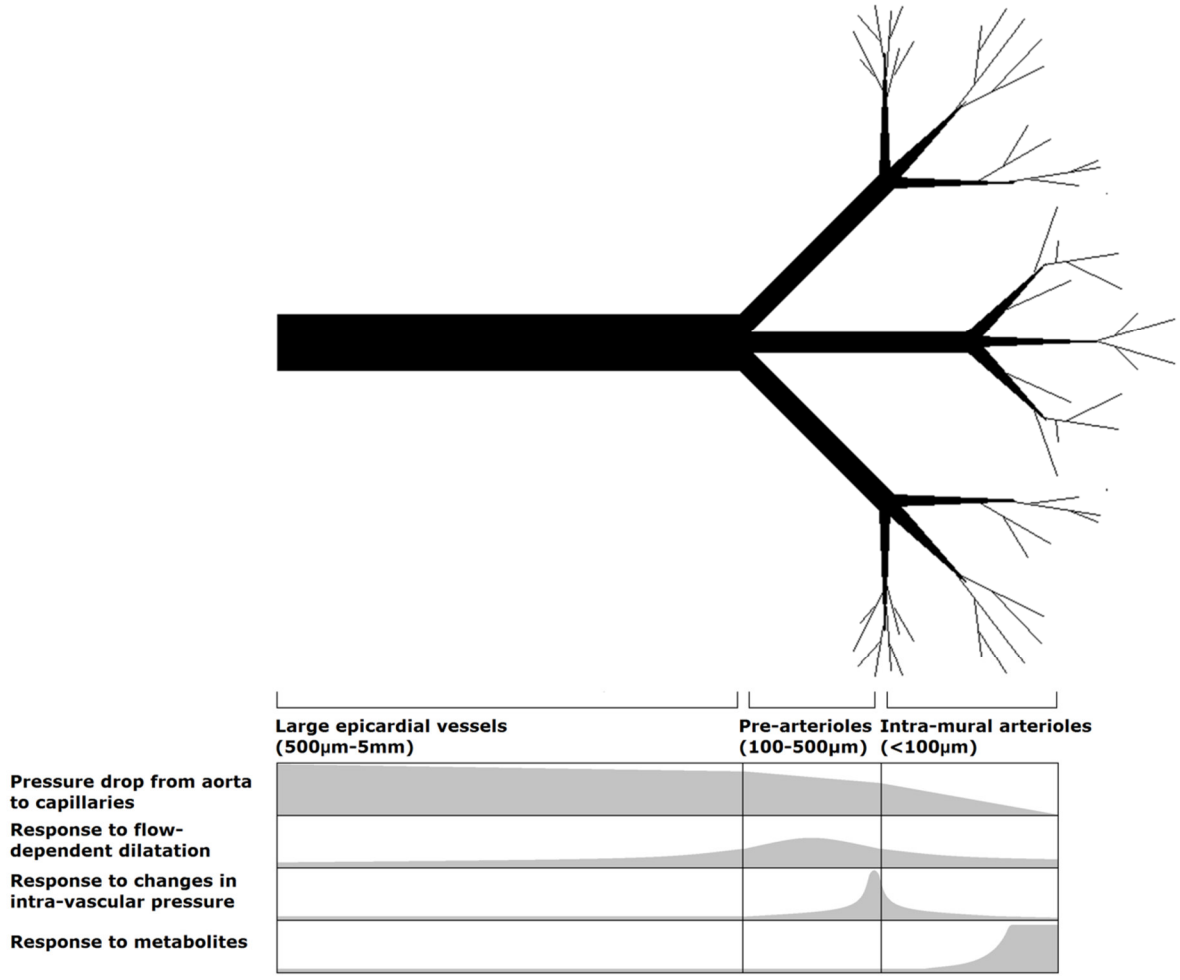

Figure 1.9. Schematic representation of the coronary circulation, with corresponding distribution within the coronary vasculature of pressure drop under normal conditions, flow-dependent vaso-dilatation, pressure dependent vaso-motor tone and response to metabolic stimuli (adapted from Crea et al[109])

Large epicardial vessels have a capacitance function and in the absence of disease, generate very little resistance to flow[109]. Small coronary arteries/pre-arterioles are not directly modulated by metabolites, but are thought to tightly regulate pressure at the origin of the intra-mural arterioles in response to flow dependent changes in endothelial shear stress[116] (endothelium dependent vasodilatation), and by a direct myogenic response to changes in pressure[117] (endothelium independent). Intra-mural arterioles dilate in response to metabolites which are produced by the myocardium as a result of increased oxygen consumption[109]. These mechanisms are summarized in **figure 1.9**. They work in synergy with one

another to match oxygen supply to demand, it is when these mechanisms are dysfunctional, or when their vasodilatory mechanisms are insufficient to maintain the required oxygen delivery to the myocardium, that myocardial ischaemia occurs.

1.3.3 Invasive assessment of the microvasculature

By measuring distal coronary pressure and coronary flow velocity, by either intra-coronary Doppler[118] or indirectly by thermodilution and using the inverse transit-time of a cold bolus of saline as a surrogate of flow velocity[115], the coronary microvasculature can be assessed by intra-coronary wire based techniques. This thesis will focus on the Doppler and combined Doppler/pressure based techniques.

Coronary flow reserve (CFR) is defined as the ratio of flow under maximal coronary and microvascular vasodilatation (hyperaemia) to flow at baseline[118]. Hyperaemia is most often achieved by either intra-coronary bolus or intra-venous infusion of adenosine. The CFR represents the extent to which the microvasculature is able to dilate over and above its baseline state. CFR can be measured non-invasively using positron emission tomography (PET)[111]; when an intra-coronary Doppler wire is used it is calculated by the ratio of mean flow velocities (average peak velocity, APV) at hyperaemia and baseline[118] (**figure 1.10**). In this circumstance, it is termed coronary flow velocity reserve (CFR).

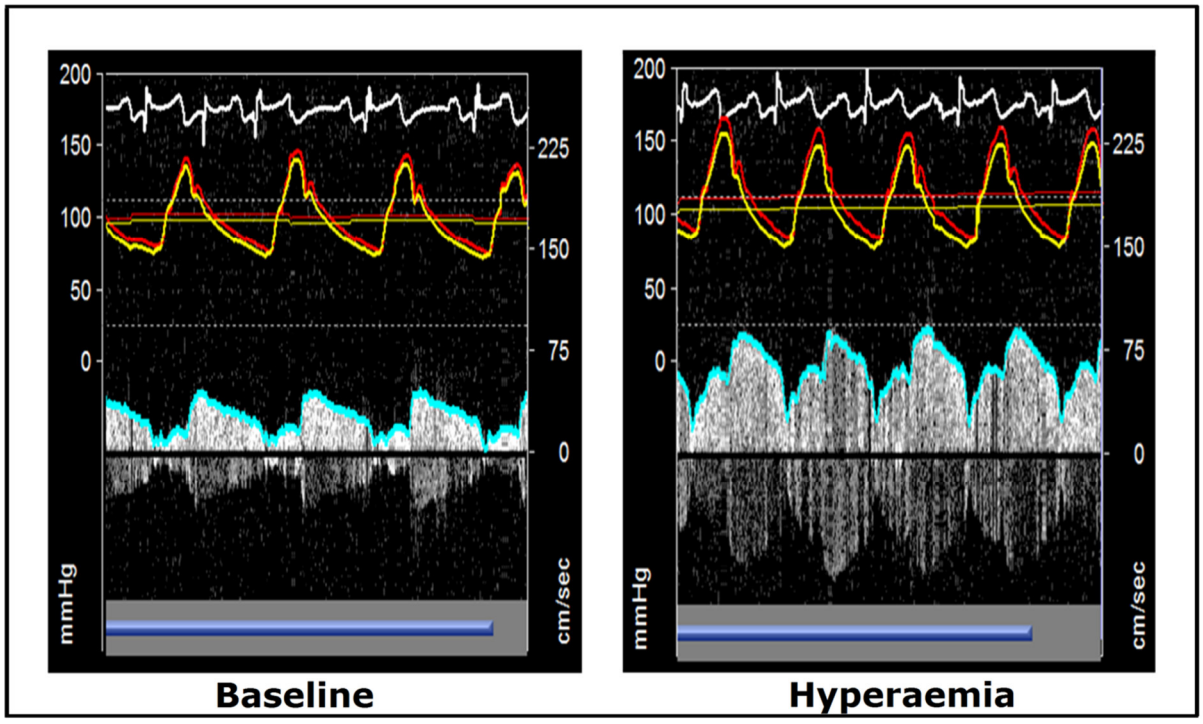

Figure 1.10. Combined intra-coronary pressure and Doppler measurement in an unobstructed vessel at baseline (left) and hyperaemia (right). The white line represents the electrocardiogram; red: aortic instantaneous and mean pressure; yellow: intra-coronary instantaneous and mean pressure; and blue: the instantaneous peak coronary flow velocity (IPV) around the Doppler envelope, the mean of which is the average peak velocity (APV). CFR= $APV_{hyperaemia}/APV_{baseline}$.

The coronary flow reserve describes the microvasculature's capacity to dilate above its level of tone at baseline. Used alone however, it does not necessarily delineate where in the coronary microvascular tree any pathology lies. In the presence of a flow limiting epicardial stenosis, the coronary microvasculature will vasodilate to maintain baseline flow up to the point where it has no capacity to dilate any further. Using PET, coronary flow reserve has been shown to be inversely related to coronary stenosis severity[93] and with very severe stenoses (≥90%), will often have a value of 1, not having any further capacity to vasodilate.

This problem can be overcome by including distal coronary pressure in the assessment of vasodilatory capacity[87]. The hyperaemic microvascular resistance (HMR_v) is calculated as the ratio of distal coronary pressure to flow velocity under hyperaemic conditions[87,119,120]. If measured under baseline conditions, an assessment of microvascular tone at baseline can be made, termed basal microvascular resistance (BMR). HMR_v provides an

assessment of minimal microvascular tone indexed for distal coronary perfusion pressure and therefore provides an assessment of the microvasculature independent of the epicardial vessel. In the presence of a critical coronary stenosis (or in the occluded segment of a CTO), the HMR can be confounded by collateral flow, however in lesions with an FFR ≥0.6 this effect appears to be minimal[88].

1.3.4 When the vessel is occluded

If a vessel is chronically occluded, although collateral flow will often preserve resting myocardial contractility, myocardial perfusion under pharmacological stress is almost universally reduced[45,92,121]. Under these conditions, the microvasculature is at or close to its maximal level of vasodilatation to accommodate for the reduced perfusion pressure. As a result, any further vasodilatation to accommodate for further metabolic demands will not be possible[46].

Indeed, this is the basis for coronary steal to occur[94]. This is demonstrated in **figure 1.11**, which illustrates coronary flow velocity and pressure in a vessel segment distal to a CTO, prior to restoration of antegrade flow. There is sufficient resistance in the donor vessel to cause a pressure drop during hyperaemia; and the collateral vessel, like most collaterals vessels, is small and tortuous generating significant resistance; in addition to this, the microvascular resistance of the collateral dependent myocardium is fixed and lacks a vasodilatory reserve[62,96]. The consequence to this is that flow is directed away from the collateral dependent myocardium resulting in a paradoxical fall in coronary flow with hyperaemia. In this example, the phenomenon is most likely contributed to by a fall in systemic pressures as a result of systemic administration of adenosine[122].

With reduced pre-arteriolar perfusion pressures and flow velocities, pre-arterioles will vasoconstrict to maintain intra-mural arterioler perfusion pressures; the intra-mural arterioles will already be maximally vaso-dilated. The end result is a vessel segment that is chronically

underperfused and underfilled, which is likely to have implications for the behaviour of both the epicardial vessel and the microvasculature if the vessel is recanalised.

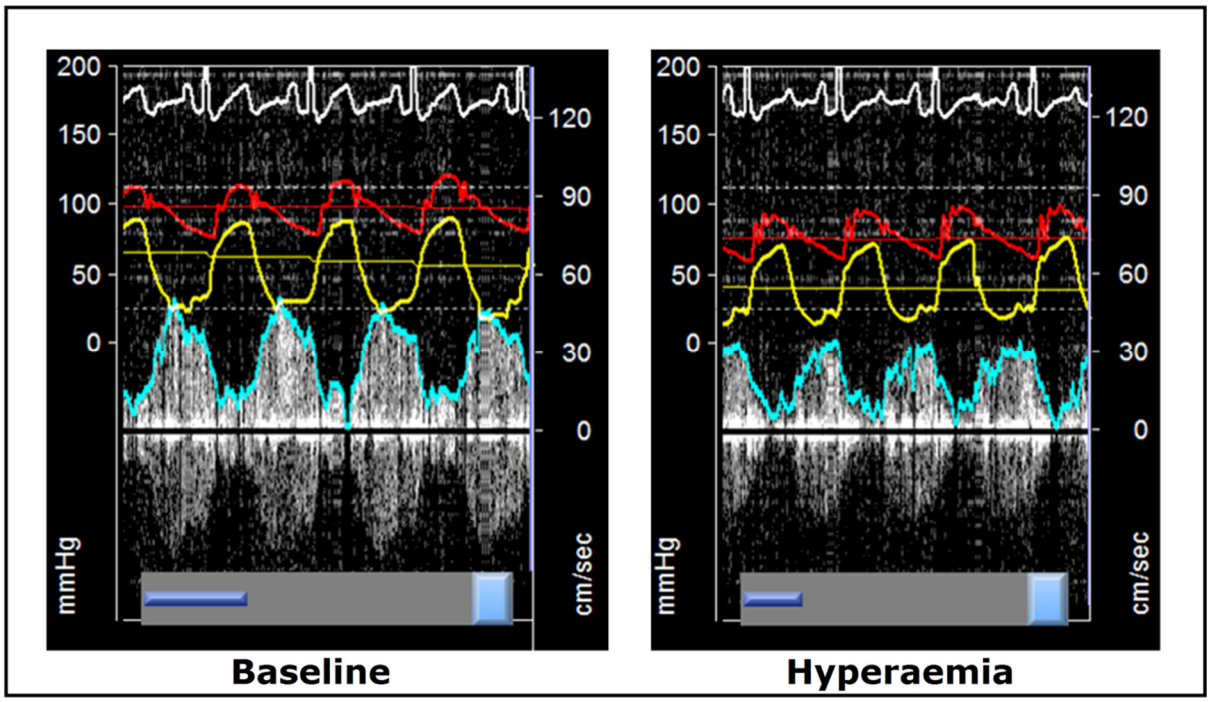

Figure 1.11. Combined intra-coronary pressure and Doppler measurement in the occluded segment distal to a CTO at baseline (left) and hyperaemia (right), showing an example of coronary steal. Note the fall in coronary flow velocity under hyperaemic conditions, in contrast to figure 1.09. The white line represents the electrocardiogram; red: aortic instantaneous and mean pressure; yellow: intra-coronary instantaneous and mean pressure; and blue: the instantaneous peak coronary flow velocity (IPV) around the Doppler envelope, the mean of which is the average peak velocity (APV).

1.3.5 When the vessel is re-opened

The state of chronic under filling of vessel segments distal to CTOs appears to lead to abnormal vascular physiology in the immediate aftermath of recanalisation of the CTO. After recanalisation, the recently occluded segment has been shown not to exhibit the expected endothelium independent vasodilatory response to intra-coronary nitroglycerin and also paradoxical vasoconstriction with atrial pacing, suggesting endothelial dysfunction[123]. In the same study, vessel diameter in what was the occluded segment increased significantly at 9-12 month follow-up and the vaso-dilatory response to intra-coronary nitroglycerin had returned. However, endothelial dysfunction in the form of vasoconstriction associated with atrial pacing at follow-up persisted[123]. A similar study examined

response to nitroglycerin in the occluded segment shortly after recanalisation and tested endothelial function by intra-coronary acetyl-choline injection[124]. Paradoxical vasoconstriction was demonstrated after acetyl-choline injection, and a blunted vaso-dilatory reponse to nitroglycerin. The numbers included in the study were very small, but an association between a better angiographic degree of collateralisation and less marked endothelial and vasodilatory dysfunction was demonstrated[124].

This can lead to a clinical dilemma, firstly choosing the appropriate size of stent after re-opening of a CTO can be a challenge, as the vessel is likely to grow. Secondly, it can be challenging to know which stenoses merit treatment and which should be left in the distal, recently occluded vessel segment. One approach would be physiological lesion assessment using FFR or iFR, however in this situation caution should be employed given the likely dynamic nature of the epicardial vessel over the ensuing months post recanalisation. Nevertheless, it is an approach which has been employed; however it is further limited by us not knowing the state of the microvasculature in this setting.

It is well accepted that reversible microvascular dysfunction occurs in a proportion of patients after PCI of non-occlusive coronary disease[125,126]. This phenomenon has been reported to be more frequent in patients who have undergone PCI to a CTO[72,127]. However, previous work in the CTO population has only used flow velocity based indices (CFR and coronary flow velocity) and has therefore not indexed for any fall in perfusion pressure which may have occurred as a result of the changes that we see in the epicardial vessel post-recanalisation.

Perhaps more importantly, microvascular function in epicardially diseased vessels is related to unobstructed reference vessels in the same patient[128], and is related to a number of patient related factors such as smoking, age, hyperlipdaemia and hypertension[109,129]. The increased prevalence of microvascular dysfunction in this setting may simply reflect the greater burden of disease in patients with CTOs and represent a global phenomenon of microvascular dysfunction rather than a finding particular

to a recently recanalised CTO. Indeed, a flaw in all previously published work examining the CTO vessel in this setting is a failure to examine a reference vessel in the same patient[72,123,124,127]. Coronary endothelial function, like microvascular dysfunction may represent a global phenomenon. It is interesting that in the study by Galassi et al[123]; examining endothelial function in the CTO segment immediately after recanalisation and at 9-12 months, endothelial function did not recover at follow-up, which would support this hypothesis.

If microvascular function in the recanalised CTO segment were compared with a reference vessel in the same patient, it would shed further light on which changes in the behaviour of the microvasculature are related to the CTO and which are global, patient related changes. It would also inform clinicians further as to potential limitations in performing physiological lesion assessment to optimise their PCI result in this setting.

1.4 What do biomarkers of collateralisation mean?

1.4.1 Introduction

The maturation of coronary collateral vessels, termed arteriogenesis, is a complex process. It is distinct from the formation of new vessels, termed angiogenesis, in that it involves the maturation and growth of networks of existing small arteries. It is likely that the mechanism is stimulated by a pressure gradient through pre-existing arterioles connecting coronary territories caused by a coronary stenosis or occlusion, resulting in shear mediated endothelial activation[130]. There is marked variation between individuals with respect to how well the collateral branches that they form are able to perfuse the myocardium[131] and an ability to form functionally superior collaterals is associated with improved clinical outcomes[131,132]. A number of studies have attempted to relate individual patient's observed ability to form collateral vessels with various biological markers (including growth factors, cytokines, chemokines and gene expression) of arteriogenic activity[133–140]. The rationale for such an approach includes further elucidation of the mechanism of arteriogenesis in

man and also the identification of potential biological deficiencies or excesses which might identify potential causes for inter-subject variability in functional collateral supply and as a consequence, possible therapeutic targets.

1.4.2 Do biomarkers relate to endothelial health rather than extent of collateralisation?

As one might expect, the level of no single biological marker has stood out as a discriminator between patients with 'good' and 'bad' collaterals. Patients with coronary disease can be shown to have varying degrees of coronary and systemic endothelial dysfunction. It is well established that the endothelium plays an important role in arteriogenesis[130,141]. It may be that an individual's ability form collateral branches may reflect their degree of coronary endothelial dysfunction.

A number of biological markers have been shown to correlate with coronary and/or systemic endothelial dysfunction[142–146]. Endothelial microparticle levels have been shown to correlate well with impairment of endothelium dependent coronary vasodilatation[143]. Their level can be increased by organ ischaemia[147] and they can induce endothelial dysfunction when exposed to rat aortic rings in vitro[148]. Intercellular adhesion molecule-1 (ICAM-1) and E-selectin are involved in the adhesion of macrophages to shear-stress activated collateral endothelium, an important step in the arteriogenic process and ICAM-1 expression has been shown to be stimulated by increased shear stress[149]. In animal models, arteriogenesis is reduced in the presence of ICAM-1 deficiency[150]. Both soluble ICAM-1 (sICAM-1) and soluble E-selectin (sE-selectin) have been shown to be at higher levels in patients with a lower angiographic grade of collateral[151]. Higher levels of sICAM-1 and interleukin-6 (IL-6) have been shown to be associated with systemic endothelial dysfunction (assessed by flow mediated dilatation)[142]. Tissue factor has been shown in vitro to be involved in the regulation of both endothelial[152] and smooth muscle cell[153] proliferation.

The association with arteriogenesis and endothelial health of a number of biological markers is unlikely to be coincidence. It seems plausible that one reflects the other and arteriogenic activity is related to endothelial health.

1.4.3 What have biomarkers been associated with?

The measure of functional collateralisation compared with the biological markers used in these studies have in the large part been measures of how well developed the coronary collateral circulation is, either measured functionally or angiographically, not the level of arteriogenic activity. In addition, often the studies have included patients with occlusions of <3 months duration[133] or non-occlusive disease, in which lesions of differing physiological severity and also duration of their current severity must have been included[134–136,139,151]. An individual therefore may have poor collaterals because of a short duration of occlusion or haemodynamically significant stenosis, a less severe non-occlusive stenosis or indeed a poor ability to form collaterals.

Some biomarkers might reflect current arteriogenic activity, whereas others may reflect an individual's ability to form collaterals. Any identified associations may reflect a direct relationship between extent of collateralisation and the biomarker in question, a relationship between biomarker levels and endothelial or circulatory health, or a relationship with active arteriogenic activity (which could mean biological factors active in the process could be higher in individuals with less well developed or less well matured collateral circulations).

We cannot measure the level of arteriogenic activity in these patients, but if we take the widely held concept that the process is stimulated by endothelial shear stress, we would expect shear stress and arteriogenic activity to correlate well. Although microvascular shear stress has been calculated in animal models[154], we cannot measure it in the collateral circulation in man. We can measure factors which we know contribute to it such as collateral resistance and absolute flow. If such a correlation does

exist, and if those biomarkers are available in the peripheral circulation, a potential use might be the serial monitoring of patients undergoing therapeutic trials aimed to stimulate collateralisation.

1.4.4 Removing some of the doubt

Collateral networks regress rapidly after CTO PCI[63,64]. By taking a population with well-established collateral networks by virtue of the existence of a chronic total occlusion, and measuring biomarkers prior to and subsequent to revascularisation; whether any measured biomarker is related to the presence of a collateral network should become clear. In that same population, a biomarker could also be compared with various indices of functional collateralisation and collateral flow.

1.5 Aims of this thesis

There remains doubt as to the long-term benefits of CTO recanalisation and the effect it has on coronary haemodynamics. The intention of this thesis is to investigate the effect of the presence of a CTO on myocardial perfusion, coronary physiology and long-term clinical outcomes. The overall aim of the thesis is to provide further evidence to guide management of patients with a CTO with or without concomitant multi-vessel disease. Using both a large cohort of patients with an identified CTO, and a small cohort undergoing detailed haemodynamic investigation around the time of CTO PCI, I hope to contribute to the existing understanding of what is best practice in the field.

In the following series of studies, I first investigate long-term clinical outcomes using a large retrospectively identified cohort of patients with a CTO on angiography. Contrary to much of the existing literature, I aim to compare outcomes between patients treated electively by medical therapy or CTO PCI. I go on to study the haemodynamic changes associated with CTO PCI. Firstly, the associated changes in physiological stenosis severity in the non-CTO vessels and how these changes might influence best revascularisation strategy. I then go on to investigate haemodynamics in

the recently recanalised CTO vessel and the effect that might have on physiological optimization of the PCI result. Finally, I compare coronary haemodynamics taken at the time of CTO PCI with biomarkers of endothelial health to investigate a link between their levels and degree of functional collateralisation.

2.0 Materials & methods

2.1 Patient inclusion, recruitment and consent

2.1.1 Retrospective study inclusion

2.1.1.1 Identification of CTOs from an angiography database

In order to study long-term clinical outcomes amongst a population with a CTO identified on angiography, patients were identified using a prospectively entered database (Patient Analysis & Tracking System, Dendrite Clinical Systems, Henley-on-Thames, United Kingdom). If a patient was recorded as having a 100% occlusion during the study period (01/01/2002 to 31/12/2007), they were identified for screening. Medical notes and angiographic images were then reviewed in order to confirm the occlusion was chronic (≥3 months in duration), with TIMI 0 antegrade flow[1].

We excluded those treated for acute MI in the territory of the occluded vessel in the preceding 3 months as this would suggest that the occlusion was not of ≥3 month's duration. Prior CABG was also an exclusion criterion as the myocardium distal to many occluded native vessels would be perfused by bypass grafts. Patients with mitral or aortic valve disease of moderate severity or greater were excluded because their prognosis and choice of revascularisation strategy were more likely to be dictated by their valve disease and the need for surgery than their coronary disease. Patients with active neoplastic disease were excluded because their prognosis was likely to be dependent on their neoplastic disease rather than their coronary disease and whether or not it was revascularised. Patients were included on the first occasion within the study period that a CTO was identified, and included only once.

2.1.1.2 Approval for inclusion

The retrospective study protocol was approved by the local research ethics committee (NRES ref:13/YH/0036). As the study was retrospective, we were unable to obtain informed consent from those patients with our primary outcome of mortality. If we had given the option to withhold consent to all patients without our primary end-point of mortality but not

those with it (because they are no longer alive), it may have resulted in bias, particularly if there is a difference in outcomes between treatment groups. We obtained approval from the National Confidentiality Advisory Group(CAG 3-06(PR3)/2013) to include clinical data and outcome measures without informed consent.

Section 251 of the NHS Act 2006 allows the Secretary of State for Health to make regulations to set aside the common law duty of confidentiality for defined medical purposes. The regulations that enable this power are called the Health Service (Control of Patient Information) Regulations 2002. The Health Research Authority (HRA) took responsibility for Section 251 in April 2013, establishing the Confidentiality Advisory Group (CAG). Section 251 was established to enable the common law duty of confidentiality to be overridden to enable disclosure of confidential patient information for medical purposes, where it was not possible to use anonymised information and where seeking consent was not practical, having regard to the cost and technology available.

The CAG granted us section 251 exemption in May 2013, giving us permission to use patient identifiable data without informed consent.

2.1.2 Prospective study inclusion, approval, recruitment and consent

The prospective haemodynamic study protocol was approved by the local research ethics committee (NRES ref: 12/YH/0360). Participants for prospective haemodynamic investigation were identified from the Hull & East Yorkshire NHS Trust PCI waiting list. If they were due to undergo PCI to a CTO, with confirmed myocardial viability in the CTO myocardial segment and no exclusion criteria they were approached. Myocardial viability was confirmed by one or more of: myocardial perfusion scintigraphy, dobutamine stress echocardiography or the absence of a wall motion abnormality in the CTO segment demonstrated by echocardiography or left ventricular angiography.

Patients were excluded if they were unable to provide consent. We also excluded patients if they had >1 occluded vessel, prior CABG with any patent grafts, or left main stem stenosis considered to be haemodynamically significant as we considered that any of these would add to the heterogeneity of any haemodynamic effect of CTO recanalisation and therefore complicate interpretation of results in such a small study. Patients with any contra-indication to adenosine were also excluded as its use in induction of hyperaemia was an essential part of the study protocol. Eligible patients were approached at the time of their pre-assessment appointment within two weeks of their scheduled procedure. At pre-assessment, patients were informed about the procedure they were scheduled to have and also invited to participate in the research study. A patient information leaflet was given describing the research study (**Appendix I**). On the day of their procedure if the patient was willing to participate in the study, a study consent form was completed (**Appendix II**).

2.2 Investigations and means of data collection

2.2.1 Angiographic assessment

2.2.1.1 Thrombolysis In Myocardial Infarction (TIMI) flow grade

The Thrombolysis In Myocardial Infarction (TIMI) flow grade was developed by the TIMI study group as a semi-quantitative grading scale for assessing coronary blood flow based on visual assessment of the rate of contrast opacification at the time of angiography of the infarct artery in myocardial infarction[155]. The scale is summarised in **table 2.1**.

For the purposes of assessment of CTOs, its importance is that antegrade flow must be TIMI grade 0 to be considered a CTO[1]. It is also used to semi-quantitatively assess coronary blood flow post-PCI.

Table 2.1 TIMI flow grade

TIMI 0	No antegrade flow beyond the point of occlusion
TIMI 1	Faint antegrade coronary flow beyond the occlusion Incomplete filling of the distal coronary bed
TIMI 2	Delayed or sluggish antegrade flow Complete filling of the distal territory
TIMI 3	Normal flow Complete filling of the distal territory

2.2.1.2 SYNTAX score

The SYNTAX score is an angiographic tool for grading the complexity of coronary artery disease[156]. A higher SYNTAX score is indicative of greater angiographic complexity and a greater therapeutic challenge, as well as a worse prognosis[157]. Each coronary segment is given a score commensurate with the proportion of left ventricular myocardium supplied downstream of it (**table 2.2**). If a lesion in a coronary segment has a stenosis of ≥50%, a score is given based upon the coronary segment plus a score for complexity of the lesion (**table 2.3**).

The SYNTAX score was calculated using a downloadable calculator using the algoryhthm described (Version 2.11 Cardialysis, Rotterdam, The Netherlands).

Table 2.2 SYNTAX score segment weighting factors

Segment No		Right dominance	Left dominance
1	RCA proximal	1	0
2	RCA mid	1	0
3	RCA distal	1	0
4	Posterior descending artery	1	n.a
16	Posterolateral branch from RCA	0.5	n.a
16a	Posterolateral branch from RCA	0.5	n.a
16b	Posterolateral branch from RCA	0.5	n.a
16c	Posterolateral branch from RCA	0.5	n.a
5	Left Main	5	6
6	LAD proximal	3.5	3.5
7	LAD mid	2.5	2.5
8	LAD apical	1	1
9	First diagonal	1	1
9a	First diagonal	1	1
10	Second diagonal	0.5	0.5
10a	Second diagonal a	0.5	0.5
11	Proximal circumflex artery	1.5	2.5
12	Intermediate artery	1	1
12a	1st Obtuse marginal	1	1
12b	2nd Obtuse marginal	1	1
13	Distal circumflex artery	0.5	1.5
14	Left posterolateral	0.5	1
14a	Left posterolaterala	0.5	1
14b	Left posterolateralb	0.5	1
15	Posterior descending	n.a	1

Adapted from Sianos et al[156].

Table 2.3 SYNTAX score stenosis complexity scoring

Diameter reduction	
- Total occlusion	x5
- Significant lesion (50-99%)	x2
Total occlusion (TO)	
- Age >3months or unknown +1	+1
- Blunt stump +1	+1
- Bridging +1	+1
- First segment visible beyond TO	+1/ per non-visible segment
- Side branch (SB)	- Yes, SB <1.5mm***** +1 - Yes, both SB < & ≥ 1.5mm +1
Trifurcations	
- 1 diseased segment	+3
- 2 diseased segments	+4
- 3 diseased segments	+5
- 4 diseased segments	+6
Bifurcations	
- Type 1·0·0, 0·1·0, 1·1·0	+1
- Type 1·1·1, 0·0·1, 1·0·1, 0·1·1******	+2
- Angulation <70° +1	+1
Aorto ostial stenosis	+1
Severe tortuosity	+2
Length > 20mm	+1
Heavy calcification	+2
Thrombus	+1

X **denotes multiplication; + addition; * If all the side branches are 1.5mm in diameter, no points are added since the lesion is considered as a bifurcation and it should be scored as such; **refers to Medina bifurcation classification**[158]**.**

Adapted from Sianos et al[156]**.**

2.2.1.3 The J-CTO score

The Multi CTO Registry in Japan (J-CTO) score is an angiographic grading score to quantify likely difficulty in successful PCI of a CTO lesion[159]. The score was derived based upon independent predictors of failure to cross a CTO lesion antegradely with a wire in <30 minutes, a point is accrued for the presence of each predictor. Its constituent parts are listed in **table 2.4**. The score is then used to put a CTO lesion into four difficulty groups: easy (J-CTO score of 0), intermediate (score of 1), difficult (score of 2), and very difficult (score of ≥3), which has been validated as predictive of successful wire crossing[159].

Table 2.4 The J-CTO score

Tapered entry tip	0
Blunt entry tip	+1
Calcification absent	0
Calcification present	+1
Bending >45° absent	0
Bending >45° present	+1
Occlusion length <20mm	0
Occlusion length ≥20mm	+1
Previous failed attempt	+1

Adapted from Morino et al[159].

To receive a score of 1 for calcification, any amount of calcium needs to be visible within the CTO lesion. Bending >45° refers to if a bend of >45° occurs within the CTO segment.

2.2.1.4 Rentrop collateral filling grade

The Rentrop collateral filling grade is a semi-quantitative measure of the extent of collateralisation to an occluded segment[89]. Using the grading system: grade 0=no collaterals; grade 1=side branch filling of the recipient artery without filling of the main epicardial artery; grade 2=partial filling of the main epicardial recipient artery; and grade 3=complete filling of the main epicardial recipient artery. The Rentrop collateral filling grade is widely reported in the literature, however it has not been shown to be related to invasive functional measures of collateral perfusion[90].

2.2.1.5 Collateral connection (CC) grade

The collateral connection (CC) grade is a semi-quantitatiive measure of the calibre of collateral connections[90] where: grade 0=no continuous connection between donor and recipient vessels, CC1=threadlike continuous connection and CC2=side branch–like connection. Pressure and flow derived measures of collateral function have been shown to be greater in those with CC2 collaterals[90].

2.2.1.6 Quantitive coronary angiography (QCA)

Quantitative coronary angiography (QCA) is a means of estimating vessel diameter size after some form of calibration, in the case of this thesis, using the guiding catheter luminal diameter as reference. The system used in the following studies was GE Centricity CA1000 (GE Healthcare). A digitised angiography image is magnified (x2) and computer-assisted definition of vessel diameter having calibrated against the guiding catheter (which is of known diameter). Using the same software, percentage diameter stenosis can be calculated using adjacent normal vessel as a reference.

2.2.2 Clinical outcome assessment

2.2.2.1 Cause of death

In order to robustly identify mortality at follow-up for the retrospectively identified cohort of patients with a CTO, national death certification records were used.

For all deaths in England and Wales, The Office for National Statistics (ONS) collects information on date of death and cause of death from civil registration records[160]. The underlying cause of death as recorded on the death certificate, in addition to any other conditions or diseases that the patient had at the time of death, which may or may not have directly contributed to the death are recorded. Certified conditions recorded on the death certificate are subsequently coded in line with the International Statistical Classification of Diseases and Related Health Problems (ICD). The Health and Social Care Information Centre (HSCIC) works with the ONS to provide a service to researchers and other professionals to access to these data.

Identifiable data in the form of a patient's 10 digit NHS number, date of birth and name are securely transferred to the HSCIC, along with a unique study identifier. The HSCIC then use an automated system to match identified participants to their records. In the small proportion of

participants in whom automated matching is unsuccessful, a manual matching process is undertaken. The matched files are transferred back to the researcher with mortality data linked to the unique study identifier only, ready for statistical analysis.

2.2.2.2 Myocardial infarction & repeat revascularisation

In a similar fashion to the collection of mortality data at follow-up for the retrospectively identified cohort of patients with a CTO; national audit data was used to for collect data for MI, PCI or CABG at follow-up. Data were obtained through linkage performed by The National Institute for Cardiac Outcomes Research (NICOR)[161], obtained from the Myocardial Infarction National Audit Project (MINAP), British Cardiovascular Intervention Society (BCIS) Central Cardiac Audit Database (CCAD) and the National Adult Cardiac Surgery Audit (NACSA).

Any event recorded in England and Wales on any of the three databases would be identified. Individual events were not adjudicated.

2.2.3 Haemodynamic assessment

2.2.3.1 ComboMap system (model 6800)

The ComboMap system (**figure 2.1**) processes the information it receives from the guidewire (ComboWire), the pressure transducer connected to the guiding catheter and the electrocardiogram (ECG). Intravascular blood pressure and flow velocity measured in the coronary and are displayed on the console screen in real time.

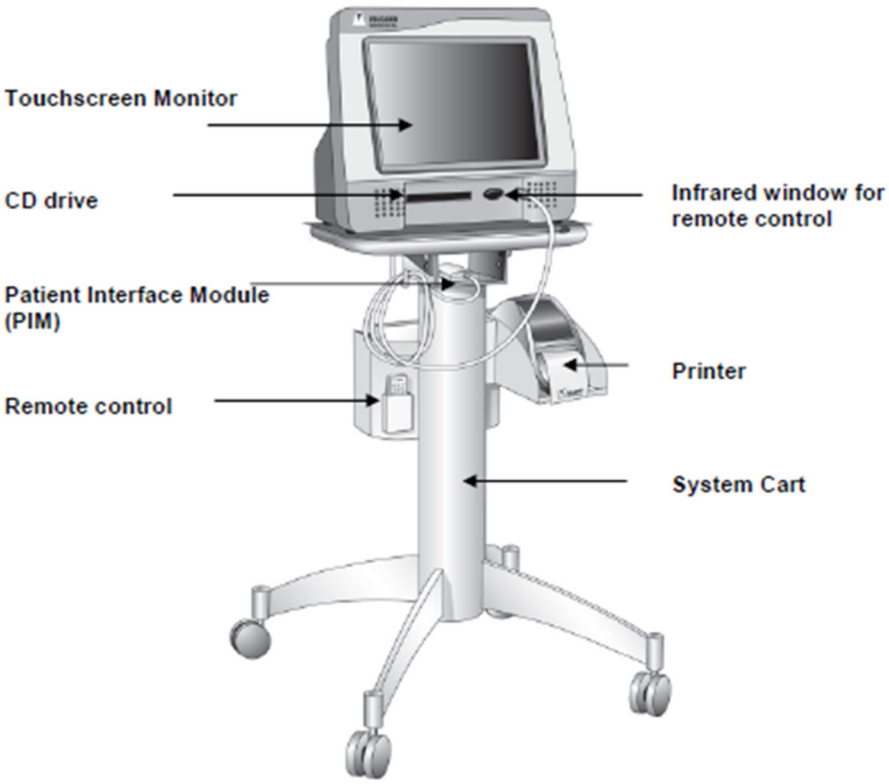

Figure 2.1 The ComboMap system (model 6800). Taken from the ComboMap user manual[162]

2.2.3.2 Combowire XT

The Combowire is a steerable guide wire which combines a pressure and a Doppler sensor. The wire has a diameter of 0.014" (0.36 mm) and a length of 185 cm (**figure 2.2**). The wires used in the studies presented in this thesis contained a pressure and ultrasound transducer mounted at the wire's tip (model reference 9500). A patient interface module was used to transmit signals from the Combowire to the ComboMap console.

The advantage of the use of a wire which can measure both pressure and flow velocity is the additional information relating to resistance of both the coronary stenosis and the coronary microvasculature that can be derived compared with pressure measurement alone. However, Combowires are challenging to manipulate and it can be difficult to align the Doppler beam to obtain a good trace. In addition, even if a good Doppler trace is obtained, flow velocity can be under-estimated if not well aligned with maximal flow velocity. As a result, repeatability of indices which incorporate flow velocity is poor compared with pressure only based indices

(such as FFR)[59]. In routine clinical practice Combowires are seldom used and remain largely a research tool.

2.2.3.3 Measurement of pressure and flow velocity

Aortic pressure (Pa) and right atrial pressure (RAP) were measured using fluid-filled hollow guiding catheters. Pressure is transmitted through a fluid column and measured using an external pressure transducer. The pressure transducer was fixed to the catheter table and prior to each procedure, the system was zeroed at the right atrial level with the patient supine.

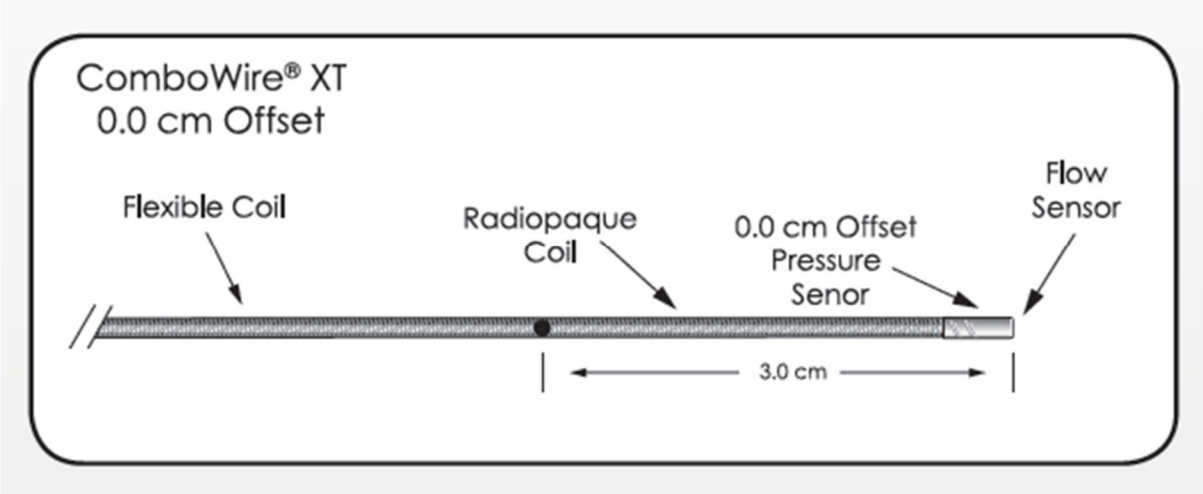

Figure 2.2 The Combowire XT (model 9500). Taken from the ComboMap user manual[162].

Aortic pressure was continuously measured through the guiding catheter positioned in the coronary ostium and connected to the ComboMap to provide Pa. Right atrial pressure was measured at the beginning and at the end of each CTO PCI procedure by positioning the guiding catheter in the right atrium. For RAP, mean pressures were used in the absence of a breath holding; measurements were recorded manually for incorporation into haemodynamic calculations.

The ComboWire was used to measure coronary pressure (Pd). Calibration of the pressure wire was performed with the wire positioned and rested on the table through an automated process. The Combowire was initially positioned at the tip of the guiding catheter to facilitate equalisation

of Pd with aortic pressure (Pa). Once Pd was equal to Pa, the Combowire was advanced to the desired segment in the coronary artery. After each measurement, the Combowire was withdrawn to the guiding catheter to ensure equalisation with Pa suggesting there had been no drift in Pd, if drift exceeded 5mmHg the measurement was repeated.

The pulsed Doppler beam of the Combowire model 9500 is directed forwards from the tip of the wire and has an angle width of approximately 45°, it samples flow velocity approximately 5mm from the wire tip. The wire was manipulated until a good Doppler trace was obtained judged by signal amplitude and density on the ComboMap. Instantaneous peak velocity (IPV) was derived by the ComboMap using an automated algorithm to detect the Doppler envelope.

Instantaneous values of the ECG trace, Pa, Pd, IPV and APV for each study were stored on compact disc. Data was recorded and stored at 200Hz. Analysis was performed using customised software (Study Manager, Academic Medical Center, University of Amsterdam, The Netherlands) which allowed individual time point values recorded at 200Hz to be extracted. Times of individual measurements and events were noted for each study.

2.2.3.4 Anticoagulation, vasodilators and induction of hyperaemia

In order to prevent guiding catheter thrombosis, for all CTO PCI procedures 100 units/kg of heparin were given at the beginning of the procedure, with further bolus doses to achieve an activated clotting time of >300 seconds.

To maximally vasodilate the epicardial coronary artery prior to physiological assessment, 100μg of intra-coronary glyceryl tri-nitrate was administered prior to each coronary haemodynamic assessment.

Hyperaemia was achieved by intravenous infusion of adenosine at a dose of 140μg·kg·minute^{-1}, administered through a central (femoral) vein. The hyperaemic period was continued until steady state hyperaemia had been achieved and a recording thereafter of ≥20 beats had been taken.

2.2.4 Measurement of Biomarkers

2.2.4.1 Biomarker sampling

Before PCI, 20ml of blood was aspirated from the catheter in the right atrium. At the same time as the pre-PCI right atrial sampling, 20ml of blood was aspirated from the femoral arterial sheath. At follow-up, 6-8 weeks after the successful procedure, 20ml of blood was sampled from a peripheral vein.

For the measurement of sICAM-1 and sE-selectin, blood samples were drawn into serum separator tubes, allowed to clot for 30 minutes prior to centrifugation and centrifuged for 15 min at 1000*g*. For the measurement of plasma TF levels, blood samples were drawn into vacutainer tubes containing 0·11 mol/l sodium citrate, and centrifuged for 15 minutes at 3,000*g.* For the measurement of plasma microparticles blood samples were drawn into vacutainer tubes containing 0·11 mol/l sodium citrate, centrifuged for 15 minutes at 1,500*g* at room temperature. Plasma supernatant was then rapidly centrifuged for 2 minutes at 13,000*g* to produce separated 'platelet free' plasma. All separated serum and plasma was stored in aliquots at −80°C to permit assay in batches.

2.2.4.2 Enzyme-linked immunosorbant assays (ELISA)

The concentration of sE-Selectin and sICAM-1 were determined using a commercially available specific sandwich enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D Systems). ELISA plates pre-coated with a specific capture antibody were used, standards and samples were added to the plate and incubated for 2 hours at room temperature. The bound soluble adhesion molecule of interest was detected by a further 2 hour incubation with a specific antibody conjugated to horseradish peroxidise followed by a 30 minute incubation with stabilized hydrogen peroxide and tetramethylbenzidine. The reaction was stopped by the addition of 1 M sulphuric acid to each well and the optical density at 450nm measured using a microplate reader (BMG Labtech, Aylesbury, United Kingdom).

Tissue factor was measured by ELISA. Briefly, microtitre ELISA plates were coated overnight with a specific TF capture antibody (sheep anti-Human Tissue Factor, Enzyme Research Laboratories, Swansea, United Kingdom) in 50 mmol carbonate buffer pH = 9.6. The plates were then incubated overnight at 4°C with PBS/Bovine serum albumen blocking buffer. After a washing step, standards (diluted recombinant tissue factor, American Diagnostica inc, Stanford, USA) and samples were added to the plate and incubated for 90 minutes at room temperature. After another washing step, anti-TF IgG conjugated to horseradish peroxidise was added to the plates and incubated for 90 minutes at room temperature. The plates were washed again and a tetramethylbenzidine substrate solution added and agitated at room temperature for 15 minutes. The reaction was stopped by the addition of 2 M sulphuric acid to each well and the optical density at 490nm measured using a microplate reader (BMG Labtech).

Microparticles concentrations were determined using a commercially available specific ELISA kit (ZYMUPHEN MP-Activity, Hyphen BioMed, Quadratech, Epsom, United Kingdom). Briefly, the diluted plasma sample or standard, supplemented with calcium, Factor Xa and thrombin inhibitors was added to a microplate pre-coated with Streptavidine and biotinylated Annexin V and incubated at 37°C for 1 hour. After a washing step, a Bovine factor Xa-Va mixture containing calcium and purified human Prothombin are added to each well and incubated for a further 10 minutes at 37°C. Thrombin specific chromogenic substrate was then added to each well, after 3 minutes 2% Citric acid was added as a stop solution to each well and optical density at 405nm measured using a microplate reader (BMG Labtech).

The results of each of the ELISA were compared to a standard curve generated by dilutions of a standardised sample for each biomarker, from which individual biomarker concentrations were derived.

2.3 Data processing & analysis

2.3.1 Propensity matching

2.3.1.1 Overview

In the absence of a randomised trial to compare two treatments, as is the case with the comparison of long-term clinical outcomes for patients treated by either CTO PCI or medical therapy; all we have is observational research to guide us. As patients receiving each treatment are not randomized, pre-treatment characteristics are likely to differ between groups and it is likely the treatment decision will to some extent be based upon patient's pre-treatment characteristics[163].

Currently in the literature observational studies of this nature tend to use either multivariable regression modelling or some form of propensity modelling[163] to address this. Propensity modelling can be used to adjust observational data in a number of ways including stratification, use of a generated propensity score as a variable within a multi-variable model or by propensity matching patients based upon a generated propensity score to match patients with similar pre-treatment characteristics.

Propensity matching has a number of advantages over multivariable regression modelling. For multi-variable models, it is generally accepted that there should be at least ten outcome events per covariate included in the model[164]. If an outcome is uncommon, and there are a large number of covariates considered to be potentially confounding, it will not be possible to include them all in the model. Propensity matching also relies on a (logistic) regression model to generate a propensity score, however the outcome of the logistic regression model used is allocation to a particular treatment, for example CTO PCI. If four hundred patients in a cohort received that treatment, then forty covariates can be included in the model. Provided there is sufficient overlap of pre-treatment characteristics, patients in each treatment group can be matched to a patient with a similar propensity score (a value between zero and one, the likelihood of receiving a particular treatment based upon the model). Ideally one group will have a larger number of patients within it to facilitate matching of as many

patients as possible. Once matched, patient groups can be compared with one another without further adjustment, analogous to a randomised trial. This is advantageous as providing the clinician interpreting the result to guide their practice has an awareness of the limitations of the technique, the raw differences presented can be more easily appreciated than an adjusted hazard ratio from a multivariable model, which does not necessarily correspond to the data presented on a Kaplan-Meier curve.

Although analogous to the results of a randomised trial, propensity matching does not account for any confounding variables which are unmeasured or not included in the propensity model.

The adequacy of the propensity matching process should be assessed by comparison of between treatment groups after matching. A generally acceptable difference between groups is a standardised difference of <0.1. If only p-values are use to make a comparison, even if imbalance still exists, the fall in sample size after the matching process may push the p-value >0.05[165]. Analysis should be performed stratified by matched pairs as matched pairs will be similar to one another and therefore not wholly independent[165].

2.3.1.2 Propensity matching applied to CTO PCI and medical therapy

For each patient in the retrospective cohort a propensity score indicating the likelihood of a CTO being treated by PCI was calculated by the use of a non-parsimonious multivariable logistic regression model. Co-variates included in the logistic regression model to calculate the propensity score were: age, gender, previous PCI, previous MI, diabetes mellitus, smoking status, peripheral vascular disease(PVD), hypertension, chronic kidney disease(CKD)stage ≥3, chronic lung disease, cerebrovascular disease, Canadian Cardiovascular Society(CCS)class, New York Heart Association(NYHA)class, presentation (stable angina, unstable angina(UA)/non-ST elevation myocardial infarction(NSTEMI), ST elevation myocardial infarction (STEMI), arrhythmia or heart failure) estimated CTO

duration, left main stem disease, SYNTAX Score, number of significantly diseased vessels, J-CTO score, proximal LAD CTO, branch vessel CTO (defined as diagonal, obtuse marginal, posterior descending artery, posterior left ventricular branch or distal circumflex artery in a right dominant circulation), Left ventricular systolic function, confirmed demonstrable ischaemia (confirmed evidence of ischaemia by non-invasive testing, the absence of which does not mean the absence of ischaemia), confirmed myocardial viability in the CTO territory (the absence of a resting left ventricular regional wall motion abnormality or confirmation by non-invasive testing in the presence of a wall motion abnormality, the absence of which does not mean the absence of viability), number of anti-anginal medications, warfarin use and loop diuretic use. To identify matched pairs of patients undergoing CTO PCI and elective medical therapy a 1:1 optimal match with a ±0.03 caliper and no replacement was used. Cumulative survival was calculated using the Kaplan–Meier method. Clinical outcomes in the matched population were analysed with Cox proportional hazards regression stratified by matched-pair.

2.3.2 Haemodynamic calculations

2.3.2.1 Fractional flow reserve

Fractional flow reserve (FFR) is defined as the ratio of maximal myocardial blood flow distal to a stenotic artery to the theoretical maximal flow in the absence of the stenosis[60]. This can be described by the following equation:

$$FFR = \frac{Q}{Q\boldsymbol{n}}$$

Where Q=flow distal to a stenotic artery and $Q\boldsymbol{n}$=the theoretical maximal flow in the absence of the stenosis. Flow can be derived as follows:

$$Q = \frac{\Delta P}{R}$$

Where Q=flow, ΔP=pressure gradient and R=resistance. Blood flow distal to a stenotic artery (Q) and theoretical maximal flow in the absence of the stenosis ($Q\boldsymbol{n}$) can therefore be expressed as follows:

$$Q\boldsymbol{n} = \frac{Pa - Pv}{Rmin\boldsymbol{n}}$$

$$Q = \frac{Pd - Pv}{Rmin\boldsymbol{s}}$$

Where Pa=aortic pressure, Pd=distal coronary pressure, Pv=right atrial pressure, $Rmin\boldsymbol{n}$=minimal microvascular resistance in the absence of a stenosis and $Rmin\boldsymbol{s}$= minimal microvascular resistance in the presence of a stenosis. The ratio of $Q/Q\boldsymbol{n}$ can therefore be derived from pressure measurements without direct measurement of coronary flow:

$$\frac{Q}{Q\boldsymbol{n}} = \frac{\frac{Pd - Pv}{Rmin\boldsymbol{s}}}{\frac{Pa - Pv}{Rmin\boldsymbol{n}}}$$

Under conditions of maximal hyperaemia $Rmin\boldsymbol{s}$ and $Rmin\boldsymbol{n}$ can be considered to be constant and equivalent (and also minimal) so the equation can be simplified to:

$$\frac{Q}{Q\boldsymbol{n}} = \frac{\frac{Pd - Pv}{Rmin\boldsymbol{s}}}{\frac{Pa - Pv}{Rmin\boldsymbol{n}}} = \frac{Pd - Pv}{Pa - Pv}$$

In fact, in routine practice, Pv is often considered to be so small relative to Pa and Pd as to be of negligible significance and therefore the ratio of Pd/Pa under hyperaemic conditions is used to derive the FFR. For the purposes of this thesis, FFR is calculated as:

$$FFR = \frac{Pd - Pv}{Pa - Pv}$$

Pd and Pa are both measured in mmHg at steady state hyperaemia and mean values are used, taken over 5 cardiac cycles[166]. Pv (or RAP) is measured at the beginning and end of each successful CTO PCI procedure,

mean pressure is used. Any measurements taken prior to PCI incorporate the initial measurement as do those taken after access to the occluded segment of the vessel, but prior to balloon dilatation. Any measurements taken after balloon dilatation of the CTO incorporate the P_V measurement taken at the end of the procedure. An FFR ≤0.80 is considered haemodynamically significant[56].

FFR is the only physiological measure of stenosis severity to be supported by randomised trials to guide revascularisation strategy[56,58,105], and its repeatability is superior to indices which incorporate flow velocity[59]. It is therefore the best, and most widely used index for these purposes in clinical practice. However, the additional indices described in the following sections provide important information in understanding factors which might influence the FFR, such as changes in microvascular resistance. This information could not be obtained by using pressure based indices alone.

2.3.2.2 Coronary flow velocity reserve (CFR)

Coronary flow velocity reserve (CFR) describes the capacity of the microvasculature to augment coronary flow with hyperaemia[118]. It is calculated as follows:

$$CFR = \frac{APV\boldsymbol{H}}{APV\boldsymbol{B}}$$

Where APV**H**=average peak velocity at steady state hyperaemia and APV**B**=average peak velocity at baseline, both measured over 5 cardiac cycles. APV is measured in $cm \cdot s^{-1}$.

2.3.2.3 Hyperaemic microvascular resistance (HMRv)

The hyperaemic microvascular resistance (HMR_v) describes the maximal vasodilatory capacity of the microvasculature, indexed for distal perfusion pressure using mean, whole cardiac cycle pressure and flow velocity[87,119,120]. It is calculated as follows:

$$HMRv = \frac{Pd}{APV\boldsymbol{H}}$$

Both pressure and flow velocity are measured at steady state hyperaemia over 5 cardiac cycles. If measured using Pd and $APV\boldsymbol{B}$ under baseline conditions, an assessment of microvascular tone at baseline can be made, termed basal microvascular resistance (BMR). $HMRv$ and BMR are measured in mmHg·cm^{-1}·s^{-1}

2.3.2.4 Minimal instantaneous microvascular resistance

During the period in the cardiac cycle from 25% into the diastolic period (taken to be from the dicrotic notch to the ECG R-wave), stopping at the onset of the qrs complex, the absolute value and the variance of microvascular resistance have been shown to be minimal[167]. This period therefore represents the period of maximal microvascular vasodilatation. Instantaneous minimal microvascular resistance is therefore calculated calculated as the mean Pd/mean IPV taken during this period, measured in mmHg·cm^{-1}·s^{-1}.

2.3.2.5 Hyperaemic stenosis resistance (HSR)

Hyperaemic stenosis resistance (HSR) is a measure using both mean coronary pressures and flow velocity to calculate the resistance across a particular stenosis or vessel segment. It has been shown to be superior to FFR or CFR at predicting myocardial ischaemia by non-invasive testing[168,169]. It is calculated as follows:

$$HSR = \frac{Pa - Pd}{APV\boldsymbol{H}}$$

All measures are taken over 5 cardiac cycles during steady state hyperaemia. HSR is measured in mmHg·cm^{-1}·s^{-1}.

2.3.2.6 Invasive measures of functional collateralisation

Fractional collateral flow reserve (FFR_{coll}) was calculated as for FFR as (Pd-RAP)/Pa-RAP), using mean pressures taken over 5 cardiac cycles at stable hyperaemia[166], with Pd measured in the occluded segment of the artery prior to restoration of antegrade flow[64]. Collateral flow velocity reserve was calculated as for CFR with flow velocities in the occluded segment measured at rest and steady state hyperaemia[95] as APV at steady state hyperaemia divided by APV at baseline, measured over 5 cardiac cycles.

2.3.2.7 Estimation of absolute coronary flow

Absolute coronary flow can be estimated if coronary diameter is known and flow velocity is measured and has been validated in animal models against an electromagnetic flow meter[170,171]. Coronary diameters are measured using quantitative coronary angiography.The calculation is as follows:

$$Q = (\pi r^2) \times \left(\frac{APV}{2}\right)$$

Where Q = absolute flow (in ml·min^{-1}), r = vessel radius; APV can be measured at baseline or hyperaemia. Rate pressure product (RPP) is defined as the product of heart rate (in beats/min) and systolic blood pressure (in mmHg), it is closely related myocardial oxygen consumption[172]. As resting absolute myocardial blood flow is closely related to RPP, values for resting absolute coronary flow are adjusted by dividing them by their respective RPP/10,000[172].

2.3.2.8 Diastolic flow velocity-pressure gradient relation (DFV-PGR)

The diastolic flow-velocity pressure gradient relation (DFV-PGR) describes the relationship between pressure and flow for a given stenosis or vessel segment[83,84]. It is calculated using continuous recordings of 30 cardiac cycles measured in the distal vessel from baseline through to

maximal hyperaemia[86]. Instantaneous pressures and flow velocities, recorded at 200Hz are extracted from the Study Manager programme and Pa timings corrected to adjust for any time delay with respect to Pd. Individual instantaneous flow velocities (200 per second) from mid-diastole (after the diastolic upstroke in coronary flow velocity) to atrial activation (identified by the beginning of the p-wave on ECG) are plotted against instantaneous pressure gradient (*Pa-Pd*). The DFV-PGR is then calculated using Stata v.12 (StataCorp, College Station, Texas), fitting the following quadratic linear regression equation:

$$\Delta P = (F \times IPV) + (S \times IPV^2)$$

Where ΔP is the pressure gradient in mmHg, F is the coefficient of pressure loss due to viscous friction and S is the coefficient of pressure loss due to flow separation or localized turbulence downstream from the stenosis[83,84] (**figure 2.3**).

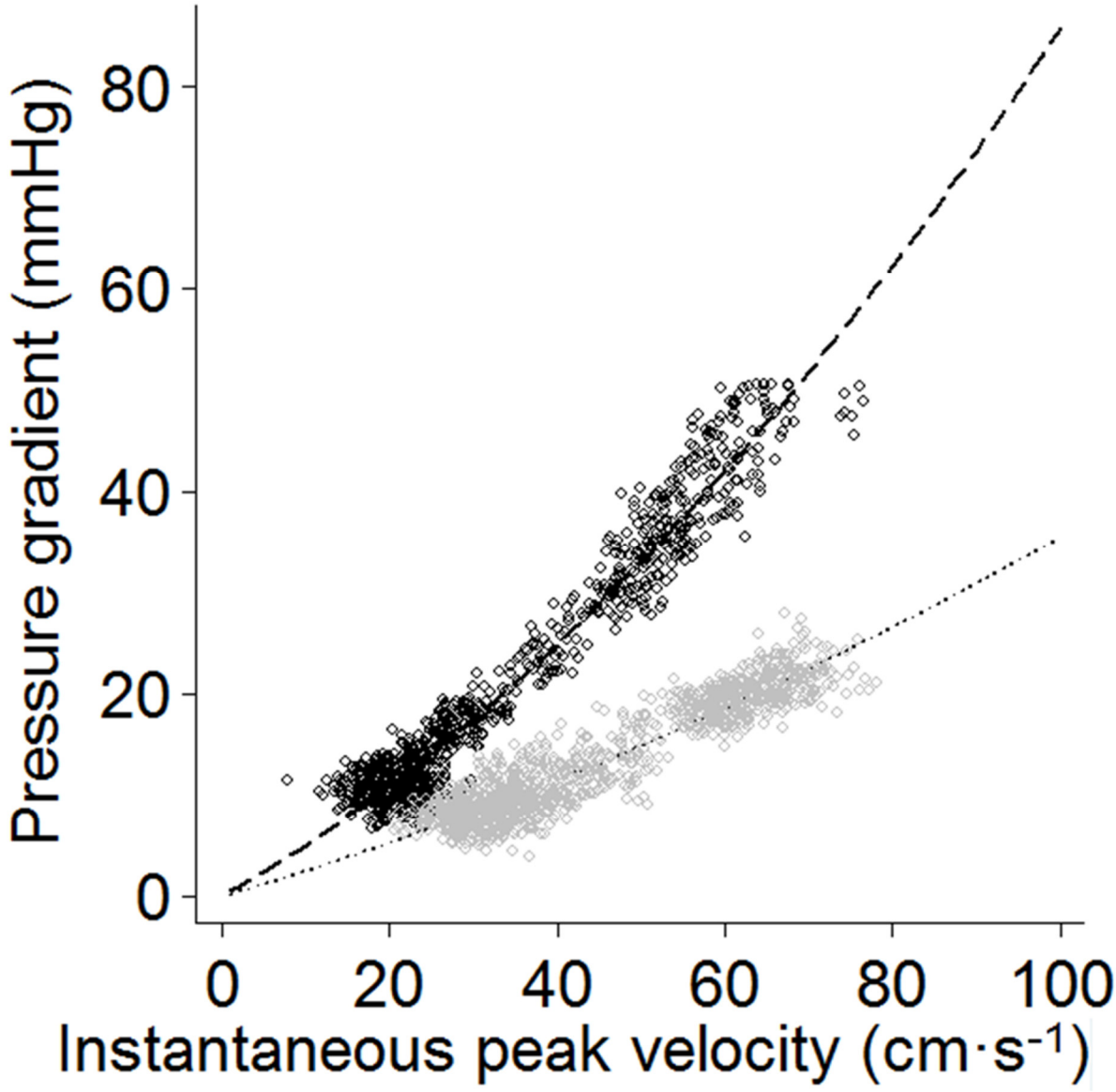

Figure 2.3. Calculation of the DFV–PGR slope using the formula $\Delta P = FV + SV^2$. Two examples are shown, a more severe stenosis (black) and a milder stenosis (grey). Each data point represents an individual instantaneous pressure and flow velocity measurement.

2.3.2.9 Wave intensity analysis

Wave intensity analysis can be used to identify both distally and proximally originating influences upon flow of fluid through a tube. Net wave intensity is calculated as the product of change in pressure (dP) and change in flow velocity (dU) for a particular interval in the cardiac cycle and is calculated in units of power ($W \cdot m^{-2} \cdot s^{-2}$). It has particular value in the coronary circulation as flow is heavily influenced by myocardial contraction both in its effect on left ventricular ejection and also its effect upon the microvasculature. Waves originating from the aorta will be positive and

those from the microvasculature negative[173]. By estimation of the speed of these waves, it is possible to separate proximal and distal influences upon coronary flow[98,173,174].

Wave speed in the coronary arteries can be estimated as follows using the sum of squares method[173,174]:

$$c = \frac{1}{\rho}\sqrt{\frac{\sum dP^2}{\sum dU^2}}$$

Where c = wave speed, ρ is the density of blood (and assumed to be 1050kg·m^{-3})[98], dP is the change in pressure for a particular interval in the cardiac cycle and dU is the change in flow velocity for a particular interval in the cardiac cycle. There is some doubt as to the accuracy of this technique; however variations in the calculated value between 25% and 200% have little effect on the subsequent calculation of wave intensity[175].

Once wave speed has been calculated, distal and proximal originating wave intensity can be calculated at each time interval within the cardiac cycle by using the following formulae[98]:

Distal originating wave intensity:

$$WI_{-} = -\frac{1}{4\rho c}\left(\frac{dP}{dt} + pc\frac{dU}{dt}\right)^2$$

Proximal originating wave intensity:

$$WI_{+} = \frac{1}{4\rho c}\left(\frac{dP}{dt} + pc\frac{dU}{dt}\right)^2$$

Net wave intensiy:

$$WI_{net} = WI_{+} + WI_{-} = \left(\frac{dP}{dt}\right)\left(\frac{dU}{dt}\right)$$

Once proximal and distal wave intensity have been calculated, whether the wave results in flow acceleration or deceleration can be determined[98]. For distal originating waves, if dP has a negative value the wave *accelerates* flow and if positive it *decelerates* flow. For proximal originating waves, if dP has a negative value the wave *decelerates* flow and if positive it *accelerates* flow.

To calculate wave intensity analysis, recorded data were analyzed using dedicated custom software. Study Manager (Academic Medical Center, University of Amsterdam, The Netherlands) was used to generate raw data at 200Hz from ComboMap files. This data was then used in a Matlab (Mathworks Inc, Natick, Massachusetts, USA) environment for wave intensity analysis; Imperial College London, London, United Kingdom). The output from which is shown in **figure 2.4**. Peak wave intensity in ($W \cdot m^{-2} \cdot s^{-2}$) can be calculated by measuring peak deflections of each wave. Cumulative wave energy can be calculated as the area under the curve, measured in $J \cdot m^{-2} \cdot s^{-2}$. Measurements of peak wave intensity and cumulative wave energy are calculated in a semi-automated fashion using Matlab, after manual definition of the time intervals of individual waves.

In order to minimise artefact from our calculations of wave intensity analysis, ensemble averaged pressure and flow velocities for 20 cardiac cycles were used. In addition, flow velocity and pressure signals were filtered using a Savitzky–Golay filter[176].

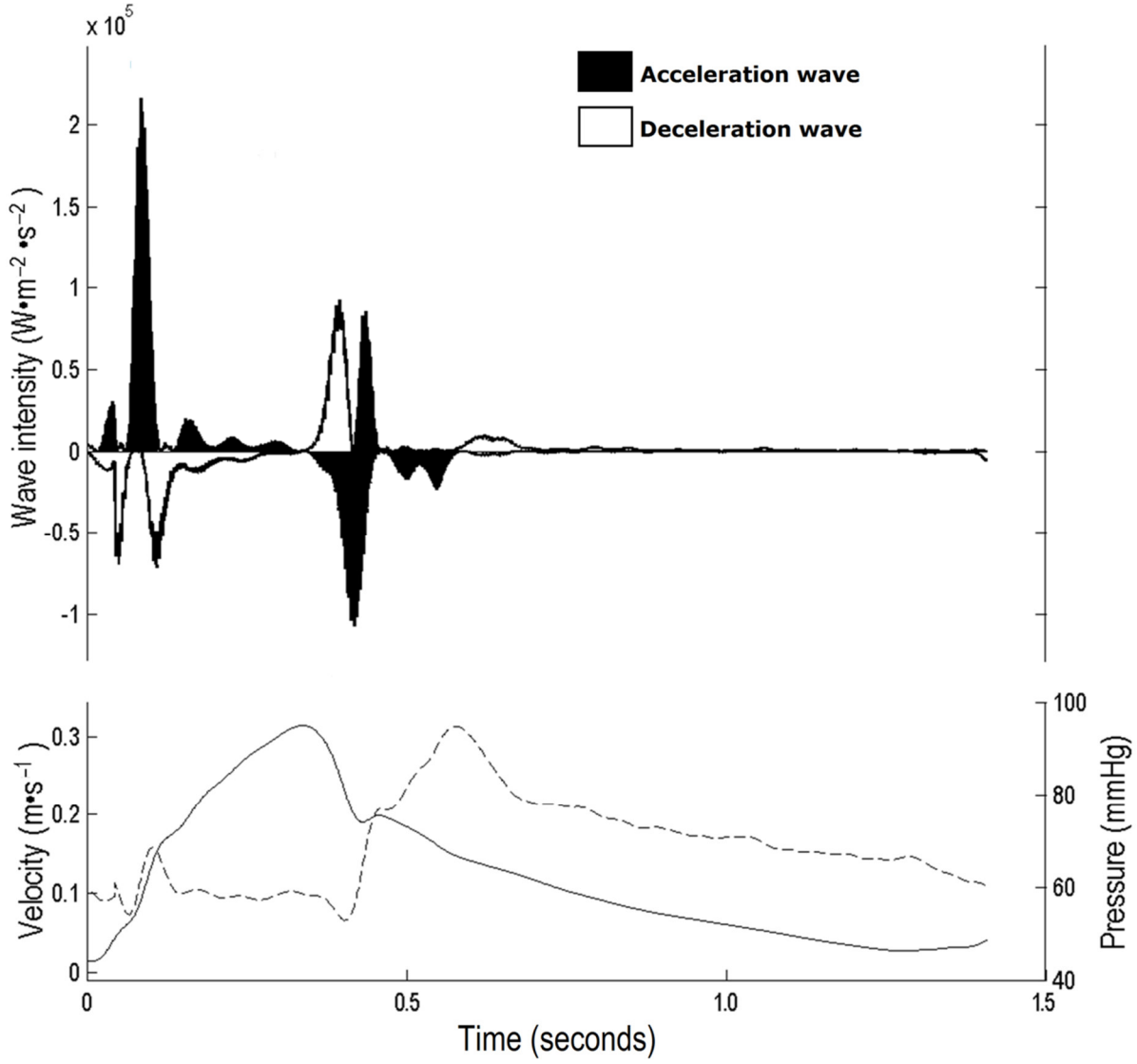

Figure 2.4. Wave intensity analysis measured in the left anterior descending coronary artery. Bottom panel: ensemble averaged coronary pressure (solid line) and flow velocity (dashed line). Top panel: wave intensity analysis measurements derived from ensemble averaged pressure and flow. Positive waves originate from the aorta and negative from the microvasculature.

3.0 Medical therapy, percutaneous coronary intervention and prognosis in patients with chronic total coronary occlusions

3.1 Abstract

3.1.1 Objective

There is little published data reporting outcomes for those found to have a chronic total coronary occlusion (CTO) which is electively treated medically versus those treated by PCI. We sought to compare long-term clinical outcomes between patients treated by PCI and elective medical therapy in a consecutive cohort of patients with an identified CTO.

3.1.2 Methods

Patients found to have a CTO on angiography between January 2002 and December 2007 in a single tertiary centre were identified using a dedicated database. Those undergoing CTO PCI and elective medical therapy to the CTO were propensity matched to adjust for baseline clinical and angiographic differences.

3.1.3 Results

In total 1957 patients were identified, a CTO was treated by PCI in 405(20.7%) and medical therapy in 667(34.1%), 885(45.2%) patients underwent CABG. Of those treated by PCI or medical therapy, propensity score matching identified 294 pairs of patients, PCI was successful in 177 patients (60.2%). All-cause mortality at 5 years was 11.6% for CTO PCI and 16.7% for medical therapy HR 0.63(0.40-1.00) p=.052. The composite of 5-year death or MI occurred in 13.9% of the CTO PCI group and 19.6% in the medical therapy group, HR 0.64(0.42 to 0.99), p=.043. Amongst the CTO PCI group, if the CTO was revascularised by any means during the study period, 5 year mortality was 10.6%, compared with 18.3% in those not revascularised in the medical therapy group, HR 0.50(0.28-0.88, p=.016).

3.1.4 Conclusions

Revascularisation, but not necessarily PCI of a CTO is associated with improved long-term survival relative to medical therapy alone.

3.2 Introduction

A chronic total coronary occlusion(CTO) is present in between one fifth and half of patients who have significant coronary artery disease[3,4]. There is a large body of observational data suggesting an association between successful CTO percutaneous coronary intervention(PCI) and improved clinical outcomes, including survival[18,22]. These findings are contrary to the findings of randomized trials comparing medical therapy with PCI of stable non-occlusive coronary disease, where except for reduced urgent revascularisation in the FAME-2 trial[177](which did include a very small proportion of CTOs), no overall outcome benefit has been demonstrated[23–27].

There is little published data reporting outcomes for those found to have a CTO which is electively treated medically versus those treated by PCI; a comparison which is more pertinent to clinical decision making than PCI success versus failure. Practice with respect to revascularisation of CTOs varies between clinicians, so it is likely that pre-treatment characteristics overlap. In addition, important confounding variables such as overall angiographic complexity cannot be accounted for if registries from which data is collected do not include them[18].

The aim of this study is to compare long-term clinical outcomes in a consecutive cohort of patients with an identified CTO on angiography between these two treatment groups. Our primary outcome was difference in 5-year all-cause mortality between groups propensity matched for clinical and angiographic pre-treatment characteristics.

3.3 Methods

3.3.1 Definitions

A CTO was defined as complete coronary occlusion of ≥3 months duration with TIMI grade 0 flow. Duration of occlusion was estimated as time from symptom onset, MI or from previous angiography (outside the study period) to angiography. CTO PCI success was defined as stenting of the target vessel with <30% residual stenosis and TIMI grade III flow to

the distal vessel. Patients were grouped according to treatment strategy (PCI/CABG/medical therapy) on an intention-to-treat basis. If a patient had multi-vessel disease and was treated by PCI but the CTO was treated medically, they were included in the medical therapy group.

3.3.2 Study population

All patients undergoing coronary angiography or PCI in a single tertiary centre were prospectively entered into a dedicated database including demographic and procedural details; each patient record was validated by a clinical audit officer. Patients with an occluded coronary artery on angiography between 1st January 2002 and 31st December 2007 were identified (n=4457). I excluded those treated for acute MI in the territory of the occluded vessel in the preceding 3 months, with prior CABG, mitral or aortic valve disease of moderate severity or greater, active neoplastic disease and those already included in the study. The final cohort included 1957 patients. Medical records and coronary angiograms were reviewed retrospectively to give additional angiographic, procedural and clinical details not routinely recorded in the database.

3.3.3 Ethics

The study complies with the Declaration of Helsinki. The study protocol was approved by the local research ethics committee (13/YH/0036). As the study was retrospective and we were unable to obtain informed consent from those patients with our primary outcome of mortality, we obtained approval from the National Confidentiality Advisory Group(CAG 3-06(PR3)/2013) to include clinical data and outcome measures without informed consent.

3.3.4 Angiographic assessment

Overall angiographic complexity was quantified by the use of the Syntax Score[156,178]. Complexity of the individual CTO was assessed by the

J-CTO score[159]. Left ventricular function was assessed by left ventriculography, and where not available by echocardiography.

3.3.5 Outcome measures

Our primary outcome was 5-year all cause mortality amongst propensity matched groups. Data on patient mortality and international classification of disease cause of death was obtained from Office of National Statistics death certification records.

Secondary outcomes included hospitalization for myocardial infarction (MI), the composite of death and MI, and PCI or coronary artery bypass graft surgery (CABG) at follow-up. Follow-up data was obtained through linkage by The National Institute for Cardiac Outcomes Research, obtained from the Myocardial Infarction National Audit Project, British Cardiovascular Intervention Society Central Cardiac Audit Database and the National Adult Cardiac Surgery Audit respectively.

3.3.6 Statistical analysis

Data is presented as percentages and mean±SD or median(inter-quartile range) as appropriate. Differences in proportions are tested with a chi-squared test or Fisher's exact test, and differences in continuous variables with a Student t-test or Mann-Whitney U-test.

Propensity matching was performed to minimize any selection bias due to the differences in clinical characteristics between PCI and elective medical therapy treatment groups. For each patient in the cohort a propensity score indicating the likelihood of a CTO being treated by PCI was calculated by the use of a non-parsimonious multivariable logistic regression model. Co-variates included in the logistic regression model to calculate the propensity score were: age, gender, previous PCI, previous MI, diabetes mellitus, smoking status, peripheral vascular disease (PVD), hypertension, chronic kidney disease (CKD) stage ≥3, chronic lung disease, cerebrovascular disease, Canadian Cardiovascular Society (CCS) class, New York Heart Association(NYHA)class, presentation (stable angina, unstable

angina(UA)/non-ST elevation myocardial infarction (NSTEMI), ST elevation myocardial infarction (STEMI), arrhythmia or heart failure) estimated CTO duration, left main stem disease, Syntax Score, number of significantly diseased vessels, J-CTO score, proximal LAD CTO, branch vessel CTO (defined as diagonal, obtuse marginal, posterior descending artery, posterior left ventricular branch or distal circumflex artery in a right dominant circulation), left ventricular systolic function, confirmed demonstrable ischaemia (confirmed evidence of ischaemia by non-invasive testing, the absence of which does not mean the absence of ischaemia), confirmed myocardial viability in the CTO territory (the absence of a resting left ventricular regional wall motion abnormality or confirmation by non-invasive testing in the presence of a wall motion abnormality, the absence of which does not mean the absence of viability), number of anti-anginal medications, warfarin use and loop diuretic use. The C-statistic for the propensity score model was 0.750 and the Hosmer-Lemeshow test for goodness of fit was 0.778. To identify matched pairs of patients undergoing CTO PCI and elective medical therapy a 1:1 optimal match with a ±0.03 caliper and no replacement was used. Cumulative survival was calculated using the Kaplan–Meier method. Clinical outcomes in the matched population were analysed with Cox proportional hazards regression stratified by matched-pair.

Two sub-group analyses amongst propensity matched patients were performed. We compared outcomes including only propensity matched-pairs in which the CTO PCI patient underwent successful PCI. The second sub-group analysis compared propensity matched patients only if the CTO territory of the CTO PCI patient had been revascularised by any means during the study period and the CTO territory of the medical therapy patient had not been revascularised.

Stata v.12(StataCorp, College Station, Texas) was used for statistical analysis. Probability values were 2-sided, and values of $p<0.05$ were considered significant.

3.4 Results

3.4.1 Overview

Of the 1957 patients identified in the cohort, a CTO was treated by PCI in 405(20.7%) and medical therapy in 667(34.1%), 885(45.2%) patients underwent CABG. In 23 patients, the only CTO identified was a non-dominant right coronary artery; these patients were excluded from analysis, leaving 1934 patients. Of those treated by PCI or medical therapy, propensity score matching identified 294 pairs of patients. **Table 3.1** lists demographic, clinical and angiographic characteristics by treatment group for the entire cohort and for propensity matched groups. After propensity matching, no significant imbalance was identified in covariates between groups.

Significant multi-variable predictors of 5-year mortality were age, elective medical therapy of the CTO, CKD$\geq$3, peripheral vascular disease, chronic lung disease, NYHA class, and ejection fraction<30% (**table 3.2**).

Table 3.1 Baseline characteristics in entire cohort and propensity matched groups.

	Entire cohort (n=1934)					Propensity matched groups (n=588)			
	CABG	**CTO PCI**	**Medical therapy**	**p-value***	**Std diff***	**CTO PCI**	**Medical therapy**	**p-value***	**Std diff***
	n=878	**n=405**	**n=651**			**n=294**	**n=294**		
Age	66.0±9.3	63.2±10.1	65.8±10.7	p<0.001	-0.257	64.3±10.0	63.9±10.2	p=0.645	0.038
Male gender	728(82.9)	301(73.1)	506(77.7)	p=0.205	-0.080	220(74.8)	220(74.8)	p=1.00	0.000
Previous MI	454(51.7)	202(49.9)	394(60.5)	p=0.001	-0.215	151(51.4)	143(48.6)	p=0.563	-0.048
Previous PCI	41(4.7)	22(5.4)	64(9.8)	p=0.011	-0.166	18(6.1)	24(8.2)	p=0.337	-0.079
Diabetes	193(22.0)	78(19.3)	137(21.0)	p=0.484	-0.044	62(21.1)	53(18.0)	p=0.349	0.077
Smoking				p=0.037				p=0.802	
Ex	471(53.6)	200(49.4)	306(47.0)		0.048	142(48.3)	141(48.0)		0.007
Current	148(16.9)	73(18.0)	160(24.5)		-0.160	56(19.1)	62(21.1)		-0.051
PVD	115(13.1)	38 (9.4)	103(15.8)	p=0.003	-0.195	28(9.5)	31(10.5)	p=0.681	-0.034
Hypertension	554(63.1)	225(55.6)	349(53.6)	p=0.537	0.039	159(54.1)	157(53.4)	p=0.869	0.014
Hypercholester-olaemia	563(64.2)	199(49.1)	293(45.0)	p=0.191	0.083	146(49.7)	135(45.9)	p=0.364	0.075
Family History	268(30.5)	152(37.5)	198(30.4)	p=0.017	0.151	107(36.4)	103(35.0)	p=0.731	0.028
CKD ≥3	235(26.8)	82(20.3)	179(27.5)	p=0.005	-0.181	59(20.1)	64(21.8)	p=0.879	-0.042
Chronic lung disease	52(5.9)	23(5.7)	51(7.8)	p=0.182	-0.086	19(6.5)	15(5.1)	p=0.480	0.058
Cerebrovascular disease	65(7.4)	22(5.4)	61(9.4)	p=0.021	-0.151	17(5.8)	20(6.8)	p=0.610	-0.042
CCS class				p<0.001				p=0.683	
No Angina	24(2.7)	1(0.3)	63(9.7)		-0.445	1(0.3)	0(0)		0.082
I	326(37.1)	192(47.4)	309(47.5)		-0.001	153(52.0)	156(53.1)		-0.020
II	269(30.6)	138(34.1)	181(27.8)		0.136	100(34.0)	92(31.3)		0.058
III	216(24.6)	72(17.8)	92(14.1)		0.100	38(12.9)	45(15.3)		-0.068
IV	43(4.9)	2(0.5)	6(0.9)		-0.051	2(0.7)	1(0.3)		-0.008
NYHA Class				p=0.001				p=0.970	
I	503(57.3)	298(73.6)	414(63.6)		0.216	217(73.8)	217(73.8)		0
II	267(30.4)	95(23.5)	155(23.8)		-0.008	67(22.8)	68(23.1)		-0.008
III	97(11.0)	12(3.0)	74(11.4)		-0.330	10(3.4)	9(3.1)		0.019
IV	11(1.3)	0(0)	8(1.2)		-0.158	0(0)	0(0)		-
Presentation				p<0.001				p=0.565	
Stable angina	645(73.5)	371(91.6)	491(75.4)		0.446	265(90.1)	268(91.2)		0.012
UA/NSTEMI	194(22.1)	32(7.9)	86(13.2)		-0.173	27(9.2)	26(8.8)		0.082
STEMI	15(1.7)	1(0.3)	11(1.7)		-0.148	1(0.3)	0(0)		0.082
Arrhythmia	11(1.3)	1(0.3)	33(5.1)		-0.303	1(0.3)	0(0)		0.082
Heart Failure	13(1.5)	0(0)	30(4.6)		-0.311	0(0)	0(0)		-
Occlusion duration (months)	16(8-52)	12(6-24)	16(8-56)	p<0.001	-0.372	12(7-26)	12(7-29)	p=0.551	0.039
LMS disease	184(21.0)	1(0.3)	23(3.5)	p<0.001	-0.243	1(0.3)	1(0.3)		0
Syntax score	24(19-29)	14.5(10-19)	14.5(9-21.5)	p=0.359	-.197	14.5(10-19)	13.3(9-18.5)	p=0.106	0.034
Diseased vessels				p<0.001				p=0.771	
1	74(8.4)	193(47.7)	293(45.0)		0.053	136(46.3)	144(49.0)		-0.054
2	276(31.4)	159(39.3)	223(34.3)		0.104	114(38.8)	106(36.1)		0.056
3	528(60.1)	53(13.1)	135(20.7)		-0.205	44(15.0)	44(15.0)		0.000
J-CTO score				p<0.001				p=1.00	
0	210(23.9)	162(40.0)	182(28.0)		0.256	109(37.1)	109(37.1)		0.000
1	355(40.4)	153(37.8)	250(38.4)		-0.013	113(38.4)	114(38.8)		-0.007
2	243(27.7)	76(18.8)	168(25.8)		-0.170	60(20.4)	59(20.1)		0.008
≥3	70(8.0)	14(3.5)	51(7.8)		-0.190	12(4.0)	12(4.0)		0.000
Proximal LAD CTO	138(15.7)	53(13.1)	90(13.8)	p=0.733	-0.022	37(12.6)	32(10.9)	p=0.522	0.053
Branch vessel CTO	114(13.0)	42(10.4)	115(17.7)	p=0.001	-0.211	38(12.9)	43(14.6)	p=0.550	-0.049
LV function				p<0.001				p=0.722	
Good (EF >50%)	527(60.0)	304(75.1)	363(55.8)		0.414	213(72.5)	220(74.8)		-0.054
Mod (EF 30-50%)	246(28.0)	88(21.7)	163(25.0)		-0.078	68(23.1)	60(20.4)		0.066
Poor (EF <30%)	105(12.0)	13(3.2)	125(19.2)		-0.524	13(4.4)	14(4.8)		-0.016
Confirmed ischaemia†	491(55.9)	279(68.9)	356(54.7)	p<0.001	0.295	204(69.4)	198(67.4)	p=0.595	0.044
Confirmed viability†	551(62.8)	322(79.5)	369(56.7)	p<0.001	0.505	222(75.5)	220(74.8)	p=0.849	0.016
N anti-anginals				p=0.001				p=0.991	
0	159(18.1)	85(21.0)	182(28.0)		-0.162	73(24.8)	72(24.5)		0.008
1	332(37.8)	150(37.0)	262(40.3)		-0.066	114(38.8)	113(38.4)		0.007
2	260(29.6)	126(31.1)	130(20.0)		0.257	76(25.9)	77(26.2)		-0.008
3	106(12.1)	38(9.4)	67(10.3)		-0.031	28(9.5)	30(10.2)		-0.023
4	21(2.4)	6(1.5)	10(1.5)		-0.004	3(1.0)	2(0.7)		0.037
Loop diuretic	121(13.8)	30(7.4)	139(21.4)	p<0.001	-0.405	26(8.8)	22(7.5)	p=0.547	0.050
Warfarin	29(3.3)	13(3.2)	41(6.3)	p=0.027	-0.145	3(1.0)	10(3.4)	p=0.050	-0.162

Std diff represents standardised difference; *p-values and standardised differences compare CTO PCI and medical therapy groups only. †Confirmed viability/ischaemia only.

Table 3.2 Multi-variable predictors of 5 year mortality for the entire cohort (n=1934)

	Multi-variable analysis		
	HR	**95% CI**	**p-value**
Medical therapy*	1.56	1.25-1.94	p<0.001
Age(years)	1.05	1.04-1.07	p<0.001
Diabetes	1.30	1.04-1.67	p=0.023
CKD ≥3	1.42	1.17-1.72	p<0.001
PVD	1.63	1.27-2.09	p<0.001
Chronic lung disease	1.72	1.26-2.37	p=0.001
Presentation with heart failure	1.70	1.09-2.66	p=0.019
NYHA class			
II	1.60	1.24-2.06	p<0.001
III	1.87	1.34-2.59	p<0.001
IV	6.01	3.30-10.95	p<0.001
Poor LV function (EF <30%)	2.05	1.56-2.68	p<0.001

***Elective medical therapy of the CTO**

3.4.2 Procedural details

PCI of the CTO was successful in 250(61.7%) of all patients in whom the CTO was attempted and 177(60.2%) of the propensity matched group. In the propensity matched CTO PCI group, 88(29.9%) patients underwent PCI to another vessel other than the CTO vessel as part of their initial revascularisation strategy, compared with 62(21.1%) in the group in which the CTO was treated medically. PCI was complicated by wire perforation in 4(1%) of CTO PCI procedures, all of which were included in the propensity matched group (1.4%). Pericardial drainage for tamponade was required in one patient, peri-procedural death occurred in one patient and two patients were managed conservatively. No wire perforations occurred in any patients in the cohort undergoing PCI to non-occlusive disease. In those in the propensity matched CTO PCI group treated by successful PCI, median total length of stented segments was 46mm(31-63mm). In those in the propensity matched medical therapy group in whom PCI to concomitant non-occlusive disease was performed, median total length of

stented segment was 24mm(16-35mm). Drug eluting stents were used in 140(79.0%) of patients undergoing successful CTO PCI and 36(58.1%) of patients in the elective medical therapy group who underwent PCI to non-occlusive disease.

3.4.3 5-year outcomes

5-year outcomes for the entire cohort and propensity matched groups are listed in **table 3.3**. Mortality at 5 years in the propensity matched CTO PCI group was 11.6%, compared with 16.7% in the medical therapy group but did not quite reach conventional statistical significance, hazard-ratio 0.63(0.40-1.00, p=0.052)(**figure 3.1**). However the composite of death or myocardial infarction at 5 years occurred in 13.9% of the CTO PCI group and 19.6% in the elective medical therapy group, hazard-ratio 0.64(0.42 to 0.99), p=0.043(**figure 3.2**). Repeat revascularisation, unplanned in the initial treatment strategy was more common in the CTO PCI group than the elective medical therapy group. The majority of CTO PCI patients (51, 73.9%) who underwent repeat, unplanned revascularisation did not undergo successful initial CTO PCI. In the propensity matched CTO PCI group, 54(18.4%) patients underwent CABG within 5 years and 15(5.1%) underwent further PCI unplanned in the initial revascularisation strategy. In the medical therapy group, 13(4.4%) underwent CABG within 5 years and 22(7.5%) underwent subsequent PCI.

Table 3.3 Outcome comparisons for the entire cohort(left) and propensity matched groups(right).

	Entire Cohort				Propensity matched groups			
	Number of events (%)				Number of events (%)			
Outcome	CTO PCI n=405	Medical Therapy n=667	Hazard-Ratio	p-value	CTO PCI n=294	Medical Therapy n=294	Hazard-Ratio (95% CI)	p-value
5-year mortality	43(10.6)	170(26.1)	0.37 (0.26-0.51)	p<0.001	34(11.6)	49(16.7)	0.63 (0.40-1.00)	p=0.052
5-year cardiac death	23(5.7)	71(10.9)	0.47 (0.30-0.76)	p=0.002	14(4.8)	19(6.5)	0.65 (0.30-1.38)	p=0.261
5-year death/MI	56(13.8)	187(28.7)	0.44 (0.32-0.59)	p<0.001	41(13.9)	57(19.6)	0.64 (0.42-0.99)	p=0.043
5-year unplanned revascularisation	96(23.7)	76(11.7)	2.06 (1.52-2.78)	p<0.001	69((23.5)	35(11.9)	1.81 (1.18-2.79)	p=0.007

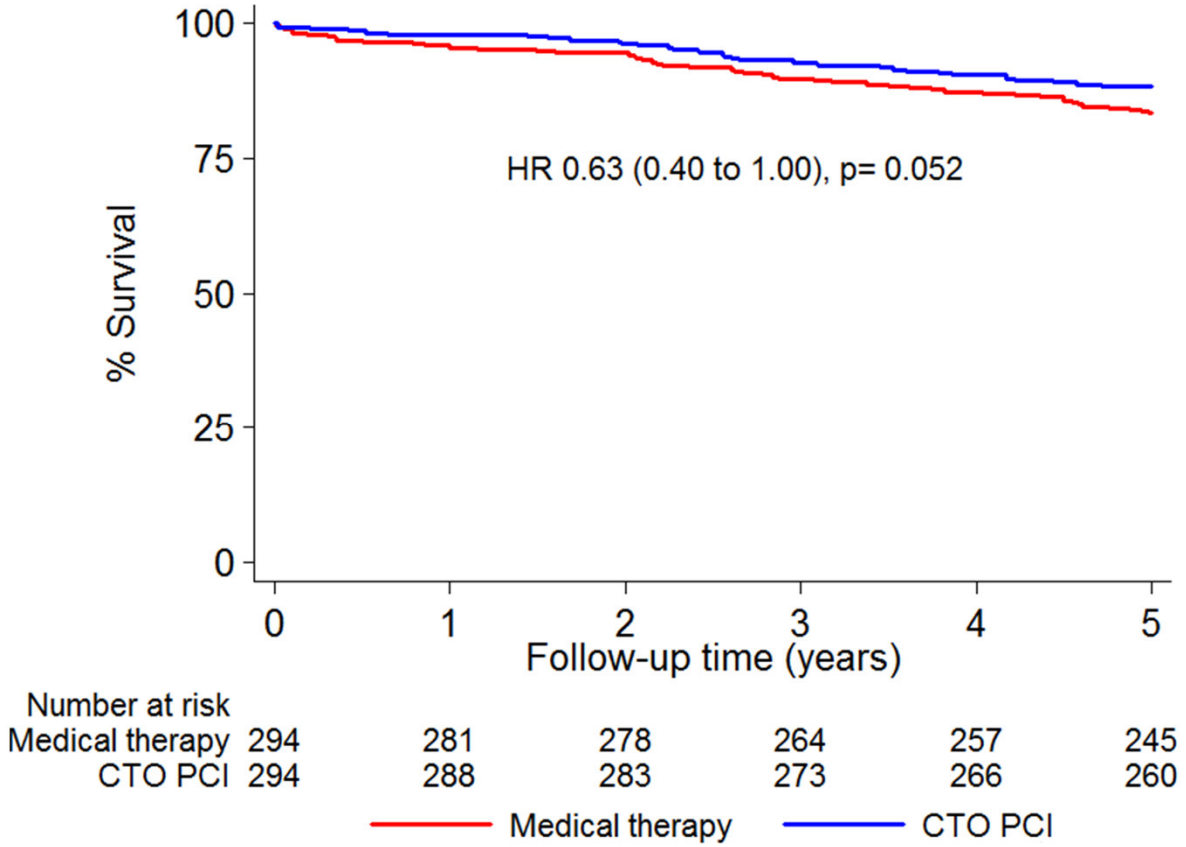

Figure 3.1 Kaplan-Meier curves showing 5-year survival for propensity matched CTO PCI and medical therapy groups

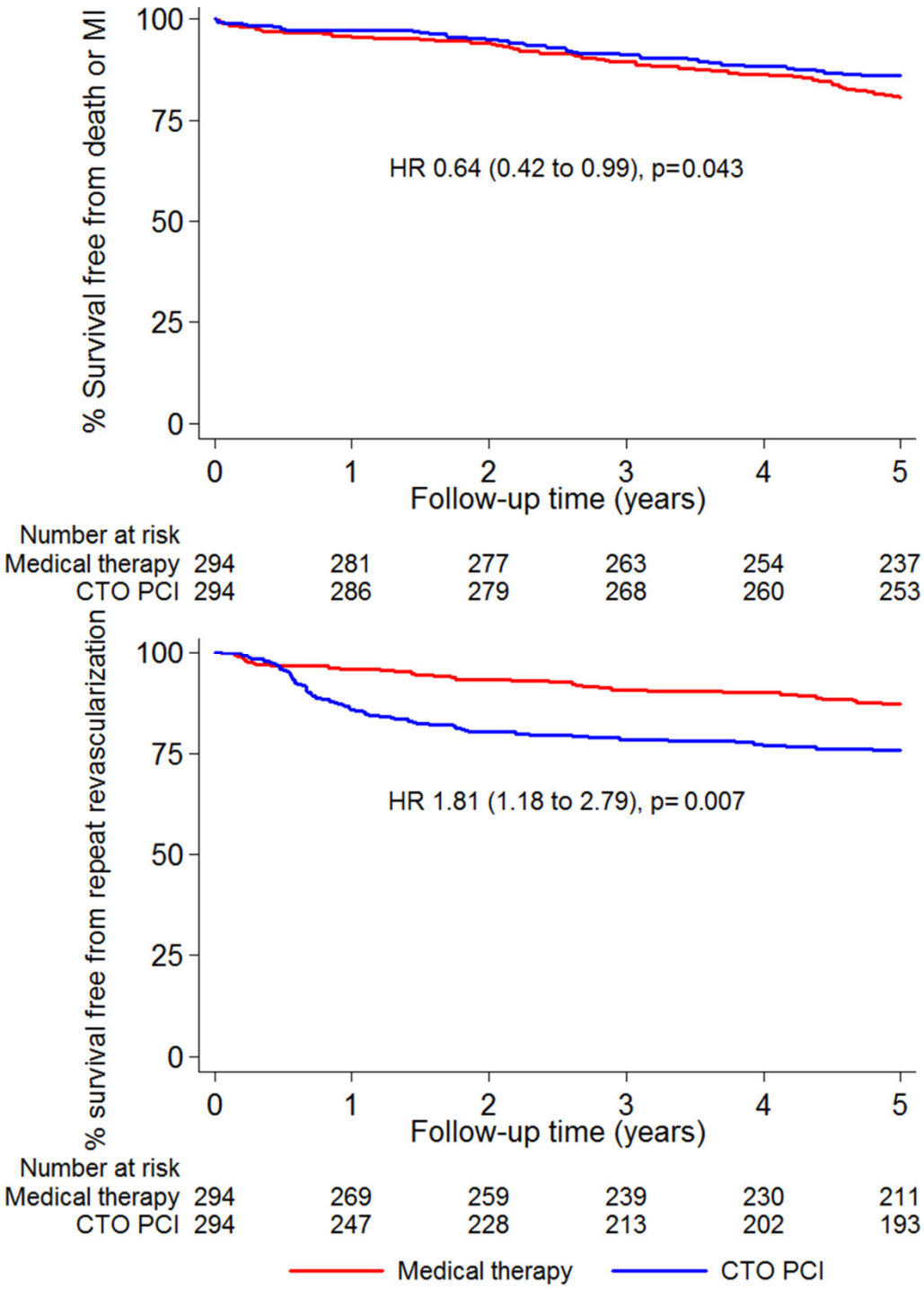

Figure 3.2 Kaplan-Meier curves showing 5-year composite of death or myocardial infarction(top) and 5-year unplanned repeat revascularisation for propensity matched CTO PCI and medical therapy groups.

3.4.4 Sub-group analyses

If only the 177 matched pairs in which CTO PCI was successful were analyzed, there was no statistically significant difference in 5-year mortality or the 5-year composite of death or MI (**figure 3.3** and **table 3.4**).

However, the excess of repeat revascularisation in the CTO PCI group was no longer evident (**figure 3.3**).

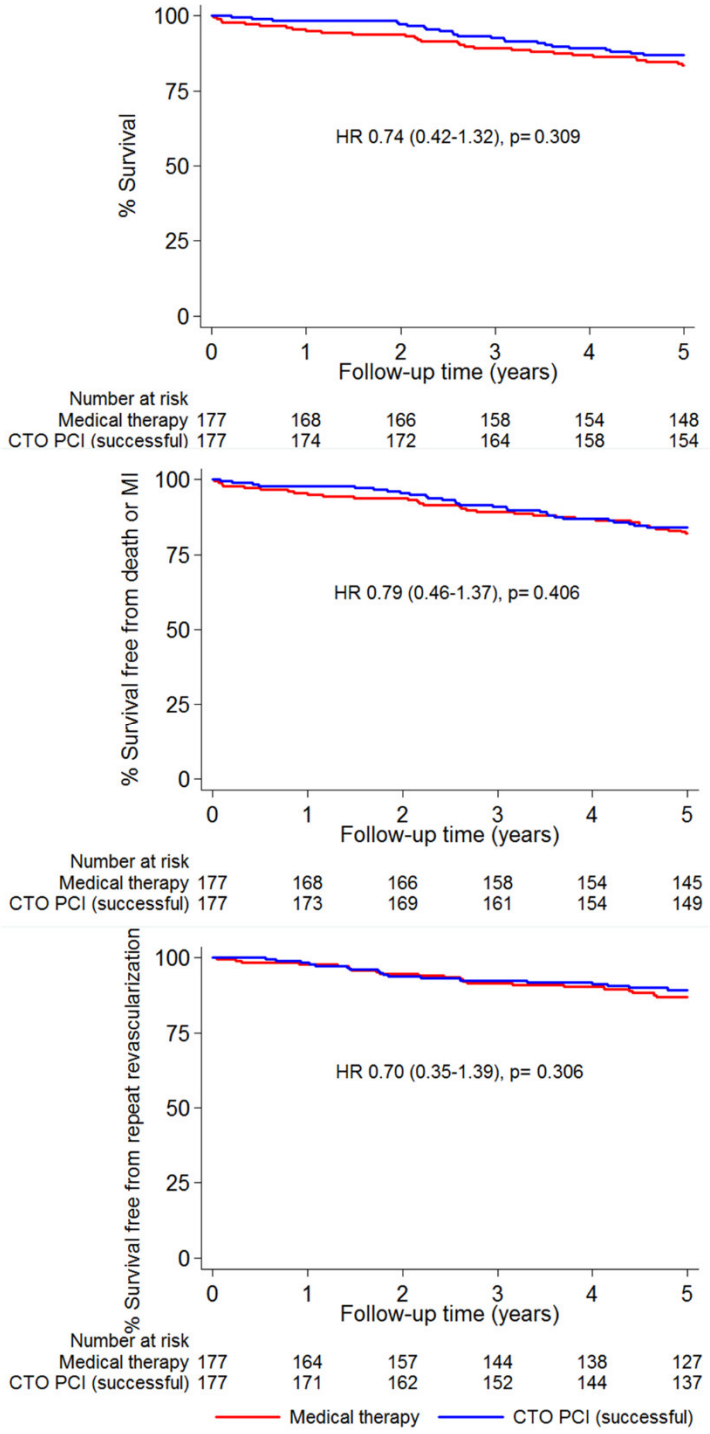

Figure 3.3. Kaplan-Meier curves showing 5-year survival(top), composite of death or MI(middle) and repeat revascularisation for propensity matched CTO PCI and medical therapy groups including only matched pairs in which CTO PCI was successful.

Table 3.4 Outcome comparisons including only propensity matched pairs in which CTO PCI was successful(left) and including only matched pairs in which during the follow-up period, the CTO was revascularised in the CTO PCI patient and the CTO was not revascularised in the medical therapy patient(right).

	Successful CTO PCI only				Revascularised CTO vs not revascularised			
	Number of events (%)				Number of events (%)			
Outcome	CTO PCI n=177	Medical Therapy n=177	Hazard-Ratio	p-value	CTO PCI n=208	Medical Therapy n=208	Hazard-Ratio (95% CI)	p-value
5-year mortality	23(13)	29(16.4)	0.74 (0.42-1.32)	p=0.309	22(10.6)	38(18.3)	0.50 (0.28-0.88)	p=0.016
5-year cardiac death	5(2.8)	12(6.8)	0.27 (0.08-0.98)	p=0.046	6(2.8)	15(7.2)	0.21 (0.06-0.75)	p=0.015
5-year death/MI	28(15.8)	32(18.1)	0.79 (0.46-1.37)	p=0.406	27(13.0)	43(20.7)	0.53 (0.31-0.89)	p=0.017
5-year unplanned revascularisation	18(10.2)	21(11.9)	0.70 (0.35-1.39)	p=0.306				

Of the 117 patients who underwent unsuccessful CTO PCI, 45(38%) underwent revascularisation to the CTO territory during the 5 year follow-up period of which 44(98%) were by CABG. Median time to unplanned revascularisation in this group was 243 days (183-359). Similarly, 20 of 294 patients treated by medical therapy underwent revascularisation to the CTO territory during the 5 year follow-up period, 13(65%) by CABG and 7(35%) by PCI.

There were 208 propensity matched-pairs in whom the CTO territory of the CTO PCI patient had been revascularised by any means during the study period and the CTO territory of the medical therapy patient had not been revascularised. There was a significant difference in mortality at 5 years, the composite of death or MI at 5 years and 5 year cardiac death, favouring the revascularised group (**figure 3.4** and **table 4**).

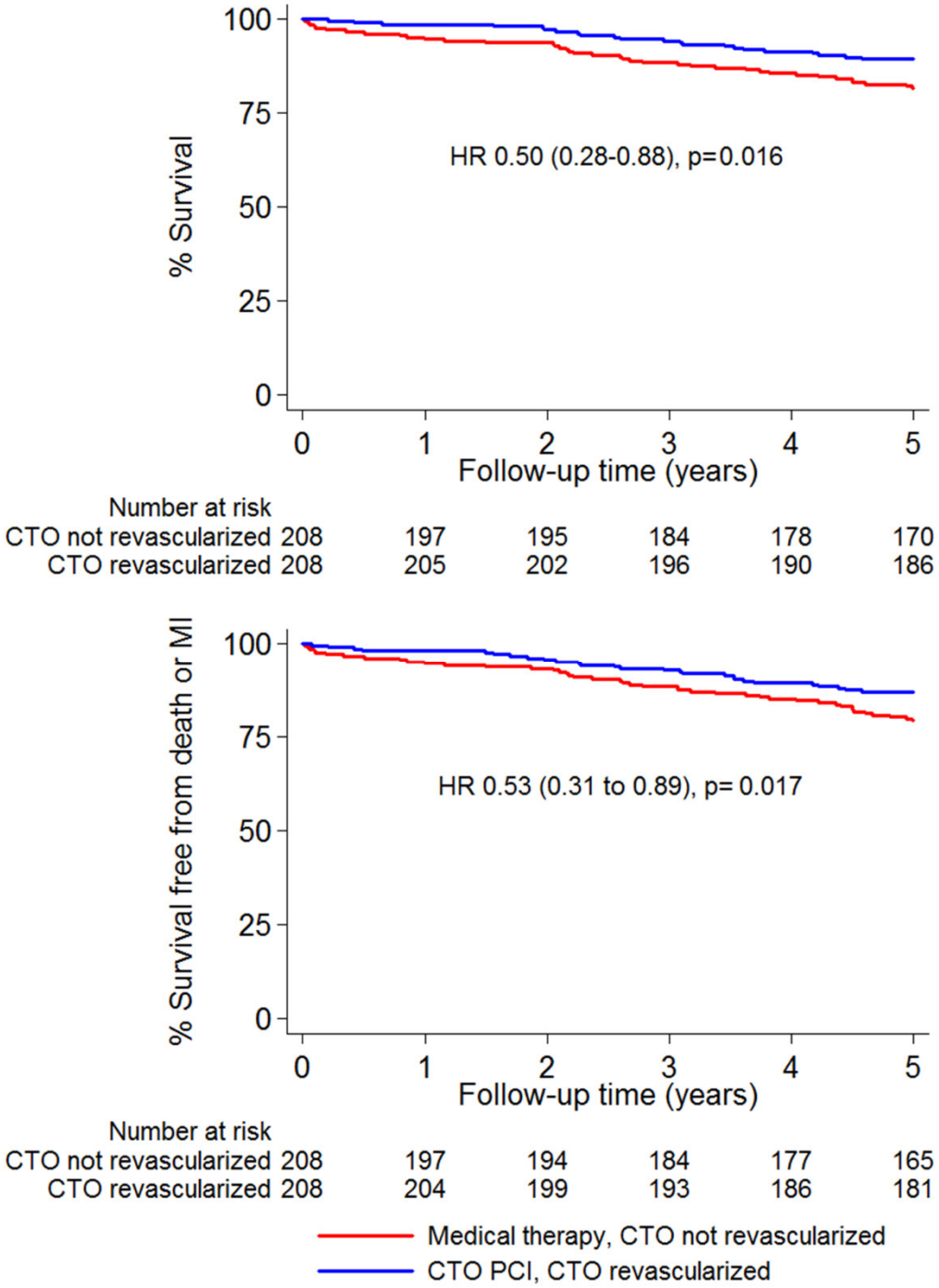

Figure 3.4 Kaplan-Meier curves showing 5-year survival(top) and composite of death or MI(bottom) for propensity matched CTO PCI and corresponding elective medical therapy groups including only matched pairs in which during the follow-up period, the CTO was revascularised in the CTO PCI patient and the CTO was not revascularised in the medical therapy patient.

3.4.5 Drug-eluting stents and target vessel revascularisation after successful CTO PCI

Of the 177 patients in the propensity matched group in whom PCI was successful, 18 (10.2%) underwent repeat, unplanned revascularisation in the 5-year study period (13 by PCI and 5 by CABG), of which 13(7.4%) patients underwent target vessel revascularisation (12 for in-stent restenosis and 1 for very late stent thrombosis). Of the 37(20.9%) patients in whom bare metal stents were used, repeat unplanned revascularisation occurred in 5(13.5%) patients during the 5-year study period (4 by PCI and 1 by CABG), 3 of which were due to in-stent restenosis in the CTO vessel and 2 due to de novo disease in another vessel. This compares with 13 (9.2%) of the 140 patients treated with drug eluting stents. Amongst the same successful CTO PCI group, 23(12.9%) patients died during the 5-year study period of whom 5 had undergone PCI with a bare metal stent (13.5% of the bare metal stent group). The other 18 deaths occurred in those treated with drug eluting stents, amounting to 12.9% of that group. Similarly, 28(15.8%) of these patients suffered the composite of death or myocardial infarction during the study period, 5 of whom had undergone PCI with a bare metal stent (13.5% of the bare metal stent group) and 23 had undergone PCI with a drug eluting stent (16.4% of the drug eluting stent group).

3.5 Discussion

3.5.1 Summary of findings

I present, in a propensity matched population well balanced for both clinical and angiographic characteristics, 5-year clinical outcomes comparing CTO PCI and elective medical therapy analysed by intention-to-treat. We are unable to reject our null hypothesis (albeit by a narrow margin) that there is no difference in our primary outcome of 5-year all-cause mortality between propensity matched CTO PCI and elective medical therapy groups. However, we do demonstrate a significant difference in the composite of 5-year death or MI favouring CTO PCI over elective medical

therapy. The difference in outcomes appears to be driven by whether the CTO was revascularised, rather than whether it was treated by successful PCI.

3.5.2 Procedural success

The evidence describing long-term outcomes associated with CTO PCI is at present entirely observational and largely involves comparison of successful versus failed procedures[18,22]. A problem peculiar to PCI of CTOs compared to PCI of other coronary lesions is that procedural success rates are much lower. Although success rates as high as 85% have been reported[15], the success rate in the UK between 2005 and 2009 was 70%[18] and a recent publication using data from The National Cardiovascular Data Registry CathPCI Registry, which included 22,365 attempts at CTO PCI in the USA between 2009 and 2013 reported a success rate of 59%[17] . This has two important implications; firstly, in applying the evidence base we cannot necessarily put our patients in the more favourable successful group. Secondly, in investigating outcomes by the principle of intention-to-treat, a sizeable proportion of patients will not receive the intended treatment because of procedural failure. This may dilute the power of the study, or complicate comparison by including other treatment modalities, in this case CABG. Randomised trials investigating outcomes of CTO PCI are likely to be beset by the same problem.

3.5.3 A survival benefit?

It may be that our failure to demonstrate a difference in all-cause mortality between those treated by CTO PCI and medical therapy (**figure 3.1**) is due to insufficient sample size. This is supported by the numerical difference in mortality and by the presence of a statistically significant survival benefit when the composite of death or MI is used (**figure 3.2**). In spite of CTO PCI success rates of 60%, 76% of propensity matched CTO PCI patients underwent revascularisation to the CTO territory compared with only 7% in the propensity matched medical therapy group. It would

appear that the increased rate of repeat revascularisation we report in the CTO PCI group (**figure 3.2**), absent if only successful cases are analysed (**figure 3.3**) was related to an intention to revascularise and subsequent referral for CABG on failure of PCI.

If it is revascularisation of the CTO territory that is important, it is somewhat surprising that when only propensity matched pairs in which CTO PCI had been successful are analysed there is a smaller numerical difference in mortality and death/MI (**figure 3.3**). This could be a true finding, or might be explained by the loss of power as a result of including only 60% of propensity matched patients, and was perhaps contributed to by the comparison with some medical therapy patients who did in fact undergo revascularisation of their CTO. In addition, it may be that CABG is associated with a greater survival benefit and the exclusion of the failed CTO PCI patients who were revascularised by CABG has removed this effect. Although the numbers are small, there was a difference in cardiac death favouring the successful PCI group over medical therapy (**table 3.4**). The clear association with improved survival if only propensity matched pairs in which the CTO was revascularised in the CTO PCI patient and not revascularised in the medical therapy patient suggests it is revascularisation of the CTO territory that is important in driving the association with improved outcomes (**figure 3.4**). This is further supported by our multi-variable analysis of the entire cohort, which shows that elective medical therapy (or the lack of revascularisation by CABG or PCI) to be independently predictive of 5-year mortality (**table 3.3**). This is consistent with the findings of a recently published retrospective cohort study using a similar population[179].

3.5.4 Why should CTOs be any different?

Why would prognostic gain be associated with PCI over medical therapy in the setting of a CTO but not in non-occlusive disease[23–27]? The presence of a CTO in a non-infarct related artery in patients who present with ST-elevation myocardial infarction is associated with inferior

outcomes[37,38]. This may be related to a greater myocardial mass at jeopardy in the infarct related artery due to an additional contribution to collateral dependent myocardium. Another possible explanation is that of a reduction in ischaemic myocardium. Unlike some non-occlusive coronary stenoses included in clinical trials, provided the myocardium is viable, CTOs almost invariably result in inducible ischaemia[45]. A larger ischaemic burden is associated with an adverse prognosis[39] and reducing that burden has been associated with improved clinical outcomes[43]. If the effect of PCI on outcome is assessed in patients only if they have demonstrable ischaemia, a clinical benefit associated with PCI seems more likely[177].

Despite the observational evidence, doubt remains as to whether CTO PCI itself confers a prognostic benefit over medical therapy. Ultimately this can only be answered by a randomised trial, two of which are ongoing (ClinicalTrials.gov numbers NCT01760083 and NCT01078051). Given the low event rates reported here, it seems unlikely that any difference in the harder clinical outcomes of death or MI would become apparent until relatively late in the follow-up period.

It may be that a randomised trial of sufficient size comparing CTO PCI with medical therapy is completed in the future and shows no evidence of prognostic gain. In such circumstances, CTO PCI's primary indication will remain what it is for all PCI in stable coronary disease; that is for the relief of symptoms of angina. This is supported by studies demonstrating an association between successful CTO PCI, improved symptoms of angina[22,180] and improved quality of life[180].

3.5.5 Limitations

This study is limited by its observational nature; however the study population was very well balanced for both clinical and angiographic characteristics. Although well conducted propensity matching can balance measured characteristics between treatment groups, unmeasured variables which would be balanced by randomization will not necessarily be equal if they are unknown or ignored. In the same vein, this study was limited by

retrospective collection of pre-treatment variables. Although CTO PCI and medical therapy groups were well balanced for symptomatic status by CCS and NYHA class, more nuanced symptomatic information, not recorded in the notes may have differed between groups. It may be that patients with slightly more severe symptoms, but the same broad CCS class tended to be revascularised and those with slightly milder symptoms treated medically. Interestingly though, this might suggest that revascularised patients are inherently higher risk. If that were the case, the benefit of revascularisation might even be greater than we have demonstrated.

The study is also limited by including only patients from a single centre. Because data were collected retrospectively, complete records of symptomatic status at follow-up were not collected and therefore control of symptoms and morbidity related to treatment could not be compared across groups. The study benefited from comprehensive national audit data collection resulting in no known censored events and collection of robust hard endpoint data. Migration outside of England or Wales could result in missed events however there is no reason to suppose that this would introduce bias between groups.

Finally, CTO PCI technique has progressed since the period of data collection and the success rates we report are lower than have been reported more recently[15,18]. As a consequence, a proportion of patients in the CTO PCI group were ultimately treated medically or by CABG. However, in an era of rapid development, to achieve 5-year clinical follow up, it is not possible for procedures to be entirely contemporary. Perhaps more importantly, a recent publication reporting over 22,000 attempts at CTO PCI in the USA between 2009-2013 reported success rates of only 59%[17], lower than reported here. Although a proportion of patients underwent PCI with bare-metal stents, rates of repeat revascularisation, and more importantly death or myocardial infarction were not sufficiently large in this group to influence our results. It seems unlikely that the use of third generation (rather than first and second generation) drug eluting stents would have greatly altered our results either.

3.5.6 Conclusions

Using an alternative approach to much of the existing literature on the subject[18,22], our findings support the suggestion that the revascularisation of a CTO is associated with improved long-term survival. However from this study, we cannot attribute this finding to successful CTO PCI at the index procedure alone.

4.0 Collateral Donor Artery Physiology and the Influence of a Chronic Total Occlusion on Fractional Flow Reserve

4.1 Abstract

4.1.1 Background

The presence of a concomitant chronic total coronary occlusion (CTO) and a large collateral contribution might alter the fractional flow reserve (FFR) of an interrogated vessel, rendering the FFR unreliable at predicting ischaemia should the CTO vessel be revascularised and potentially affecting the decision regarding optimal revascularisation strategy. We tested the hypothesis that donor vessel FFR would significantly change following percutaneous coronary intervention (PCI) of a concomitant CTO.

4.1.2 Methods and Results

In consecutive patients undergoing PCI of a CTO, coronary pressure and flow velocity were measured at baseline and hyperaemia in proximal and distal segments of both non-target vessels, before and after PCI. Haemodynamics including FFR, absolute coronary flow and the coronary flow velocity-pressure gradient relation were calculated. After successful PCI in 34 of 46 patients, FFR in the predominant donor vessel increased from 0.782 to 0.810 (difference 0.028(0.012-0.044,p=0.001)). Mean decrease in baseline donor vessel absolute flow adjusted for rate-pressure product: 177.5 to 139.9ml·min^{-1} (difference -37.6(-62.6 to -12.6, p=.005)), mean decrease in hyperaemic flow: 306.5 to 272.9 ml·min^{-1} (difference -33.5(-58.7 to -8.3, p=0.011)). Change in predominant donor vessel FFR correlated with angiographic(%) diameter stenosis severity (r=0.44, p=0.009) and was strongly related to stenosis severity measured by the coronary flow velocity-pressure gradient relation(r=0.69, p<0.001).

4.1.3 Conclusions

Recanalisation of a CTO results in a modest increase in the FFR of the predominant collateral donor vessel associated with a reduction in coronary flow. A larger increase in FFR is associated with greater coronary stenosis severity.

4.2 Introduction

The presence of a chronic total coronary occlusion (CTO) is a strong predictor of treatment strategy[3] and is found in between one in five and half of patients with significant coronary artery disease on angiography[3,4]. In the presence of a CTO, collateral blood supply originating from a major epicardial vessel other than the occluded vessel is usually present and is often sufficient to maintain resting perfusion and contractility in the collateral dependent myocardium[53]. In this setting, we would expect coronary flow to be increased relative to the same vessel in the absence of collateral donation. Restoration of antegrade flow by PCI of a CTO has been shown to be associated with a rapid reduction in received collateral supply in the treated vessel[63,64]and is likely to be coupled by an associated rapid reduction in flow in the collateral donor vessel amounting to the flow donated to the collateral dependent myocardium prior to PCI.

In the setting of both single and multi-vessel coronary disease, randomised trials support the use of fractional flow reserve to guide PCI[56,58,105] with an established treatment threshold of ≤0.8[56,58]. Revascularisation strategy based upon angiographic assessment is frequently altered by FFR assessment[78]. Although FFR is reported to be independent of changing haemodynamics[59], it is intimately related to total coronary flow through a stenosis which in turn is related to perfused myocardial mass[60]. In keeping with this, there have been a number of reports of large increases in collateral donor vessel FFR associated with PCI of a concomitant CTO and therefore reduction in perfused myocardium[65–69]. However, there is inherent variability to FFR measurement[59], and therefore selective reporting and publication bias might have exaggerated the magnitude (or even presence) of this phenomenon in the reported cases.

The purpose of this study is to serially investigate the changes in collateral donor vessel physiology, before and after successful PCI of a CTO

and to test the hypothesis that there will be an associated significant change in collateral donor vessel FFR.

4.3 Methods

4.3.1 Study patients

Forty-seven patients scheduled for PCI to a CTO for symptoms of angina (Canadian Cardiovascular Society (CCS) class 1-3) were recruited consecutively in a single tertiary centre between January 2013 and June 2014. A CTO was defined as complete coronary occlusion of ≥3 months duration with TIMI grade 0 flow[1]. Exclusion criteria were inability to provide consent, >1 occluded vessel, prior CABG with any patent grafts, left main stem stenosis considered to be haemodynamically significant and contra-indications to adenosine. Patient's usual medications were continued and they were asked to abstain from caffeine for 48 hours prior to the procedure.

4.3.2 Ethics

The study protocol was approved by the local research ethics committee (12/YH/0360). All subjects provided written informed consent.

4.3.3 Catheter laboratory protocol

Dual arterial access was used for all procedures. Femoral venous access was obtained for central administration of adenosine and measurement of right atrial pressure (RAP) at the beginning and end of the procedure using a catheter positioned in the right atrium. Patients were anti-coagulated with 100 U/kg of unfractionated heparin to maintain an activated clotting time of >300 seconds. After a 200mcg bolus of intra-coronary glyceryl trinitrate (GTN), iso-centred coronary angiograms of both non-target vessels were taken.

A dual sensor pressure-velocity 0.014" intracoronary wire (Combowire, Volcano Corp, San Diego, CA)[87] was connected to a ComboMap console (Volcano Corp) and used for haemodymamic

measurements. The wire was normalised to aortic pressure at the tip of the catheter, advanced to the distal segment of each non-target vessel and manipulated to obtain a good Doppler trace. After administration of 100mcg intra-coronary GTN, once the hyperaemic response had settled, continuous recordings from the ComboMap were taken. Hyperaemia was achieved by central venous administration of adenosine at 140μg/kg/minute. Once steady state hyperaemia had been reached and a continuous recording of ≥20 beats taken, adenosine infusion was ceased. The Combowire was withdrawn into the segment of the vessel proximal to any major side-branches and measurements repeated as described. Samples were recorded at 200Hz and stored on disk for offline analysis.

After initial haemodynamic recordings, PCI of the CTO was undertaken at the discretion of the treating interventional cardiologist using an antegrade or retrograde approach. Once access to the vessel lumen distal to the point of occlusion was achieved, prior to restoration of antegrade flow, a microcatheter was placed into the distal vessel to facilitate delivery of the ComboWire. The ComboWire was positioned in a vessel segment angiographically free of a significant stenosis, then baseline and hyperaemic measurements taken as described. PCI success was defined as stenting of the target vessel with <30% residual stenosis and thrombolysis in myocardial infarction(TIMI) grade III flow.

If PCI was successful, non-target vessel haemodynamic measurements were repeated as described pre-procedure, including repeated RAP measurement.

Recorded data was analysed using dedicated custom software (Study Manager, Academic Medical Center, University of Amsterdam, The Netherlands; and a Matlab(Mathworks Inc., Natick, Massachusetts, USA) environment for wave-intensity analysis, Imperial College London, UK).

4.3.4 Angiographic assessment

Maximal non-target vessel diameter stenosis(%) and proximal non-target vessel diameters (at the point of proximal haemodynamic

measurement) measured in two orthogonal views were calculated by two independent observers using quantitative coronary angiography(QCA)(GE Centricity CA1000, GE Healthcare) using the guiding catheter luminal diameter as reference. Mean values from both observers were used for analysis. The non-target vessel making the largest collateral contribution (the predominant collateral donor vessel), vessel collateral connection(CC) grade[90] and modified Rentrop score[89] were assessed by two independent observers blinded to haemodynamic measurements and agreed by consensus. The non-target vessel donating angiographically least/no collaterals to the occluded segment was considered the minor collateral donor vessel.

4.3.5 Data analysis

FFR was calculated as (Pd-RAP)/Pa-RAP), using mean pressures taken over 5 cardiac cycles at stable hyperaemia[166]. An FFR of ≤0.80 was considered haemodynamically significant. Flow velocity was measured in $cm \cdot s^{-1}$, mean values are expressed as average peak velocity (APV) and instantaneous values as instantaneous peak velocity (IPV). Hyperaemic microvascular resistance (HMR_v) was calculated as Pd/APV and hyperaemic stenosis resistance (HSR) as (Pa-Pd)/APV, both measured over five beats at stable hyperaemia. Absolute coronary flow was estimated as (π x proximal vessel radius^2)x(proximal vessel APV/2)[170,171]. As resting absolute myocardial blood flow is closely related to rate pressure product(RPP), values for resting absolute coronary flow were divided by the respective RPP/10,000[172]. Coronary flow velocity reserve (CFR) was calculated as APV at steady state hyperaemia divided by APV at baseline, measured over 5 cardiac cycles.

Fractional collateral flow reserve was calculated as for FFR, with Pd measured in the occluded segment of the artery, prior to restoration of antegrade flow. Collateral flow velocity reserve was calculated as for CFR

with flow velocities in the occluded segment measured at rest and steady state hyperaemia.

The diastolic flow-velocity pressure gradient relation (DFV-PGR) describes the relationship between pressure and flow for a given stenosis or vessel segment[83,84]. It was calculated using continuous recordings of 30 cardiac cycles measured in the distal vessel from baseline through to maximal hyperaemia[86]. Instantaneous pressures and flow velocities were extracted from the Study Manager programme and Pa timings corrected to adjust for any time delay with respect to Pd. Instantaneous flow velocities from mid-diastole (after the diastolic upstroke in coronary flow velocity) to atrial activation (identified by the beginning of the p-wave on ECG) were plotted against instantaneous pressure gradient(Pa-Pd). DFV-PGR was then calculated using Stata v.12 (StataCorp, College Station, Texas), fitting the quadratic linear regression equation: $\Delta P=(FxIPV)+(SxIPV^2)$ where ΔP is the pressure gradient in mmHg, F is the coefficient of pressure loss due to viscous friction and S is the coefficient of pressure loss due to flow separation or localized turbulence downstream from the stenosis[83,84]. The peak slope was defined as the gradient of the fitted values over the highest 10cm/s of measured IPV.

Wave intensity represents the rate of energy per unit area transported by travelling waves in arteries and is derived from phasic changes in local pressure and flow velocity. The blood pressure and Doppler velocity recordings were filtered with a Savitzky-Golay filter[176] and ensemble averaged using the ECG R-wave for timing. Wave intensity was calculated from simultaneous baseline pressure and flow measurements taken in the proximal non-target vessels over 20 cardiac cycles. The change in pressure was separated into wave components originating from the proximal vessel and from the microvasculature assuming the density of blood to be 1050 kg/m^3, and estimating wave speed using the sum of squares method[98,173]. Cumulative wave energy was calculated for each wave by measuring the area under the curve. Coronary flow is predominantly diastolic and is proportional to perfused myocardial mass[60].

Because we were interested in the mechanism of any change in donor vessel flow, we focused our analysis on the change in cumulative wave intensity of the backwards expansion wave (BEW).

4.3.6 Measurement repeatability

Based upon analysis of 26 repeated flow measurements at baseline and hyperaemia without any intervening treatment, coefficient of variation for average peak coronary flow velocity measurements was 17.4%. Analysis of 10 repeated measurements taken from repeated adenosine infusions gave a coefficient of variation for FFR, CFR and HMR of 3.6%, 19.7% and 8.6% respectively.

4.3.7 Statistical analysis

Stata v.12(StataCorp) was used for statistical analysis. Continuous values are expressed as means±SD, or median (25^{th} percentile-75^{th} percentile) as appropriate. Assuming a standard deviation of the difference(SDD) of 0.04 and success rate of CTO PCI of 70%; for the study to have 80% power to detect a two-tailed change in FFR of 0.02, we estimated that 48 participants were required with procedural success in 33. Continuous variables were compared using a paired t-test or Wilcoxon signed-rank test. Correlations were quantified using Pearson's correlation coefficient. Probability values were 2-sided, and values of $p<0.05$ considered significant.

4.4 Results

4.4.1 Overview

Of 47 patients recruited, 34 underwent successful CTO angioplasty, completed the study protocol and were included in analysis. One was excluded because of significant left main stem disease found at the time of PCI not apparent on initial angiography. The presence of viable myocardium in the CTO territory was confirmed in all patients by myocardial perfusion scintigraphy (n=26, 76.5%), dobutamine stress echocardiography (n=1,

2.9%) or by the absence of a wall motion abnormality by echocardiography or left ventricular angiography without additional confirmation(n=7, 20.6%). Drug-eluting stents were used for all procedures. Demographics, angiographic and procedural details are shown in **table 4.1**.

Table 4.1 Baseline Characteristics, Angiographic, and Procedural Details

Demographics(n=34)	
Male, n(%)	27(79.4)
Age, years	60.8±9.6
Left ventricular ejection fraction(%)	56.2±11
Estimated occlusion duration(weeks)	53(30-104)
CCS class I/II/III/IV	8/20/6/0
Previous PCI, n(%)	9(26.5)
Previous myocardial infarction, n(%)	10(29.4)
Hypertension, n(%)	6(17.7)
Diabetes Mellitus, n(%)	5(14.7)
Current smoker, n(%)	10(29.4)
Angiographic details	
CTO vessel(RCA/LCx/LAD)	21/4/9
Predominant donor vessel(RCA/LCx/LAD)	10/11/13
Rentrop collateral grade(1/2/3)	0/12/22
Predominant donor vessel CC grade(0/1/2)	0/18/16
Minor donor vessel CC grade(0/1/2)	17/15/2
Predominant donor vessel stenosis severity(%)	39.1(25.2-47.7)
Minor donor vessel stenosis severity(%)	39.4(26.8-46.1)
Procedural details	
Number of stents 1/2/3/4/5	6/11/10/6/1
Length of stent(mm)	74.5(44-101)
Means of recanalisation	
Antegrade lumen-lumen, n(%)	19(55.9)
Antegrade dissection re-entry, n(%)	9(26.5)
Retrograde lumen-lumen, n(%)	3(8.8)
Retrograde dissection re-entry, n(%)	3(8.8)

PCI indicates percutaneous coronary intervention; ACE-inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; RCA, right coronary artery; LCx, left circumflex artery; LAD, left anterior descending artery

4.4.2 Haemodynamic indices

Mean time in minutes from restoration of antegrade flow in the CTO vessel to post-PCI FFR measurement was 70.1±23.1 for the predominant donor vessel and 71.5±25.3 for the minor donor vessel. Pre and post-

procedural haemodynamic measurements for the predominant and minor donor vessels are detailed in **table 4.2**.

Table 4.2 Haemodynamic assessment pre and post CTO PCI

	Pre-procedure	Post procedure	Difference (95% CI)	p-value
RAP (mmHg)	5.6±2.9	6.1±3.1	0.5 (-1.7 to 0.7)	p=0.39
MAP (mmHg)	121.7±18.8	124.2±19.7	-2.5 (-9.9 to 4.9)	p=0.50
Heart rate (beats/minute)	69.7±12.4	70.4±11.2	-0.6 (-3.5 to 2.3)	p=0.67
Predominant donor vessel				
FFR	0.782±0.117	0.810±0.095	0.028 (0.012 to 0.044)	p=0.001
Baseline flow (ml/min)*	177.5±87.2	139.9±68.2	-37.6 (-62.6 to -12.6)	p=0.005
Hyperaemic flow (ml/min)†	306.5±149.0ml	272.9±151.1	-33.5 (-58.7 to -8.3)	p=0.011
CFR†	2.24±0.93	2.33±0.78	0.10 (-0.24 to 0.44)	p=0.57
HMR_v (mmHg/cm/s)†	1.92±0.71	2.47±1.35	0.55 (0.12 to 0.99)	p=0.014
HSR (mmHg/cm/s)†	0.50±0.37	0.50±0.30	-0.002 (-0.09 to 0.09)	p=0.95
Minor donor vessel				
FFR	0.808±0.104	0.813±0.110	0.005 (-0.015 to 0.023)	p=0.63
Baseline absolute flow (ml/min)*†	157.6±80.3	141.4±98.1	-16.2 (-43.3 to 11.0)	p=0.23
Hyperaemic flow (ml/min)†	274.4±147.7	270.6±185.3	-3.7 (-29.9 to 22.4)	p=0.77
CFR†	2.25±0.67	2.24±0.72	-0.01 (-0.24 to 0.27)	p=0.91
HMRv (mmHg/cm/s)†	2.28±0.95	2.47±1.32	0.19 (-0.11 to 0.49)	p=0.20
HSR (mmHg/cm/s)†	0.48±0.28	0.54±0.43	0.06 (-0.03 to 0.15)	p=0.20

CI indicates confidence interval; RAP, right atrial pressure; MAP, men aortic pressure;FFR fractional flow reserve; CFR, coronary flow reserve; HMRv, hyperaemic microvascular resistance; HSR, hyperaemic stenosis resistance. *adjusted for rate pressure product. †Satisfactory flow measurements were obtained in 32 patients

Pre-procedural predominant donor vessel FFR measured 0.782±0.117, which increased to 0.810±0.095 after CTO angioplasty (difference 0.028, 95% CI 0.012 to 0.044, p=0.001). We found no significant difference in the minor donor vessel. Individual FFR measurements are detailed in **figure 4.1**. The treatment threshold for the predominant donor vessel was crossed from ≤0.8 to >0.8 in 4 patients

(11.8%), however 4 patients also crossed in the opposite direction from an FFR of >0.8 to ≤0.8.

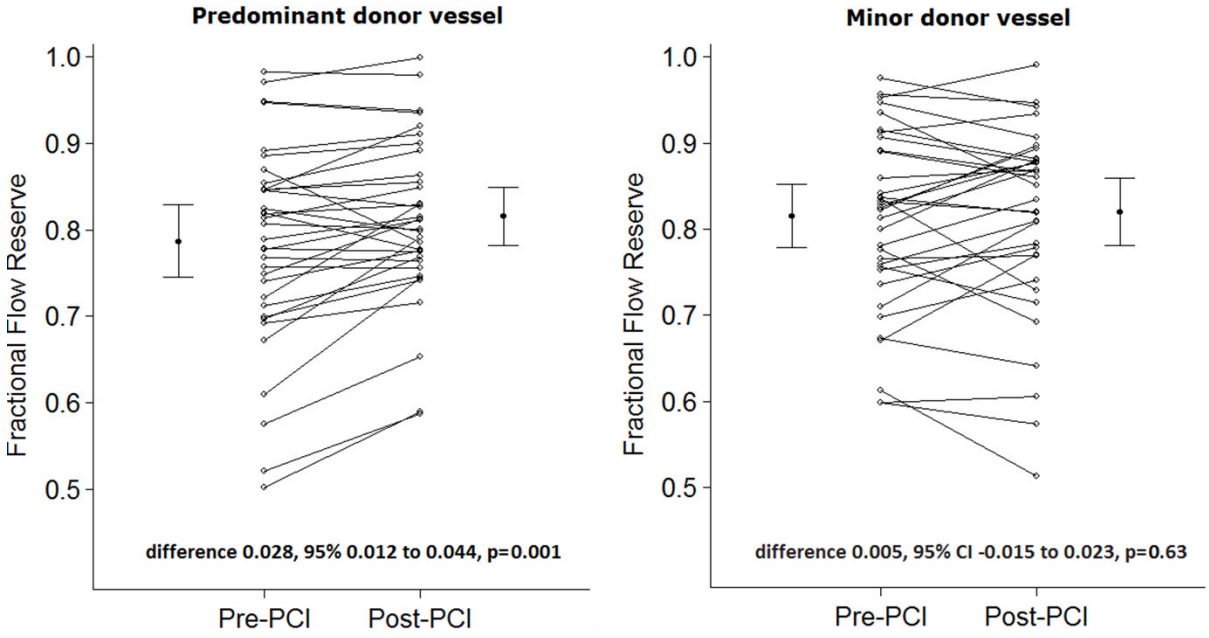

Figure 4.1 The change in non-target vessel FFR before and after CTO PCI. Mean values are presented either side of the link-plots, error-bars represent 95% confidence intervals.

4.4.3 Coronary flow

Satisfactory flow measurements were obtained in 32 of 34 subjects completing the study protocol. Changes in baseline and hyperaemic absolute coronary flow are depicted in **figure 4.2**. Predominant donor vessel absolute coronary flow under baseline conditions, adjusted for RPP was 177.5±87.2ml/min pre-procedure, and reduced to 139.9±68.2ml/min post-procedure(difference -37.6ml/min, 95% CI -62.6 to -12.6, p=0.005). Predominant donor vessel hyperaemic absolute coronary flow also reduced, pre-procedure: 306.5±149.0ml/min; post-procedure: 272.9±151.1ml/min (difference -33.5ml/min, 95% CI -58.7 to -8.3ml/min p=0.011). We found no statistically significant difference in baseline or hyperaemic absolute coronary flow in the minor donor vessel. There was no statistically significant difference in the absolute size of reduction in coronary flow in the predominant donor vessel at baseline compared with hyperaemia (difference 4ml/min, 95% CI -29.6 to 37.7ml/min, p=0.60). There was also

no statistically significant change in mean CFR or HSR in either the predominant donor vessel or minor donor vessel.

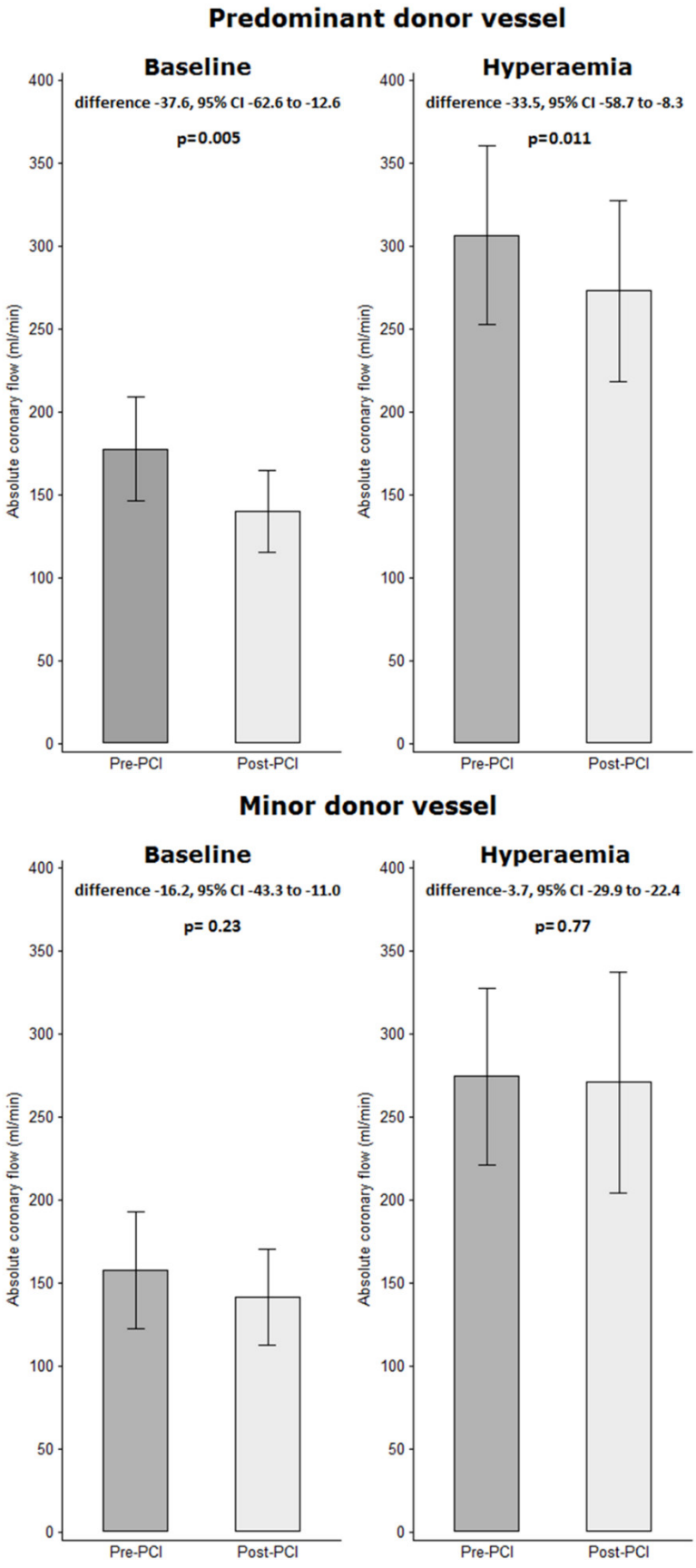

Figure 4.2 Mean absolute coronary flow pre and post-PCI at baseline(adjusted for RPP) and hyperaemia for the predominant donor vessel(above) and the minor donor vessel(below).

HMR did increase after CTO PCI in the predominant donor vessel; pre-procedure: 1.92±0.71 mmHg·cm·s^{-1}, post-procedure 2.47±1.35 mmHg·cm·s^{-1} (difference 0.55 mmHg·cm·s^{-1}, 95% CI 0.12 to 0.99, p=0.014); there was no statistically significant change in the minor donor vessel.

It was possible to measure coronary flow velocity distal to the point of occlusion in 30 patients, 4 of which through a retrograde approach. Mean collateral flow velocity reserve measured 1.09±0.25 (excluding retrograde measurements: 1.08±0.26), with coronary steal evident in 9 patients (26%). We found no correlation between change in predominant donor vessel FFR and invasive measures of collateral perfusion measured distal to the occlusion; fractional collateral flow reserve: r=-0.08, p=0.66, collateral flow velocity reserve: r=-0.10, p=0.62; or change in coronary flow velocity at the point of FFR measurement: r=0.11, p=0.55. In the predominant donor vessel, there was a trend to a smaller reduction in flow in more severe stenoses measured by DFV-PGR(r=0.33, p=0.068). We did find a relationship between maximal angiographic stenosis severity in the predominant donor vessel and change in FFR in the predominant donor vessel: r=0.44, p=0.009 (**figure 4.3**).

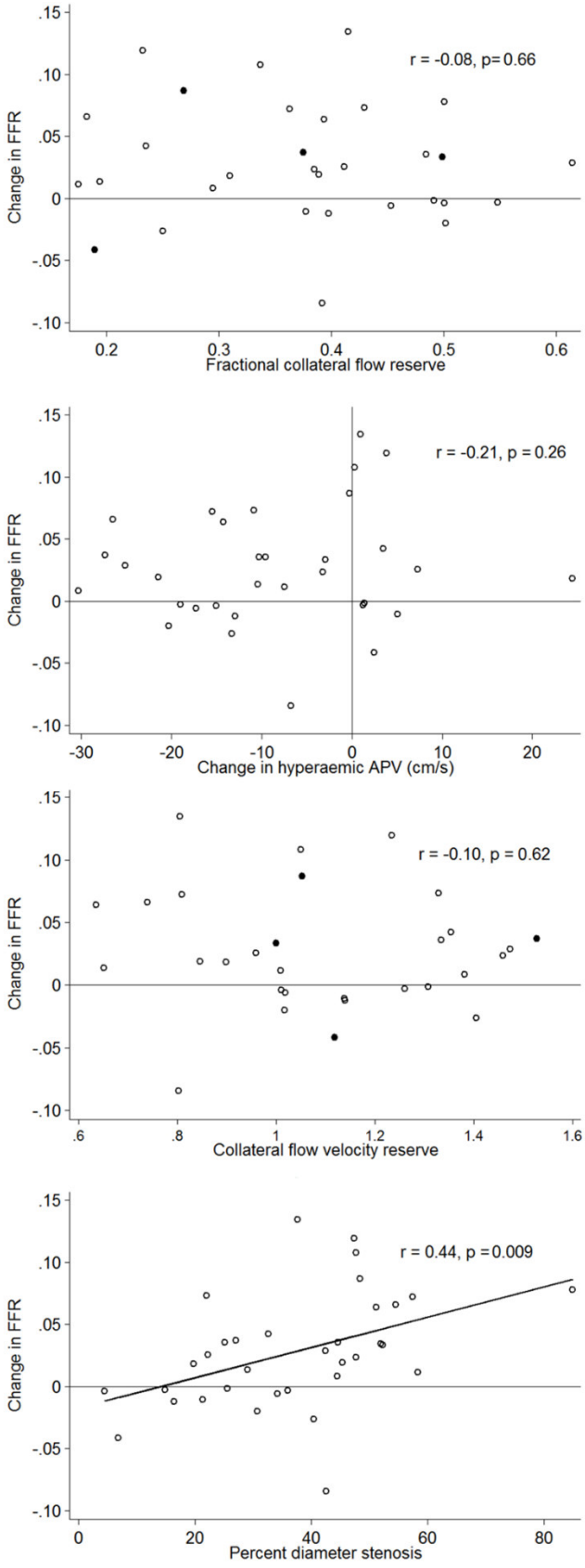

Figure 4.3 Relationships with change in predominant donor vessel FFR. Top : fractional collateral flow reserve distal to the chronic occlusion(n=31). Second top : change in distal hyperaemic APV(n=32). Second bottom: collateral flow velocity reserve distal to the chronic occlusion(n=30). Solid markers represent measures taken by a retrograde approach. Bottom: angiographic(percent diameter) stenosis severity(n=34).

Figure 4.4 shows an example of measurement and calculation of the DFV-PGR. There was a strong correlation between peak DFV-PGR slope in the predominant donor vessel and change in predominant donor vessel FFR; r=0.69, p<0.001(**figure 4.5**).

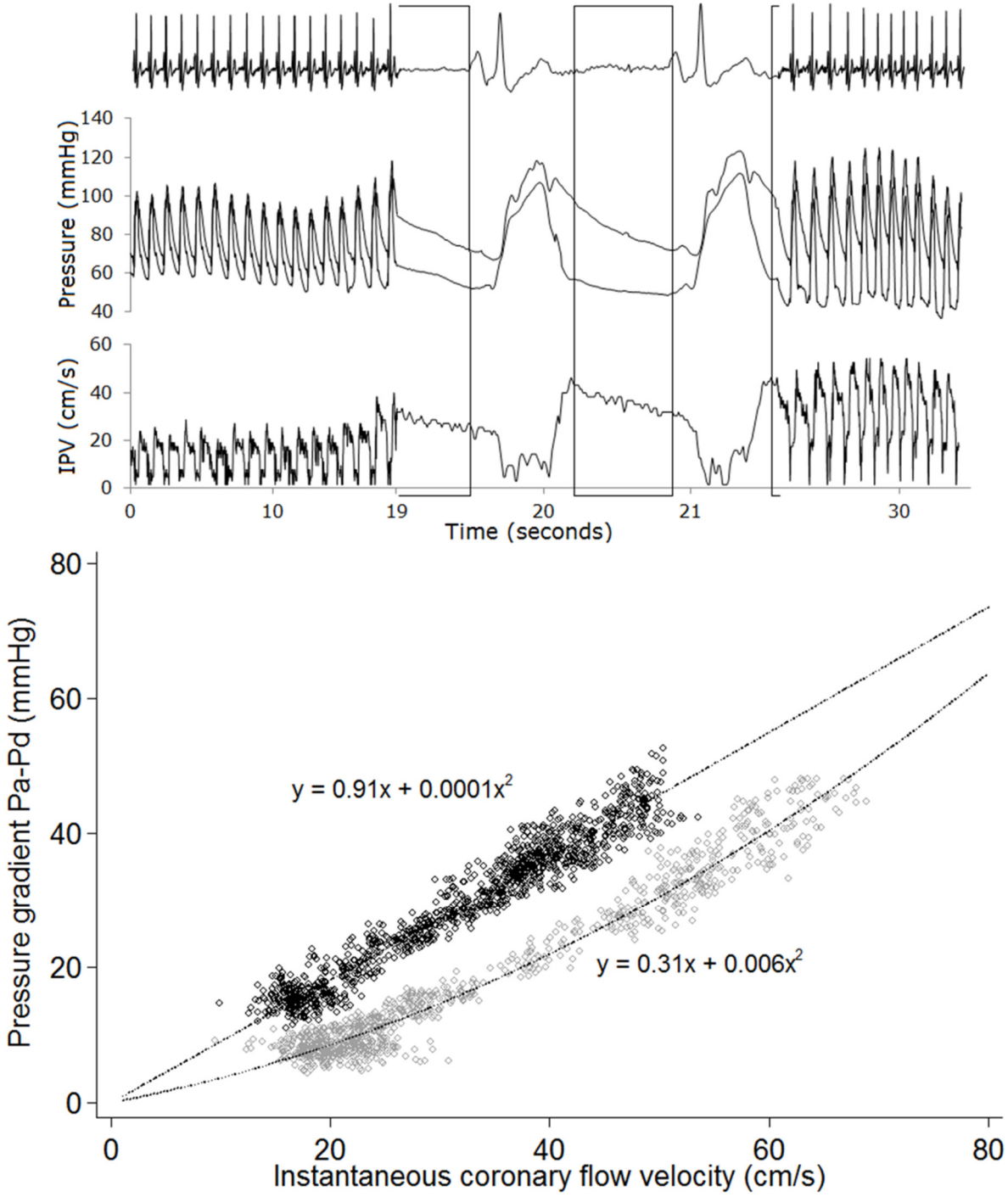

Figure 4.4 Top panel: simultaneous pressure and flow measurement for calculation of DFV-PGR, measurements are taken during the boxed diastolic periods. Bottom: calculation of the DFV-PGR slope using the formula $\Delta P=FV+SV^2$. In the above example F=0.91 and S=0.0001(black circles). Another example is shown with different coefficient values, but a similar peak gradient: F=0.31 and S=0.006(grey circles).

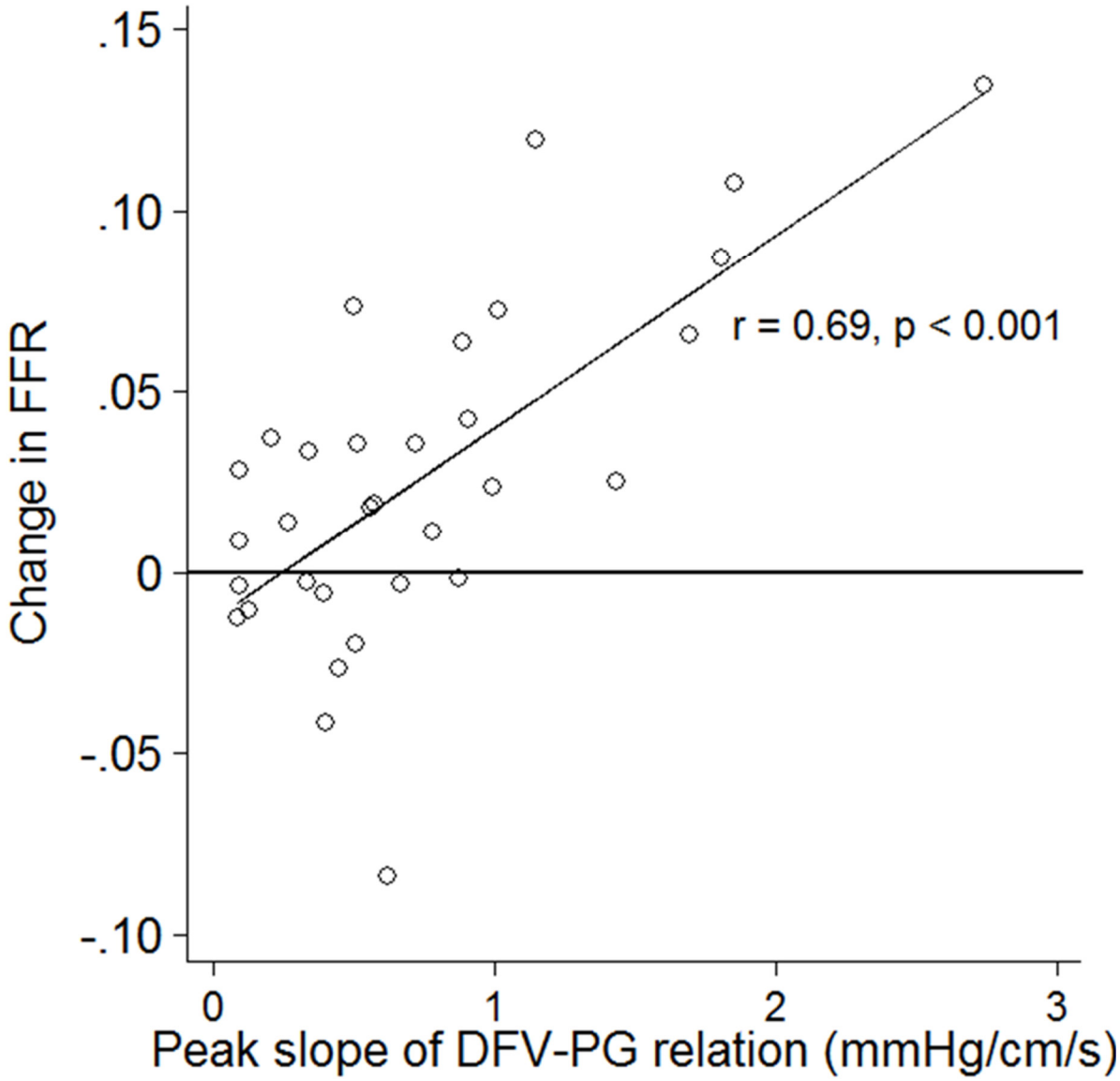

Figure 4.5 Relationship between peak DFV-PGR and change in predominant donor vessel FFR, before and after CTO PCI(n=32).

4.4.4 Wave intensity analysis

Wave intensity analysis was performed in 32 of 34 patients using measurements taken from the proximal non-target vessels prior to any major branch; **figure 4.6** shows typical examples. In the predominant donor vessel, mean cumulative wave energy of the BEW decreased from 79.7±44.3x10^5 J·m^{-2}·s^{-2} before PCI to 65.3±43.6x10^5 J·m^{-2}·s^{-2} after PCI (difference -14.3x10^5 J·m^{-2}·s^{-2}, 95% CI -25.9x10^5 to -2.9x10^5, p=0.016). We found no statistically significant difference in the minor donor vessel;

Pre-PCI: 71.9±39.9x10^5 J·m^{-2}·s^{-2}, post-PCI: 67.1±42.3x10^5 J·m^{-2}·s^{-2} (difference -4.8x10^5 J·m^{-2}·s^{-2}, 95% CI -18.5x10^5 to 9.0x10^5, p=0.49). Change in cumulative wave energy correlated with change in resting coronary flow, unadjusted for RPP: r=0.43, p=0.014.

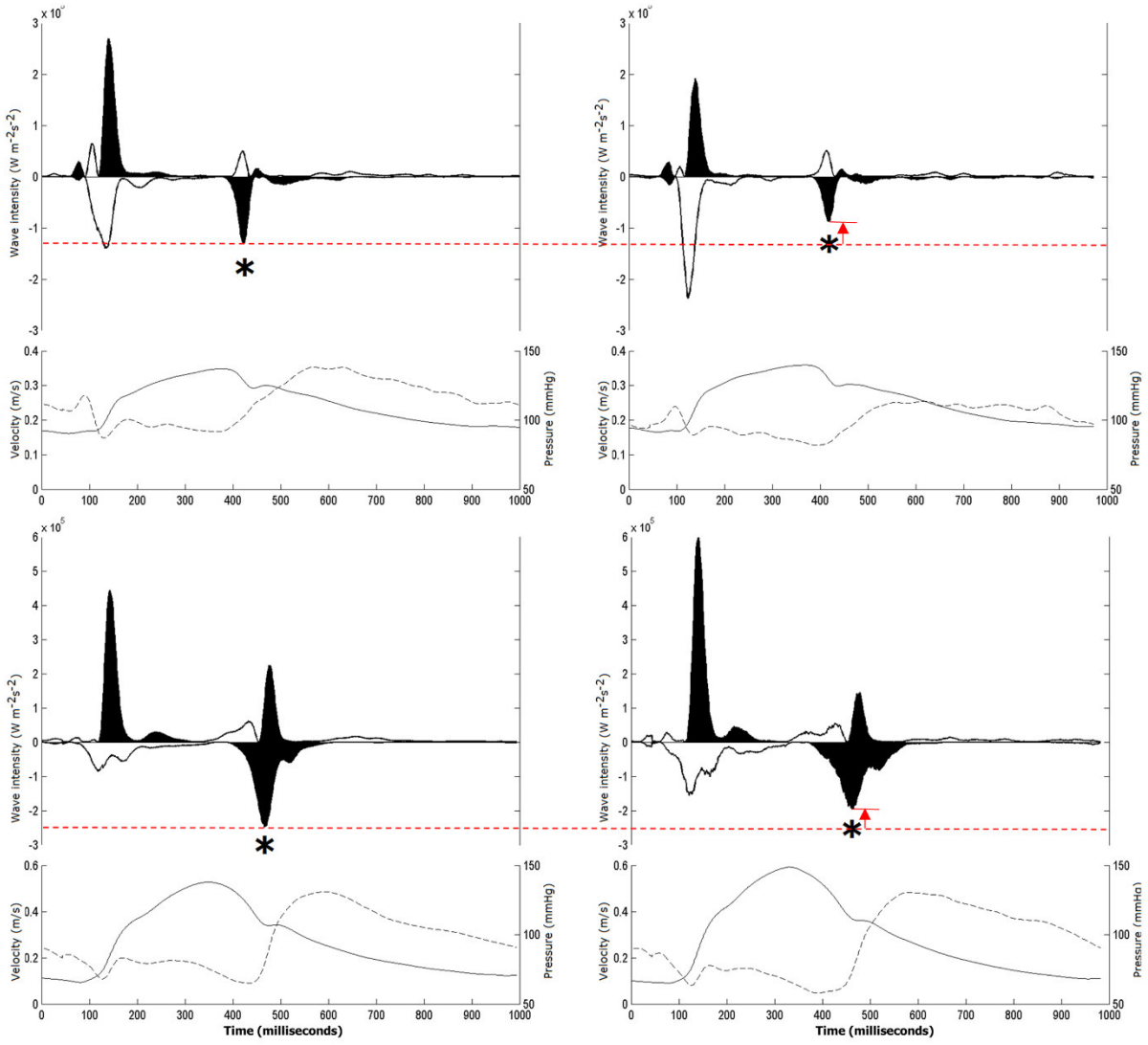

Figure 4.6 Wave intensity analysis, ensemble averaged coronary pressure (solid line) and flow velocity (dashed line) measured in the proximal predominant donor vessel pre(left) and post(right) PCI of a CTO. Top two panels: proximal RCA donating collaterals to a chronically occluded LAD. Bottom two panels: proximal LAD donating collaterals to a chronically occluded RCA. BEW are asterisked. Note the reduction in backwards expansion wave intensity post CTO PCI (red lines).

4.5 Discussion

4.5.1 Summary of findings

My findings support the hypothesis that in a patient with a CTO, measurement of the FFR in an artery providing collateral supply to the myocardium beyond the occlusion is significantly lower than it would otherwise be in the absence of the CTO. Our estimate of the size of the effect in a group of unselected patients is smaller than suggested by case reports of the phenomenon[65–69]. A number of our findings are suggestive of a possible physiological mechanism and reasons for variation in size of change, they are as follows: (1)there is an associated reduction in absolute coronary flow and increase in HMRv; (2)the reduction in coronary flow is associated with a reduction in size of the BEW; (3)the magnitude of reduction in flow is similar at baseline and hyperaemia; (4) change in FFR is strongly related to donor vessel coronary stenosis severity;(5)there is no demonstrable association between invasive indices of collateral function and change in FFR.

4.5.2 Effect size

The increase in predominant donor vessel FFR associated with CTO PCI of approximately 0.03 is consistent with a smaller study examining the same phenomenon[70,71] however case reports suggest the expected increase should be closer to 0.10[65–69]. This may be because measurement and re-measurement of an index such as the FFR is vulnerable to confounding by regression to the mean. If measurements are considered as a whole and not selected based upon their values, regression to the mean will not influence overall effect size. However individual measurement changes are much more likely to involve a contribution by regression to the mean[107]. It can be estimated that the SDD of repeated FFR measurements is 0.032 and coefficient of repeatability 0.063[105]; this measurement variability is sufficient for regression to the mean to explain the disparity if the case reports are subject to selective reporting and/or publication bias. In addition, there appears to be a greater change in FFR in more severely

diseased vessels. Including angiographically unobstructed vessels is likely to have reduced our effect size. However it has been suggested that the phenomenon exists in unobstructed vessels[62] and the mean pre-PCI FFR in the predominant donor vessel was 0.78, close to the widely practiced treatment threshold of 0.80 at which the phenomenon is most relevant.

It is conceivable that in the weeks following CTO recanalisation, the return of normal vasomotion in the previously occluded segment of the CTO vessel[123] and further closure of collateral channels might result in a further fall in donor vessel flow and increase in FFR. Given that successful recanalisation of a CTO should result in no pressure gradient between collateral donor and recipient vessels, it seems unlikely that this would result in a major change. Repeat measurement at an interval after initial CTO PCI would be required to investigate this further.

4.5.3 Reduction in donor vessel flow

A likely explanation for the change in predominant donor vessel FFR is the associated reduction in absolute coronary flow (**figure 4.2**) and increase in HMRv. Previous studies have shown a rapid reduction in recruitable collateral flow distal to the point of occlusion after CTO PCI[63,64], we have shown a reduction in coronary donor vessel flow at a similar interval. The absence of this finding in the vessel donating no/fewer collaterals suggests the change is related to reduced collateral donation. A generalised effect of PCI on microvascular function seems less likely, whether mediated through an adrenergic effect[181] or through myocardial stunning and an elevation in left ventricular end-diastolic pressure[182,183]. An alternative mechanism that may work in synergy with the reduction in flow is that collateral contribution to distal pressure in the donor vessel might increase once the CTO is recanalised in the reverse direction to collateral flow prior to PCI.

4.5.4 The mechanism of a reduction in donor vessel flow

In support of the hypothesis that the observed reduction in donor vessel coronary flow is related to a reduction in collateral donation and perfused myocardial mass, we demonstrate a reduction in the size of the BEW in the predominant collateral donor vessel associated with CTO PCI. Moreover, the size of that reduction is related to the size of reduction in flow. A predominant pattern of 6 coronary waves measured by wave intensity analysis has been described. The BEW, caused by the relief of myocardial microcirculatory compression in early diastole, is responsible for the large increase seen in coronary flow in early diastole[98](**figure 4.6**). Increased left ventricular contractility is associated with an increase in the size of the early backwards compression wave(eBCW)[99]. The size of the BEW, being driven by the reverse of the mechanism of the eBCW is likely to be related to the mass of myocardium relaxing in early diastole. A reduction in its size associated with a change in flow supports the hypothesis that a change in donor vessel antegrade flow is related to reduced collateral donation, rather than an increase in received collateral supply.

We describe a similar fall in predominant donor vessel absolute flow after CTO PCI at baseline and hyperaemia. This is consistent with the fall in coronary flow being the component of pre-PCI flow donated to the collateral dependent myocardium. Flow in well collateralised occluded vessel segments responds to an arteriolar vasodilatory stimulus in a similar fashion to flow beyond a severe stenosis[46]. The microcirculation beyond a severe stenosis is already maximally vasodilated, so a further vasodilatory stimulus is unlikely to increase flow[93]. Coronary flow distal to a CTO can actually diminish with adenosine infusion (a coronary flow reserve of <1), a phenomenon known as coronary steal[62,121]. The mean collateral flow velocity reserve measured in the occluded segment in this study was 1.09, with coronary steal evident in 9 patients (26%). The small relative proportion of donor vessel absolute flow attributable to the collateral circulation at hyperaemia may explain the relatively small increase in FFR.

4.5.5 The change in FFR is related to stenosis severity

We report an association between predominant donor coronary stenosis severity and change in FFR in the predominant donor vessel associated with CTO PCI, assessed both angiographically and haemodynamically (**figures 4.3 & 4.5**). Although functional stenosis severity assessment by angiography is limited, it is independent of individual variation in measurement of FFR and therefore the relationship with pre/post measurement should not be confounded by regression to the mean[107].

The DFV-PGR describes the pressure gradient as a result of overall lesion severity, encompassing lesion length, diameter stenosis and induced turbulence as coronary flow velocity changes (**figure 4.4**). The slope of the curve is independent of the absolute difference in Pd and Pa and so in addition to describing the effect of a change in flow on pressure based physiological lesion indices, it should also be less susceptible to confounding by regression to the mean compared with indices dependent upon absolute values of Pa and Pd. The observed strong association between a steeper predominant donor peak DFV-PGR slope and a greater change in predominant donor FFR (**figure 4.5**) is supportive of the hypothesis that any change in pressure gradient (and therefore FFR) is related to reduced flow.

4.5.6 Relation of change in FFR and indices of collateral function in the occluded segment

The absence of a relationship between the change in predominant donor flow and measured indices of collateral function is surprising. It may be that overall collateral dependent myocardial mass is more important than the measurement of collateral function for a given myocardial mass. This could be evaluated by comparing LAD with non-LAD CTOs, but would require a study population larger than reported here. The absence of a correlation between change in predominant donor vessel flow and change in predominant donor FFR may reflect an interaction between the effect of

donor vessel stenosis severity on collateral flow and the effect of the change of flow. Coronary steal, and therefore reduced collateral flow at hyperaemia, is more prevalent if a collateral donor vessel has a lower FFR[62]. We report a trend towards a smaller change in hyperaemic flow associated with CTO PCI in predominant donor vessels with more severe stenoses. A smaller change in flow may therefore be associated with a steeper DFV-PGR slope, masking any relationship.

4.5.7 Clinical implications

This study confirms that the presence of a CTO is associated with a lower FFR in the predominant collateral donor vessel than if the CTO were absent. The change is smaller than might be expected and is closely related to lesion severity such that greater changes are largely confined to stenoses of severities that remain below the treatment threshold of ≤0.8 in spite of a large increase in FFR. The number of patients in the study population that cross the treatment threshold is small. A small number have also crossed the FFR treatment threshold in the opposite direction to that expected, most likely because of measurement variation and possibly short term PCI related effects upon the microvasculature. When planning multi-vessel revascularisation in the presence of a concomitant CTO, physiological lesion assessment by FFR is reliable. If measurements are close to the current established treatment threshold of ≤0.80, a probable small increase in FFR should be considered when deciding upon treatment strategy.

If a collateral donor vessel supplying the collateral dependent myocardium distal to a CTO has a stenosis with an FFR ≤0.80, some might argue that treating the donor vessel stenosis alone and leaving the CTO would be a reasonable approach. Whilst this may have a small effect on the extent of ischaemia distal to the CTO, the almost universal presence of myocardial ischaemia distal to a CTO if the myocardium is viable, even in patients with single vessel disease, in this and other published studies[45,92,121], would suggest that it would not eliminate ischaemia

completely. As consensus grows that we should be aiming for complete (rather than incomplete) revascularisation, it is difficult to recommend this approach.

4.5.8 Limitations

This is a single centre study, and the number of patients with a significant lesion in the predominant donor vessel was small. The study population had a preponderance of right coronary CTOs, with fewer LAD CTOs. This is a reflection of practice and is in keeping with other publications in the field, but may have reduced the size of the observed effect.

Measurements were repeated early after PCI, therefore transient procedural related changes such as microvascular dysfunction due to distal embolization, catecholamine release[181], left ventricular stunning[182,183] or a hyperaemic stimulus related to side-branch occlusion may have influenced donor vessel physiology. However, if the observed effect were due to transient global effects of PCI, we would expect a similar effect on the vessel donating no/less collaterals angiographically. In addition, other than the hyperaemic effect of side branch occlusion, these mechanisms would result in a larger reduction in donor vessel flow and larger increase in FFR. Given the smaller than expected change we observed, it seems unlikely that these additional mechanisms are contributing greatly to the overall change, however they may have contributed to individual variation.

4.5.9 Conclusions

Recanalisation of a CTO results in a modest increase in the FFR of the collateral donor vessel associated with a reduction in coronary flow. The magnitude of the change is closely related to lesion severity such that the largest changes are observed across stenoses which remain haemodynamically significant in spite of a large increase in FFR. In vessels with less severe stenoses, the effect is likely to be so small that it is masked

by variations in physiology both related and unrelated to PCI as well as measurement variation.

5.0 Microvascular Dysfunction in the Immediate Aftermath of Chronic Total Coronary Occlusion Recanalisation

5.1 Abstract

5.1.1 Objectives

The aim of this study was to compare microvascular resistance under both baseline and hyperaemic conditions immediately after PCI of a CTO with an unobstructed reference vessel in the same patient

5.1.2 Background

Microvascular dysfunction has been reported to be prevalent immediately after CTO PCI. However previous studies have not made comparison with a reference vessel. Patients with a CTO may have global microvascular and/or endothelial dysfunction, making comparison with established normal values misleading.

5.1.3 Methods

After successful CTO PCI in 21 consecutive patients, coronary pressure and flow velocity were measured at baseline and hyperaemia in distal segments of the CTO/target vessel and an unobstructed reference vessel. Haemodynamics including hyperaemic microvascular resistance (HMRv), basal microvascular resistance (BMR) and instantaneous minimal microvascular resistance at baseline and hyperaemia were calculated and compared between reference and target/CTO vessels.

5.1.4 Results

After CTO PCI, BMR was reduced in the target/CTO vessel compared with the reference vessel: 3.58mmHg/cm/s vs. 4.94mmHg/cm/s, difference -1.36 $mmHg \cdot cm^{-1} \cdot s^{-1}$ (-2.33 to -0.39,p=0.008). We did not detect a difference in HMR: 1.82 $mmHg \cdot cm^{-1} \cdot s^{-1}$ vs. 2.01 $mmHg \cdot cm^{-1} \cdot s^{-1}$, difference -0.20(-0.78 to 0.39,p=0.49). Instantaneous minimal microvascular resistance correlated strongly with length of stented segment at baseline (r=0.63, p=0.005) and hyperaemia (r=0.68, p=0.002).

5.1.5 Conclusions

Basal microvascular resistance is reduced in a recanalised CTO in the immediate aftermath of PCI compared to an unobstructed reference vessel; however hyperaemic microvascular resistance appears to be preserved. A longer stented segment is associated with increased microvascular resistance.

5.2 Introduction

Even in the absence of flow-limiting epicardial coronary disease, an intact microvascular vasodilatory reserve is important in preventing myocardial ischaemia under stress[111] and is associated with improved prognosis[112]. Transient target vessel microvascular dysfunction can occur in a proportion of patients in the immediate aftermath of PCI of non-occlusive coronary disease[125,126] and has been reported to be more frequent in patients who have undergone PCI to a CTO[72,127]. Microvascular function in epicardially diseased vessels is related to unobstructed reference vessels in the same patient[128], so the increased prevalence of microvascular dysfunction in this setting may simply reflect the greater burden of disease in patients with CTOs and represent a global phenomenon of microvascular or endothelial dysfunction.

Microvascular resistance in the immediate aftermath of CTO PCI is not well described with respect to the now well established combined pressure and flow indices of microvascular resistance[87]. In addition, previous studies examining the effect of PCI to a CTO on microvascular function and coronary physiology have examined the target vessel, but not made comparison with a reference vessel[72,124,127]. The aim of this study was to describe the immediate effect of CTO PCI on the coronary microvasculature distal to the occlusion in comparison with an unobstructed reference vessel in the same patient. Using instantaneous measures of microvascular resistance we aimed to measure instantaneous minimal microvascular resistance to estimate maximal microvascular dilatation at rest and hyperaemia. We also investigated whether there was a relationship

between post-PCI microvascular resistance and pre-PCI measures of collateral perfusion as well as procedural factors which might influence post-PCI microvascular resistance such as length of stented segment and means of CTO recanalisation.

5.3 Methods

5.3.1 Study population

I included 21 patients from a cohort of 34 patients recruited consecutively into a coronary haemodynamic study (described in **chapter 4.0**) in a single tertiary centre between July 2013 and June 2014 who underwent successful PCI to a CTO for symptoms of angina (Canadian Cardiovascular Society (CCS) class 1-3). We excluded 2 patients due to sub-optimal coronary flow measurements and 11 because of non-target angiographic coronary stenosis severities of >50% diameter. A CTO was defined as complete coronary occlusion of ≥3 months duration with TIMI grade 0 flow[1]. The presence of viable myocardium in the CTO territory was confirmed in all patients by myocardial perfusion scintigraphy(n=16, 76%), dobutamine stress echocardiography(n=1, 5%) or by the absence of a wall motion abnormality by echocardiography or left ventricular angiography without additional confirmation(n=4, 19%). Exclusion criteria were inability to provide consent, >1 occluded vessel, disease of >50% angiographic severity in both other major epicardial coronary arteries, prior CABG with any patent grafts, left main stem stenosis considered to be haemodynamically significant and contra-indications to adenosine. Patient's usual medications were continued and they were asked to abstain from caffeine for 48 hours prior to the procedure.

5.3.2 Ethics

The study protocol was approved by the local research ethics committee (12/YH/0360). All subjects provided written informed consent.

5.3.3 Catheter laboratory protocol

Dual arterial access was used for all procedures. Femoral venous access was obtained for central administration of adenosine and measurement of right atrial pressure (RAP) at the beginning and end of the procedure using a catheter positioned in the right atrium. Patients were anti-coagulated with 100 U/kg of unfractionated heparin to maintain an activated clotting time of >300 seconds. After a 200μg bolus of intra-coronary glyceryl trinitrate(GTN), iso-centred coronary angiograms of both non-target vessels were taken.

The protocol for coronary haemodynamic assessment is summarised in **figure 5.1**. A dual sensor pressure-velocity 0.014" intracoronary wire (Combowire XT 9500, Volcano Corp, San Diego, CA)[87], with pressure and Doppler sensors at its tip, was connected to a ComboMap console (Volcano Corp) and used for haemodymamic measurements. PCI of the CTO was undertaken at the discretion of the treating interventional cardiologist using an antegrade or retrograde approach. Once access to the vessel lumen distal to the point of occlusion was achieved, prior to restoration of antegrade flow, a microcatheter was placed into the distal vessel to facilitate delivery of the Combowire. The Combowire was normalised to aortic pressure at the tip of the catheter alongside the microcatheter, removed, and passed through the microcatheter into the occluded segment and positioned in a vessel segment angiographically free of a significant stenosis. After administration of 100μg GTN in the vessel donating collaterals to the CTO vessel and once any hyperaemic response had settled, continuous recordings from the ComboMap were taken. Hyperaemia was achieved by central venous administration of adenosine at 140μg/kg/minute. Once steady state hyperaemia had been reached and a continuous recording of ≥20 beats taken, adenosine infusion was ceased. Samples were recorded at 200Hz and stored on disk for offline analysis.

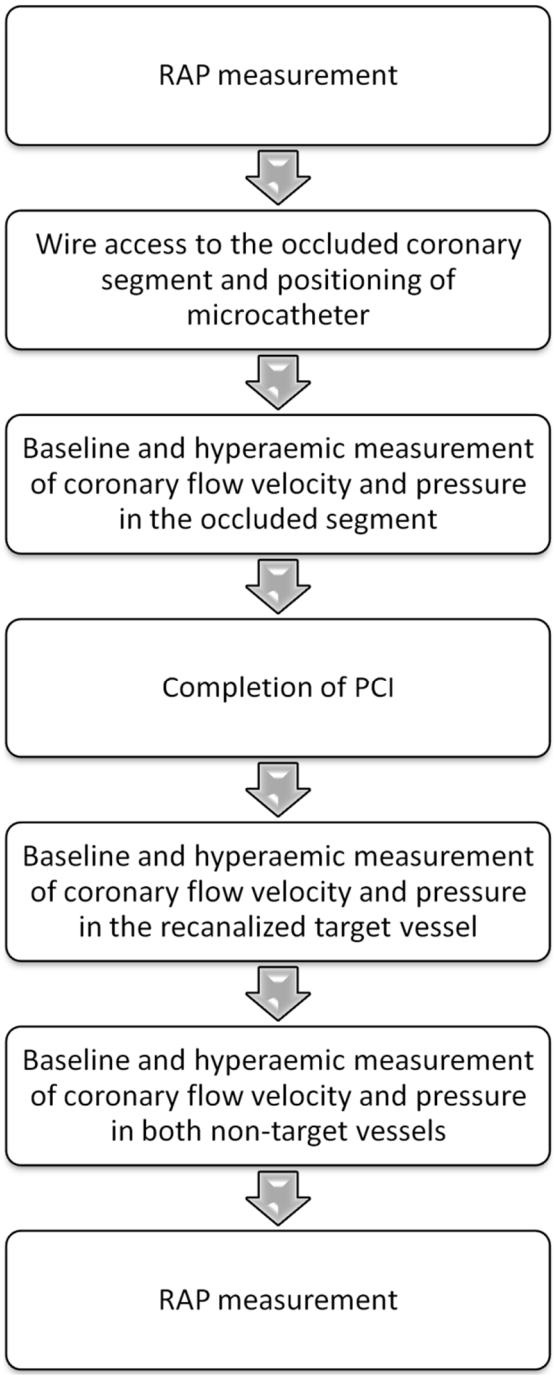

Figure 5.1. Flowchart summarizing the order of haemodynamic measurements taken as part of the study protocol. RAP = right atrial pressure.

PCI success was defined as stenting of the target vessel with <30% residual stenosis and thrombolysis in myocardial infarction(TIMI) grade III flow. After successful PCI, The Combowire was normalised to aortic pressure at the tip of the catheter, advanced to the distal segment of the target vessel at the point of measurement in the occluded segment prior to

PCI and manipulated to obtain a good Doppler trace. After administration of 100μg intra-coronary GTN, once the hyperaemic response had settled, continuous recordings from the ComboMap were taken as described. Haemodynamic measurements were then repeated in each non-target vessel as described for the target vessel post-PCI, including repeated RAP measurement.

Recorded data was analysed using dedicated custom software (Study Manager, Academic Medical Center, University of Amsterdam, The Netherlands).

5.3.4 Angiographic assessment

Maximal non-target vessel diameter stenosis(%) was calculated by two independent observers using quantitative coronary angiography(QCA)(GE Centricity CA1000, GE Healthcare) using the guiding catheter luminal diameter as reference. Mean values from both observers were used for analysis. The non-target vessel making the largest collateral contribution was identified, vessel collateral connection(CC) grade[90] and modified Rentrop score[89] were assessed by two independent observers blinded to haemodynamic measurements and agreed by consensus.

The non-target vessel selected as the reference vessel was selected based upon an angiographic diameter stenosis severity of <50%. Where possible, the non-target vessel making the smallest collateral contribution to the CTO was selected.

5.3.5 Data analysis

Flow velocity was measured in cm/s, mean values are expressed as average peak velocity(APV) and instantaneous values as instantaneous peak velocity(IPV). Hyperaemic microvascular resistance (HMRv) was calculated as Pd/APV under hyperaemic conditions and basal microvascular resistance(BMR) as Pd/APV under baseline conditions. FFR was calculated as (Pd-RAP)/Pa-RAP), using mean pressures taken over 5 cardiac cycles at stable hyperaemia[166]. Coronary flow velocity reserve (CFR) was calculated

as APV at steady state hyperaemia divided by APV at baseline, measured over 5 cardiac cycles.

In addition, we also investigated the instantaneous minimal microvascular resistance which was calculated by sampling instantaneous flow velocity and Pd from 25% into the diastolic period (taken from the dicrotic notch to the ECG R-wave), stopping at the onset of the qrs complex) over three beats. It is during this period in the cardiac cycle that the absolute value and the variance of microvascular resistance have been shown to be minimal[167]. Instantaneous minimal microvascular resistance was then calculated as the mean Pd/mean IPV taken during this period (**figure 5.2**).

Fractional collateral flow reserve (FFR_{coll}) was calculated as for FFR, with Pd measured in the occluded segment of the artery, prior to restoration of antegrade flow. Collateral flow velocity reserve was calculated as for CFR with flow velocities in the occluded segment measured at rest and steady state hyperaemia.

5.3.6 Measurement repeatability

Based upon analysis of 26 repeated flow measurements at baseline and hyperaemia without any intervening treatment, coefficient of variation for average peak coronary flow velocity measurements was 17.4%. Analysis of 10 repeated measurements taken from repeated adenosine infusions gave a coefficient of variation for FFR, CFR and HMR_v of 3.6%, 19.7% and 8.6% respectively. Using 10 repeated baseline measurements and 10 repeated hyperaemic measurements, the coefficient of variation for instantaneous minimal microvascular resistance was 12.8%. If just repeated baseline measurements were assessed it was 11.5% and if only hyperaemic measurements were assessed it was 14.0%.

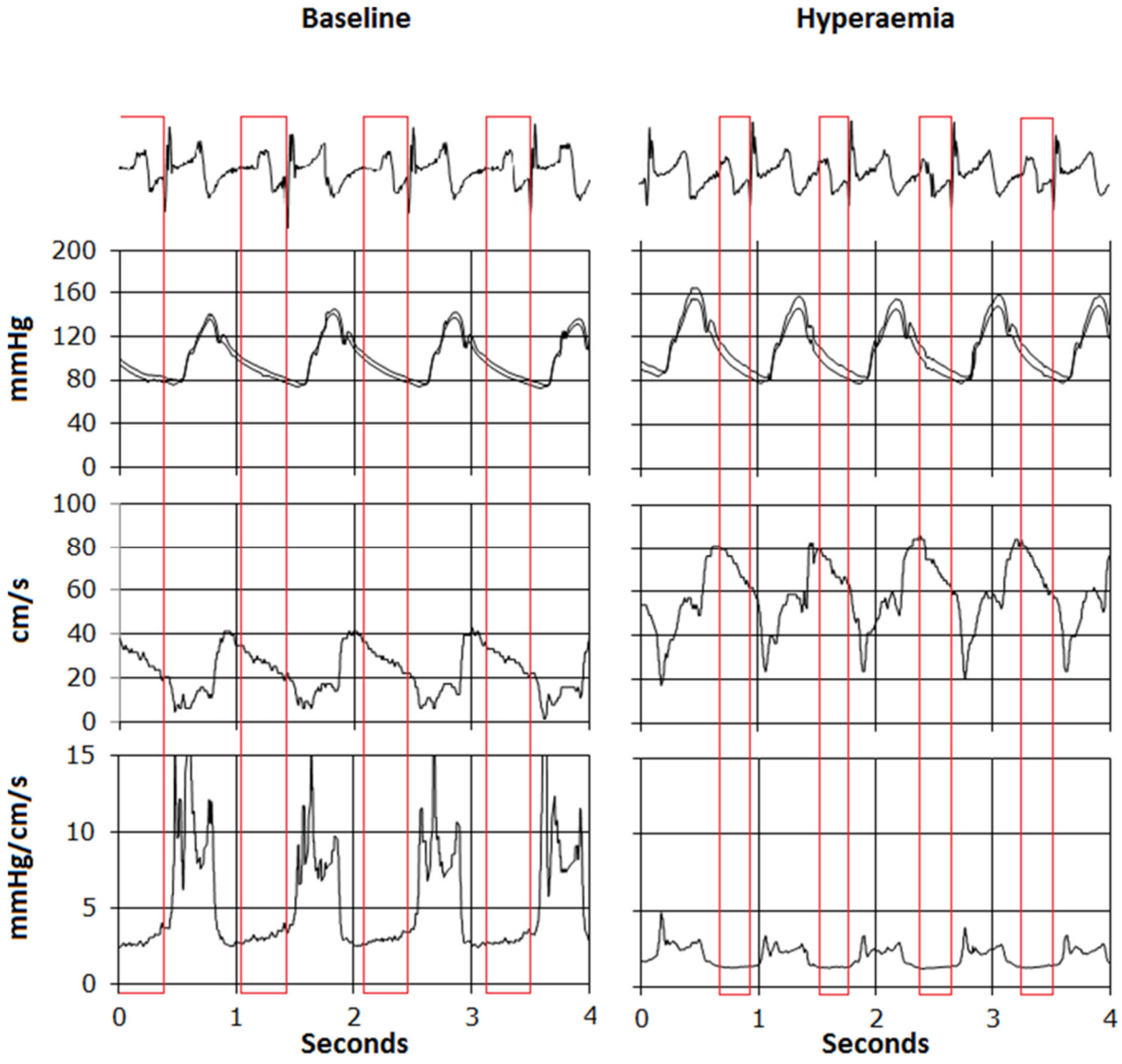

Figure 5.2 Assessment of minimal instantaneous microvascular resistance. Coronary haemodynamics measured in a recently recanalised right coronary artery. Instantaneous coronary pressure (top, Pa and Pd), flow velocity (middle) and derived microvascular resistance (bottom) under baseline conditions(left) and at maximal hyperaemia (right). The segments within red boxes represent the sampled period for minimal instantaneous microvascular resistance.

5.3.7 Statistical analysis

Stata v.12(StataCorp, College Station, Texas) was used for statistical analysis. Continuous values are expressed as means±SD, or median(25^{th} percentile-75^{th} percentile) as appropriate. Continuous variables were compared using a paired t-test or Wilcoxon signed-rank test. Correlations were quantified using Pearson's correlation coefficient. Probability values were 2-sided, and values of $p<0.05$ considered significant.

5.4 Results

5.4.1 Overview

Of the 21 patients who underwent successful CTO PCI, mean age was 60.9±10.9 years, 18 (86%) were male and mean LV ejection fraction was 57.3±10.2%. Median estimated duration of occlusion was 54 weeks (30-87) and all patients had Rentrop[89] ≥2 and CC[90] ≥1 grade collateralisation. Drug-eluting stents were used for all procedures. Demographics, angiographic and procedural details are summarized in **table 5.1**.

5.4.2 Microvascular assessment

Mean time in minutes from restoration of antegrade flow in the CTO vessel to post-PCI microvascular assessment was 54.4±20.1 for the CTO/target vessel and 68.9±21.2 for the reference vessel. Post-PCI haemodynamic indices for the CTO vessel and reference vessel are detailed in **table 5.2.** We did not demonstrate a significant difference in HMR, however BMR was significantly lower in the target vessel compared with the reference vessel: difference -1.36mmHg/cm/s (-2.33 to -0.39, p=0.008). Although not well established, if an HMRv is ≤2.0 is considered normal[119], 14(67%) patients had a 'normal' target vessel HMR and 14(67%) patients had a 'normal' reference vessel HMR post CTO PCI. If a CFR of ≥2 is considered normal[111,112,114,128], 6(29%) patients had a 'normal' target vessel CFR and 10(48%) had a 'normal' reference vessel CFR.

Table 5.1. Baseline Characteristics, Angiographic, and Procedural Details

Demographics(n=21)	
Male, n(%)	18(85.7)
Age, years	60.9±10.9
Left ventricular ejection fraction(%)	57.3±10.2
Estimated occlusion duration(weeks)	54(30-87)
CCS class	
I	6(28.6)
II	11(52.4)
III	4(19.0)
Previous PCI, n(%)	9(26.5)
Previous myocardial infarction, n(%)	5(23.8)
Hypertension, n(%)	5(23.8)
Diabetes Mellitus, n(%)	2(9.5)
Current smoker, n(%)	7(33.3)
Angiographic details	
CTO vessel, n(%)	
RCA	15(71.4)
LCx	3(14.3)
LAD	3(14.3)
Reference vessel, n(%)	
RCA	1(4.8)
LCx	12(57.1)
LAD	8(38.1)
Reference vessel diameter stenosis(%)	30.6±12.9
Rentrop collateral grade, n(%)	
1	0(0)
2	8(38.1)
3	13(61.9)
CTO vessel CC grade, n(%)	
0	0(0)
1	14(66.7)
2	7(33.3)
Procedural details	
Number of stents, n(%)	
1	3(14.3)
2	6(28.6)
3	6(28.6)
4	6(28.6)
Length of stent(mm)	86(53-114)
Means of recanalisation	
Antegrade lumen-lumen, n(%)	12(57.1)
Antegrade dissection re-entry, n(%)	6(28.6)
Retrograde dissection re-entry, n(%)	3(14.3)

PCI indicates percutaneous coronary intervention; RCA, right coronary artery; LCx, left circumflex artery; LAD, left anterior descending artery.

Table 5.2 Haemodynamic assessment post CTO PCI

	Target Vessel	Reference Vessel	Difference (95% CI)	p-value
MAP (mmHg)	94.9±17.9	92.0±17.6	2.9 (-1.5 to 7.2)	p=0.19
Heart rate (beats/minute)	70.4±12.1	71.0±14.4	-0.61 (-3.7 to 2.5)	p=0.68
BMR ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	3.58±1.37	4.94±1.54	-1.36 (-2.33 to -0.39)	p=0.008
HMR ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	1.82±0.84	2.01±0.96	-0.20 (-0.78 to 0.39)	p=0.49
CFR	1.85±0.66	2.08±0.85	-0.23 (-0.76 to 0.29)	p=0.36
FFR	0.886±0.065	0.865±0.075	0.021 (-0.021 to 0.063)	p=0.30
Minimal instantaneous BMR ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	2.57±1.13	3.40±1.26	-0.83 (-1.62 to -0.04)	p=0.04
Minimal instantaneous HMRv ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	1.31±0.84	1.29±0.79	0.01 (-0.36 to 0.39)	p=0.94

CI indicates confidence interval; MAP, mean aortic pressure; BMR, basal microvascular resistance; HMRv, hyperaemic microvascular resistance; CFR, coronary flow vrlocity reserve; FFR fractional flow reserve.

Figure 5.2 depicts an example of the calculation of minimal instantaneous microvascular resistance at baseline and at hyperaemia. **figure 5.3** shows individual measurements for target/CTO and reference vessels. Under baseline conditions, instantaneous minimal microvascular resistance in the recently recanalised CTO vessel measured in $mmHg \cdot cm \cdot s^{-1}$ was 2.57±1.13 and was significantly lower when compared with a paired unobstructed reference vessel, which measured 3.40±1.26; difference -0.83 (95% CI -1.62 to -0.04, p=0.04). We did not detect a statistically significant difference in minimal instantaneous microvascular resistance between target and reference vessels at hyperaemia **(table 5.2).** The reduction in minimal instantaneous microvascular resistance as a result of adenosine infusion was greater in the reference vessel: $2.10±1.07 mmHg \cdot cm \cdot s^{-1}$ than the target vessel: $1.26±0.68 mmHg \cdot cm \cdot s^{-1}$; difference 0.84(95% CI 0.23 to 1.45, p=0.009).

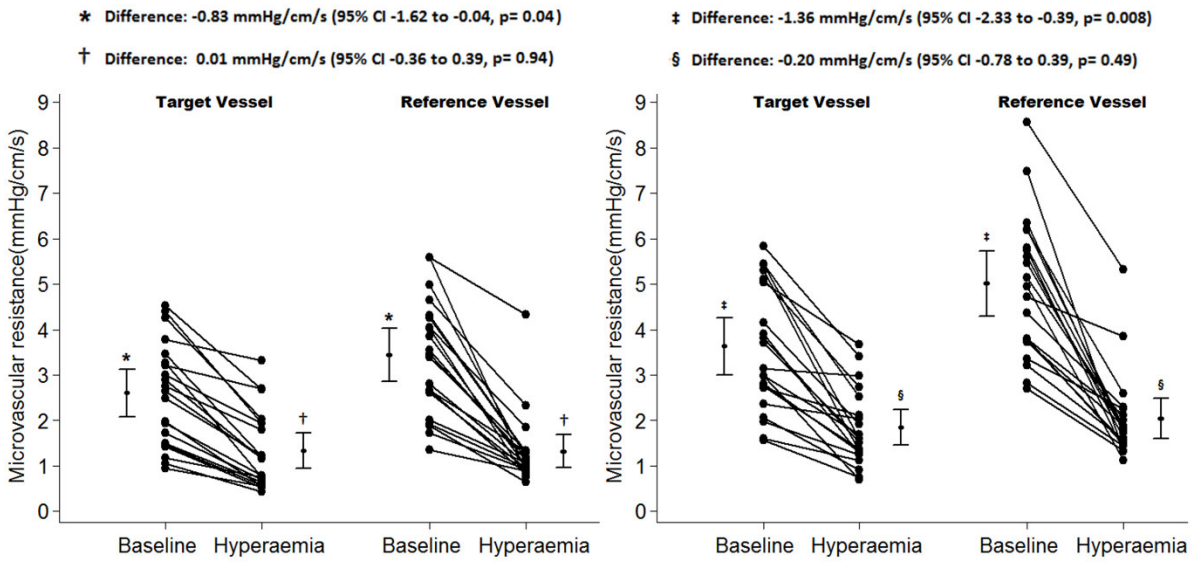

Figure 5.3 CTO/target vessel basal and hyperaemic microvascular resistance after PCI. Left graph: Instantaneous minimal microvascular resistance at baseline and hyperaemia for the CTO/target vessel (left) and an unobstructed reference vessel (right). Right graph: Using mean values, baseline and hyperaemic microvascular resistance (BMR and HMRv) for the CTO/target vessel (left) and an unobstructed reference vessel (right). Error bars represent 95% confidence intervals.

5.4.3 Determinants of post-PCI microvascular function

Any determinant of microvascular resistance should be related to the maximal vasodilatory capacity under either baseline or hyperaemic conditions, and therefore the minimal instantaneous microvascular resistance. We therefore examined the relationship between minimal instantaneous microvascular resistancve and invasively derived indices of pre-PCI collateral perfusion to the occluded segment, as well as length of stented segment (as a possible predictor of procedural microvascular injury).

It was possible to measure invasive indices of collateral function distal to the occlusion, prior to PCI in 19 of 21 patients, of which one was measured through a retrograde approach (FFR_{coll}: 0.50, Collateral flow velocity reserve: 1.02). We found no correlation between microvascular indices in the target vessel and invasive measures of collateral perfusion measured distal to the occlusion (**figure 5.4**).

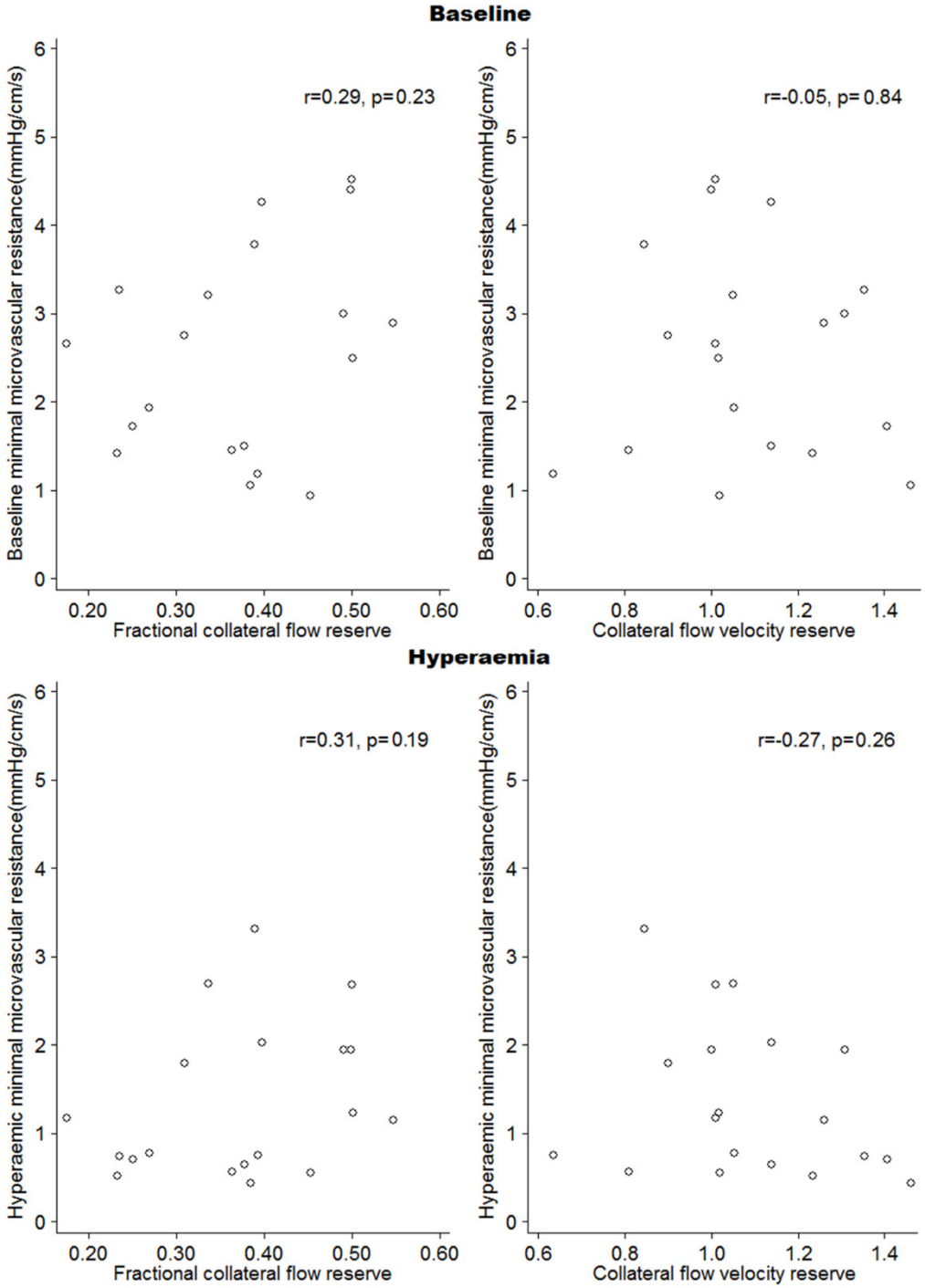

Figure 5.4 Relationship between pre-PCI collateral perfusion and post-PCI microvascular resistance CTO/target vessel instantaneous minimal microvascular resistance at baseline (top) and hyperaemia (bottom).

Target vessel minimal instantaneous microvascular resistance at both baseline and hyperaemia strongly correlated with length of stented segment in millimetres: baseline r=0.63, p=0.005; hyperaemia r=0.68, p=0.002 **(figure 5.5)**. A similar relationship was found using mean values

of microvascular resistance: BMR r=0.58, p=0.005; HMR_V r=0.58, p=0.005. There was no relationship between minimal instantaneous microvascular resistance and length of stented segment in the reference vessel: baseline r=0.21, p=0.36; hyperaemia r=0.36, p=0.11.

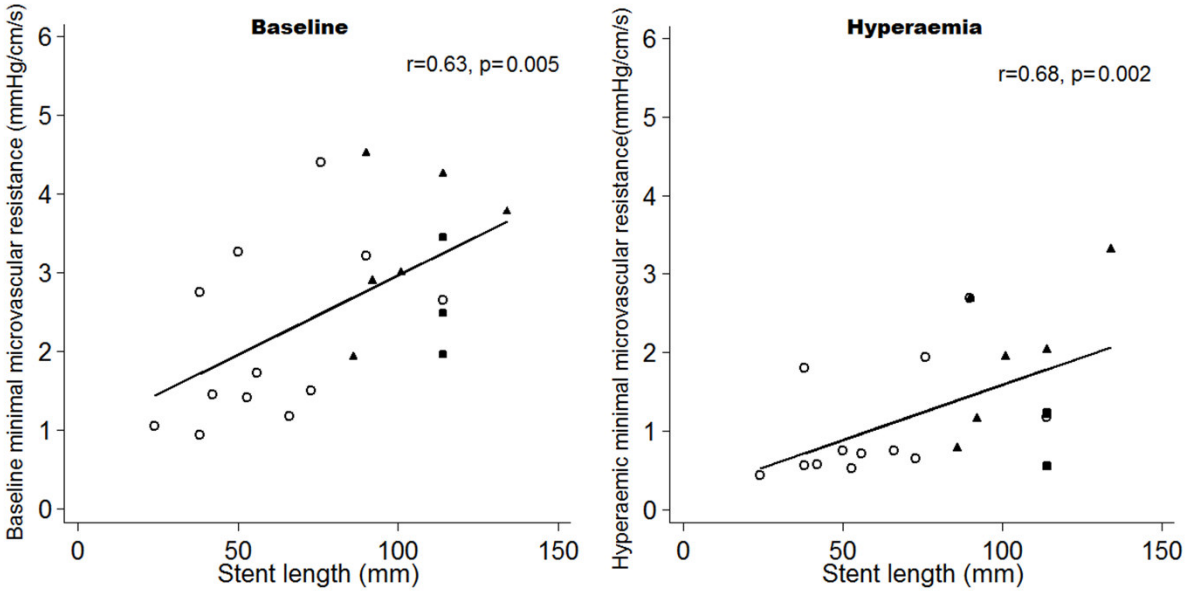

Figure 5.5 Relationship between length of stented segment in mm and CTO/target vessel instantaneous minimal microvascular resistance at baseline (left) and hyperaemia (right). Circles represent an antegrade lumen-lumen approach, triangles: antegrade dissection re-entry and squares: retrograde dissection re-entry.

5.5 Discussion

5.5.1 Summary of findings

By conventional values of microvascular normality the results of this study, consistent with previous studies[72,127], suggest that microvascular dysfunction is prevalent amongst recently recanalised CTOs. By the same definitions, this study demonstrates that high prevalence extends to global coronary microvascular dysfunction amongst patients with a CTO. We demonstrate that the microvasculature distal to a recently recanalised CTO behaves differently to the microvasculature in an angiographically unobstructed reference vessel in the same patient. In particular, the microvascular resistance under baseline conditions is lower in the recanalised CTO vessel relative to the reference vessel, however maximal vasodilatory capacity in response to adenosine appears to be preserved. There is a strong association between length of stented segment and

increased microvascular resistance both under baseline conditions and in response to adenosine infusion. We were not able to demonstrate any relationship between invasively derived indices of collateralisation prior to recanalisation of the CTO and target vessel microvascular resistance post PCI.

5.5.2 Prevalance of microvascular dysfunction

If a CFR <2 is used to define microvascular dysfunction, 71% of CTO vessels had abnormal microvascular function after PCI. A larger study by Werner et al reported a CFR of <2 in 46% of a total of 120 patients[72]. However, by the same definition 53% of patients in our study had microvascular dysfunction (CFR<2) in an angiographically unobstructed reference vessel. Werner reported that in a sizeable proportion of patients (26% of 120) CFR did not return to ≥2 at 5 month follow up[72]. Our results would suggest that these patients are likely to have had global coronary microvascular dysfunction, possibly in conjunction with diffuse but non-obstructive atheroma and had it been measured, would also have had a CFR <2 in a reference vessel at the time of PCI.

If the definition of an HMR_v >2 is used to define microvascular dysfunction, we found a lower prevalence of 33% in CTO vessels. Interestingly, the prevalence of an HMR_v >2 was also 33% in reference vessels. We did not find a statistically significant difference in HMR_v or instantaneous minimal microvascular resistance between target/CTO and reference vessels. It might be argued that the increased prevalence of reference vessel microvascular dysfunction is due to a global effect of PCI[181–183], however we have recently shown that in the non-target vessel donating least/no collaterals there is little change in indices of microvascular function before and after CTO PCI (**chapter 4.0**).

The HMR_v and CFR measure different aspects of the microvasculature. HMR measures the microvasculature's maximal vasodilatory capacity, which does not seem to be reduced relative to a reference vessel. Except for in the event of idiosyncratic peri-procedural

microvasculature insult, maximal vasodilatory capacity appears to return to what is normal for the patient immediately after CTO PCI. This is consistent with previous findings that hyperaemic absolute coronary flow in the CTO myocardial segment is no different at 24 hours compared with 6 months post PCI[29]; and maximal coronary flow velocity is no different immediately after PCI compared with at 5 months follow-up[72].

CFR incorporates baseline flow and therefore basal microvascular tone. The most striking abnormality we demonstrate of the behaviour of the CTO/target vessel microvasculature relative to the reference vessel microvasculature is the significant relative reduction in basal microvascular resistance.

5.5.3 Reduced basal microvascular tone

We report a significant reduction in BMR, minimal basal instantaneous microvascular resistance and the relative reduction in microvascular resistance in response to adenosine (as a result of lower basal microvascular resistance) in a recently recanalised CTO vessel compared with an unobstructed reference vessel (**table 5.2 & figure 5.4**)**.** This is in keeping with previous reports of increased coronary flow velocity immediately after CTO PCI[72,127], and is likely to represent impairment of the normal auto-regulatory mechanisms of the microvasculature to regulate coronary flow. Although collateral vessels are frequently sufficient to preserve myocardial viability[130], they are seldom (if ever) sufficient to prevent ischaemia under stress[45,92]. This is most likely because the microvasculature distal to a CTO is in a state of maximal vasodilatation in order to preserve basal myocardial perfusion, and is unable to dilate any further[46,94,95]. Both endothelium dependent and independent vasodilatation have been shown to be impaired in CTO vessels in the immediate aftermath of PCI[123,124], and return to normal at follow-up[123]. It is likely that the state of reduced basal microvascular tone that we demonstrate is related to the endothelial dysfunction of a chronically under-perfused vessel and we would expect it to normalise at a similar interval post-PCI. This state of

reduced basal microvascular tone results in increased coronary flow under baseline conditions and therefore increased pressure gradient across any epicardial stenosis. The instantaneous wave free ratio (iFR)[167], is a measure of stenosis severity taken as the mean ratio of Pd/Pa during the period of minimal microvascular resistance during diastole under baseline conditions only. Notwithstanding the dysfunctional nature of vasodilatory mechanisms in the recanalised epicardial segment of a CTO, optimization of the result using iFR[167], a pressure based index of stenosis severity measured under baseline conditions, is therefore likely to lead to an erroneous result given the (most likely transient) increased basal flow. In the absence of microvascular embolisation however, hyperaemic indices of stenosis severity are likely to be unaffected.

5.5.4 Relationship with pre-PCI collateral perfusion

We have not identified a relationship between indices of collateral perfusion prior to CTO PCI and target vessel/CTO microvascular resistance after PCI (**figure 5.4**). A previous study demonstrated greater coronary endothelial dysfunction in the recently recanalised segment of CTOs in patients who had previously had less well collateralised occluded segments as assessed by the CC grade[124]. However, this finding may have reflected individual patient's ability to form collaterals by arteriogenesis, which is an endothelium dependent process[130]. One might imagine that improved collateral perfusion prior to recanalisation might result in less marked microvascular abnormalities post-recanalisation. The effect of other variables, in particular the length of the stented segment on microvascular resistance makes this difficult to investigate further in such a small patient population.

5.5.5 Relationship with length of stented segment

We demonstrate a strong relationship between length of stented segment and microvascular resistance both at baseline and hyperaemia with higher microvascular resistance associated with a longer stented

segment (**figure 5.4**). It seems likely that this is related to increased risk of micro-embolization and microvascular injury as the treated segment increases in length. The finding is consistent with a previous study which demonstrated increased risk of peri-procedural myocardial infarction (MI4a) with a longer stented segment in non-occlusive disease[184]. Contemporary techniques of dissection/re-entry tend to result in longer stented segments, but also involve greater disruption to the vascular architecture than a lumen-lumen approach which could conceivably lead to more distal embolization. The greater vascular disruption of the dissection re-entry approach might explain this finding; however the same trend with stent length seems to occur amongst the lumen-lumen group. In either case, it would appear that a dissection re-entry approach (usually involving a longer stented segment) is associated with peri-procedural microvascular impairment which may not be apparent angiographically (all study patients had TIMI III flow at the time of haemodynamic measurement). It remains to be seen if this finding has any implication with respect to clinical outcomes.

The total number of study participants was small with a low rate of diabetes. We have therefore not been able to examine clinical characteristic such as smoking, hypertension and diabetes that might be related to microvascular dysfunction as previously reported[127].

5.5.6 Clinical implications

Microvascular resistance under baseline conditions remains abnormally low approximately 1 hour after recanalisation of a CTO, but maximal vasodilatory capacity appears to remain unchanged. If physiological lesion assessment is considered in this setting, the use of 'resting' indices such as iFR are likely to overestimate lesion severity due to relative hyperaemia. Longer stented segments and dissection re-entry techniques may be associated with greater microvascular injury, larger studies are required to investigate this further and establish any clinical significance.

5.5.7 Limitations

This is a single centre study, with a small number of patients. However, it is the first to make comparison of microvascular resistance after CTO PCI in the target and an unobstructed reference vessel. The study population had a preponderance of right coronary CTOs and the reference vessel was more commonly a branch of the left coronary artery. Although this could have introduced confounding into our results, it does not seem plausible that it could account for our major findings.

By protocol, target/CTO vessel haemodynamics were assessed earlier than the reference vessel, with a mean interval of 14.5 minutes between measurements. Although it would be preferable to have taken measurements at the same interval after restoration of antegrade flow, we consider it unlikely to have altered our results.

We did not incorporate collateral flow into our calculation of microvascular resistance. However it has been shown previously that in vessels with an FFR >0.60, the effect of collateral flow on HMRv is minimal[88]. All interrogated vessels in this study had an FFR well in excess of 0.60.

Where possible we selected the vessel angiographically donating least/no collaterals to the CTO vessel. Receipt of collaterals rapidly diminishes after CTO PCI[63,64] and collateral donation falls at a similar interval with little effect on the microvasculature of the non-target vessel donating least/no collaterals (**chapter 4.0**). It is plausible that the presence of minimal collaterals may have had some effect on our measurements, however we consider it unlikely to have altered our results significantly.

We would imagine that microvascular resistance under baseline conditions in the CTO/target vessel would increase to a level similar to the reference level after an interval. However, we did not repeat assessments at follow-up and further studies would be required to confirm and describe this.

5.5.7 Conclusions

Microvascular resistance under baseline conditions is reduced in a recanalised CTO in the immediate aftermath of PCI when compared to an unobstructed reference vessel; however hyperaemic microvascular resistance appears to be similar. Although microvascular dysfunction is common in CTO vessels after recanalisation, the dysfunction appears to be abnormal autoregulation of microvascular tone (or a continued hyperaemic response to coronary occlusion in spite of recent recanalisation) with a preserved maximal vasodilatory capacity. A longer stented segment is associated with increased microvascular resistance both under baseline conditions and at hyperaemia. The abnormal microvascular conditions in this setting should be considered prior to considering physiological lesion assessment in the immediate aftermath of CTO PCI.

6.0 Biomarkers of Coronary Endothelial health: correlation with invasive measures of collateral function, Flow and Resistance in Chronically Occluded Coronary Arteries and the Effect of Recanalisation

6.1 Abstract

6.1.1 Objectives

In the presence of a chronically occluded coronary artery, the collateral circulation matures by a process of arteriogenesis, however there is considerable variation between individuals as to the functional capacity of that collateral network. This could be explained by differences in endothelial health and function. We endeavoured to examine the relationship between functional extent of collateralisation and levels of biomarkers which have been shown to relate to endothelial health.

6.1.2 Methods

We measured four potential biomarkers of endothelial health in 34 patients with mature collateral networks who underwent successful percutaneous coronary intervention (PCI) to a chronic total coronary occlusion (CTO) prior to PCI and 6-8 weeks post-PCI and examined the relationship of biomarker levels with physiological measures of collateralisation.

6.1.3 Results

We did not demonstrate a significant change in systemic levels of sICAM-1, sE-selectin, microparticles or tissue factor 6-8 weeks after PCI. We did demonstrate an association between estimated retrograde collateral flow prior to CTO recanalisation and lower levels of sICAM-1 ($r=0.39, p=0.026$), sE-selectin ($r=0.48$, $p=0.005$) and microparticles ($r=0.38$, $p=0.03$).

6.1.4 Conclusions

Recanalisation of a CTO and resultant regression of a mature collateral circulation does not alter systemic levels of sICAM-1, sE-selectin, microparticles or tissue factor. The possible relationship of retrograde collateral flow with sICAM-1, sE-selectin and microparticles is likely to

represent an association with an ability to develop collaterals rather than their presence and extent.

6.2 Introduction

In response to a chronically occluded coronary artery, coronary collaterals develop which supply the myocardium distal to the occlusion and are often sufficient to preserve resting left ventricular systolic function in spite complete coronary occlusion[50,53]. Our current understanding is that growth of a network of pre-existing anastamotic arterioles, termed arteriogenesis, occurs independently of an ischaemic stimulus by means of mechanical transduction of endothelial shear stress, stimulating a cascade of growth factors and inflammatory mediators[130]. However there is considerable variability to the extent of functional collateral supply that individuals develop[131]. A number of studies have suggested associations between biomarkers thought be involved in the arteriogenic process and functional collateralisation[133–136,139,151]. However, the significance of any difference in biomarkers levels between individuals with 'good' and 'poor' functional collateralisation is not clear. Any identified associations may reflect a direct relationship between extent of collateralisation and the biomarker in question, a relationship between biomarker levels and endothelial or circulatory health, or a relationship with active arteriogenic activity (which could mean biological factors active in the process could be higher in individuals with less well developed or less well matured collateral circulations).

Both ICAM-1 and E-selectin have been implicated in the arteriogenic process[149–151]. Microparticles are thought to play an important role in endothelial function, and may relate to endothelial health[185], and tissue factor has been shown in vitro to be involved in the regulation of both endothelial[152] and smooth muscle cell[153] proliferation, both of which are thought to be integral to the arteriogenic process.

Previous studies have examined biomarkers such as vascular endothelial growth factor-1 (VEG-F) or hypoxia inducible factor 1 alpha (HIF-1α) more associated with angiogenesis; that is the formation of new blood vessels, rather than arteriogenesis; the maturation of pre-existing connections. Our current understanding of the formation of collaterals is that it is an arteriogenic process[186] and I therefore elected not to study these.

We endeavoured to investigate the relationship of these four potential biomarkers with indices of collateral flow, resistance and functional collateralisation in a population undergoing successful PCI to a CTO, all with well established collateral networks. Serum biomarkers were measured at the time of the procedure and at follow-up to investigate the effect of the presence of an active collateral network on biomarker levels.

6.3 Methods

6.3.1 Study patients

A cohort of 34 patients recruited consecutively into a coronary haemodynamic study (described in **chapter 4.0**), who underwent successful PCI to a CTO for symptoms of angina (Canadian Cardiovascular Society (CCS) class 1-3) in a single tertiary centre between January 2013 and June 2014. A CTO was defined as complete coronary occlusion of ≥3 months duration with TIMI grade 0 flow[1]. Exclusion criteria were inability to provide consent, >1 occluded vessel, prior CABG with any patent grafts, left main stem stenosis considered to be haemodynamically significant and contra-indications to adenosine. All included patients had right dominant coronary anatomy. The presence of viable myocardium in the CTO territory was confirmed in all patients by myocardial perfusion scintigraphy(n=26, 76.5%), dobutamine stress echocardiography(n=1, 2.9%) or by the absence of a wall motion abnormality by echocardiography or left ventricular angiography without additional confirmation(n=7, 20.6%). Patient's usual medications were continued and they were asked to abstain from caffeine for 48 hours prior to the procedure.

6.3.2 Ethics

The study protocol was approved by the local research ethics committee (12/YH/0360). All subjects provided written informed consent.

6.3.3 Catheter laboratory protocol

Dual arterial access was used for all procedures. Femoral venous access was obtained for central administration of adenosine and measurement of right atrial pressure(RAP) at the beginning and end of the procedure using a catheter positioned in the right atrium.

Before PCI and at the end of the procedure, 20ml of blood was aspirated from the catheter in the right atrium. At the same time as the pre-PCI right atrial sampling, 20ml of blood was aspirated from the femoral arterial sheath. At follow-up, 6-8 weeks after the successful procedure, 20ml of blood was sampled from a peripheral vein.

After initial blood sampling, patients were anti-coagulated with 100 U/kg of unfractionated heparin to maintain an activated clotting time of >300 seconds. A 200mcg bolus of intra-coronary glyceryl trinitrate(GTN) was given and iso-centred coronary angiograms of both non-target vessels were taken.

Briefly, a dual sensor pressure-velocity 0.014” intracoronary wire (Combowire, Volcano Corp, San Diego, CA)[87] was connected to a ComboMap console (Volcano Corp) and used for haemodymamic measurements. In order to estimate absolute coronary flow in the non-target vessels, the wire was advanced to the segment of the non-target vessel proximal to any major side-branches and manipulated to obtain a good Doppler trace. After administration of 100mcg intra-coronary GTN, once the hyperaemic response had settled, continuous recordings from the ComboMap were taken. Samples were recorded at 200Hz and stored on disk for offline analysis.

PCI of the CTO was undertaken at the discretion of the treating interventional cardiologist using an antegrade or retrograde approach. Once access to the vessel lumen distal to the point of occlusion was

achieved, prior to restoration of antegrade flow, a microcatheter was placed into the distal vessel to facilitate delivery of the Combowire. The wire was normalised to aortic pressure at the tip of the catheter, alongside the microcatheter, removed and advanced through the microcatheter to the distal segment of the target (CTO) vessel and manipulated to obtain a good Doppler trace. After administration of 100mcg intra-coronary GTN, once the hyperaemic response had settled, continuous recordings from the ComboMap were taken. Hyperaemia was achieved by central venous administration of adenosine at 140mcg/kg/minute. Once steady state hyperaemia had been reached and a continuous recording of ≥20 beats taken, adenosine infusion was ceased. Samples were recorded at 200Hz and stored on disk for offline analysis.

PCI success was defined as stenting of the target vessel with <30% residual stenosis and thrombolysis in myocardial infarction (TIMI) grade III flow. After successful PCI, non-target and target vessel haemodynamic measurements were repeated as described pre-procedure, at the site of the previous measurement.

Recorded data was analysed using dedicated custom software (Study Manager, Academic Medical Center, University of Amsterdam, The Netherlands)

6.3.4 Angiographic assessment

Proximal non-target vessel diameters (at the point of proximal haemodynamic measurement) measured in two orthogonal views were calculated by two independent observers using quantitative coronary angiography (QCA)(GE Centricity CA1000, GE Healthcare) using the guiding catheter luminal diameter as reference. Mean values from both observers were used for analysis. The vessel collateral connection(CC) grade[90] and modified Rentrop score[89] were assessed by two independent observers blinded to haemodynamic measurements and agreed by consensus.

6.3.5 Data analysis

Fractional collateral flow reserve was calculated as (Pd-RAP)/Pa-RAP), using mean pressures taken over 5 cardiac cycles at stable hyperaemia[166], with Pd measured in the occluded segment of the artery, prior to restoration of antegrade flow[64]. Collateral flow velocity reserve was calculated with flow velocities in the occluded segment measured at rest and steady state hyperaemia[95] as APV at steady state hyperaemia divided by APV at baseline, measured over 5 cardiac cycles. The resistance index of the collateral supply pathway, incorporating both the resistance of the collateral vessel and of the donor segment proximal to the collateral take-off was calculated as: $R_{Coll}=(P_{Ao}-P_d)/APV_d$ ($mmHg \cdot cm^{-1} \cdot s^{-1}$)[133,187]. The peripheral resistance index, or the resistance in the target (collateral recipient) vessel downstream of the collateral supply was calculated as: $R_P=P_d/APV_d$ ($mmHg \cdot cm^{-1} \cdot s^{-1}$)[133,187].

The difference in combined non-target vessel absolute flow before and after successful CTO PCI should approximate to absolute flow through the retrograde collateral network (although would not include the contribution of antegrade bridging collaterals). For the purposes of estimating non-target vessel flow, absolute coronary flow was estimated as (π x proximal vessel radius^2)x(proximal vessel APV/2)[170,171]. As resting absolute myocardial blood flow is closely related to rate pressure product(RPP), values for resting absolute coronary flow were divided by the respective RPP/10,000[172].

6.3.6 Biomarker assays

For the measurement of sICAM-1 and sE-selectin, blood samples were drawn into serum separator tubes, allowed to clot for 30 minutes prior to centrifugation and centrifuged for 15 min at 1000*g*. The separated serum was stored in aliquots at −80°C to permit assay in batches. The concentration of sE-selectin and sICAM-1 were determined using a commercially available specific sandwich enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D Systems). Briefly, ELISA plates pre-

coated with a specific capture antibody were used, standards and samples were added to the plate and incubated for 2 hours at room temperature. The bound soluble adhesion molecule of interest was detected by a further 2 hour incubation with a specific antibody conjugated to horseradish peroxidise followed by a 30 minute incubation with stabilized hydrogen peroxide and tetramethylbenzidine. The reaction was stopped by the addition of 1 M sulphuric acid to each well and the optical density at 450nm measured using a microplate reader (BMG Labtech, Aylesbury, United Kingdom).

For the measurement of plasma microparticles blood samples were drawn into vacutainer tubes containing 0·11 mol/l sodium citrate, centrifuged for 15 minutes at 1,500*g* at room temperature. Plasma supernatant was then rapidly centrifuged for 2 minutes at 13,000*g.* The separated 'platelet free' plasma was stored in aliquots at −80°C to permit assay in batches. Microparticles concentrations were determined using a commercially available specific ELISA kit (ZYMUPHEN MP-Activity, Hyphen BioMed, Quadratech, Epsom, United Kingdom). Briefly, the diluted plasma sample or standard, supplemented with calcium, Factor Xa and thrombin inhibitors was added to a microplate pre-coated with Streptavidine and biotinylated Annexin V and incubated at 37°C for 1 hour. After a washing step, a Bovine factor Xa-Va mixture containing calcium and purified human Prothombin are added to each well and incubated for a further 10 minutes at 37°C. Thrombin specific chromogenic substrate was then added to each well, after 3 minutes 2% Citric acid was added as a stop solution to each well and optical density at 405nm measured using a microplate reader (BMG Labtech).

For the measurement of plasma TF levels, blood samples were drawn into vacutainer tubes containing 0·11 mol/l sodium citrate, centrifuged for 15 minutes at 3,000*g* and stored in aliquots at −80°C to permit assay in batches. TF was measured by ELISA. Briefly, microtitre ELISA plates were coated overnight with a specific TF capture antibody (sheep anti-Human Tissue Factor, Enzyme Research Laboratories, Swansea, United Kingdom)

in 50 mmol carbonate buffer pH = 9.6. The plates were then incubated overnight at 4°C with PBS/Bovine serum albumen blocking buffer. After a washing step, standards (diluted recombinant tissue factor, American Diagnostica inc, Stanford, USA) and samples were added to the plate and incubated for 90 minutes at room temperature. After another washing step, anti-TF IgG conjugated to horseradish peroxidise was added to the plates and incubated for 90 minutes at room temperature. The plates were washed again and a tetramethylbenzidine substarte solution added and agitated at room temperature for 15 minutes. The reaction was stopped by the addition of 2 M sulphuric acid to each well and the optical density at 490nm measured using a microplate reader (BMG Labtech).

The intra-assay coefficient of variation of the ELISA measurements was 9.2% for sICAM-1, 9.3% for sE-selectin, 9.5% for microparticles and 13.8% for TF. Inter-assay coefficients were 7.1% for sICAM-1, 12.9% for sE-selectin, 11.6% for microparticles and 14.7% for TF.

6.3.7 Biomarker comparisons and correlations

To establish if biomarkers levels in the right atrium were elevated relative to sample sites remote from the heart, right atrial biomarker levels were compared with levels taken from the femoral arterial sheath prior to PCI. To investigate if there had been a change in biomarker levels between prior to PCI and follow-up, right atrial biomarker levels taken prior to PCI were compared with those from a peripheral venous sample taken at follow-up. Correlations were tested between right atrial biomarker levels and FFRcoll, CollFVR, the change in non-target baseline flow after PCI (Δcombined non-target vessel baseline flow, an approximation of retrograde collateral flow after PCI), R_{Coll} and R_P.

6.3.8 Statistical analysis

Stata v.12(StataCorp) was used for statistical analysis. Continuous values are expressed as means±SD, or median(25^{th} percentile-75^{th} percentile) as appropriate. Continuous variables were compared using a

paired t-test or Wilcoxon signed-rank test. Correlations were quantified using Pearson's correlation coefficient. Probability values were 2-sided, and values of $p<.05$ considered significant.

6.4 Results

6.4.1 Overview

Pre-procedural biomarker levels were taken in all 34 successful cases, follow-up levels were taken at between 6 and 8 weeks post PCI in 30. Drug-eluting stents were used for all procedures. Demographics, angiographic and procedural details are shown in **table 6.1**.

Table 6.1 Baseline Characteristics, Angiographic, and Procedural Details

Demographics(n=34)	
Male, n(%)	27(79.4)
Age, years	60.8±9.6
Left ventricular ejection fraction(%)	56.2±11
Estimated occlusion duration(weeks)	53(30-104)
CCS class I/II/III/IV	8/20/6/0
Previous PCI, n(%)	9(26.5)
Previous myocardial infarction, n(%)	10(29.4)
Hypertension, n(%)	6(17.7)
Diabetes Mellitus, n(%)	5(14.7)
Current smoker, n(%)	10(29.4)
Angiographic details	
CTO vessel(RCA/LCx/LAD)	21/4/9
Rentrop collateral grade(1/2/3)	0/12/22
Maxiumum CC grade(0/1/2)	0/18/16
Procedural details	
Number of stents 1/2/3/4/5	6/11/10/6/1
Length of stent(mm)	74.5(44-101)
Means of recanalisation	
Antegrade lumen-lumen, n(%)	19(55.9)
Antegrade dissection re-entry, n(%)	9(26.5)
Retrograde lumen-lumen, n(%)	3(8.8)
Retrograde dissection re-entry, n(%)	3(8.8)

PCI indicates percutaneous coronary intervention; ACE-inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; RCA, right coronary artery; LCx, left circumflex artery; LAD, left anterior descending artery

6.4.2 Haemodynamic measures of functional collateralisation

Table 6.2 lists summary statistics for measures of functional collateralisation, collateral flow and resistance. Measures of collateral perfusion are only included if the vessel distal to the occlusion was accessed antegradely (n=28). As one of those patients had inadequate flow traces, combined pressure and flow measurements taken distal to the occlusion are only included in 27 patients. Pre and post PCI donor vessel flow measurements were possible in 32 of 34 patients. Mean combined absolute baseline flow in both non-target vessels, adjusted for rate pressure product reduced from 335.0 $ml \cdot min^{-1}$ prior to PCI to 281.3 $ml \cdot min^{-1}$ post-PCI (difference -53.8 $ml \cdot min^{-1}$, 95% CI -93.9 to -13.6, p=.01). Mean combined absolute hyperaemic flow in both non-target vessels reduced from 580.8

ml· min^{-1} prior to PCI to 543.6 ml· min^{-1} post-PCI (difference -37.2 ml· min^{-1}, 95% CI -75.5 to 0.1, p=0.056).

Table 6.2 Haemodynamic measures of functional collateralisation

FFRcoll*	**0.38±0.12**
CollFVR†	**1.07±0.25**
R_{Coll}† ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	**4.87 (2.82-7.04)**
R_P† ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	**4.85 (3.71-6.66)**
R_{Coll} Hyperaemia† ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	**4.94 (2.88-7.77)**
R_P Hyperaemia† ($mmHg \cdot cm^{-1} \cdot s^{-1}$)	**3.39 (2.09-5.26)**
Δcombined non-target vessel baseline flow§ (ml· min^{-1})	**-53.8±117.8**
Δcombined non-target vessel hyperaemic flow§ (ml· min^{-1})	**-37.2±106.0**

***n=28, †n=27, §n=32; FFRcoll indicates Fractional collateral flow reserve; CollFVR, collateral flow velocity reserve; R_{Coll}, resistance of the collateral vessel; R_P, resistance in the collateral recipient vessel downstream of the collateral supply.**

6.4.3 Biomarker levels

Levels of all four measured biomarker levels are depicted in **figure 6.1**. We found no veno-arterial gradient in levels of any of the biomarkers. Biomarker levels taken at follow up from a peripheral vein were also not significantly different from arterial samples taken immediately prior to PCI when the collateral pathway was still 'active'.

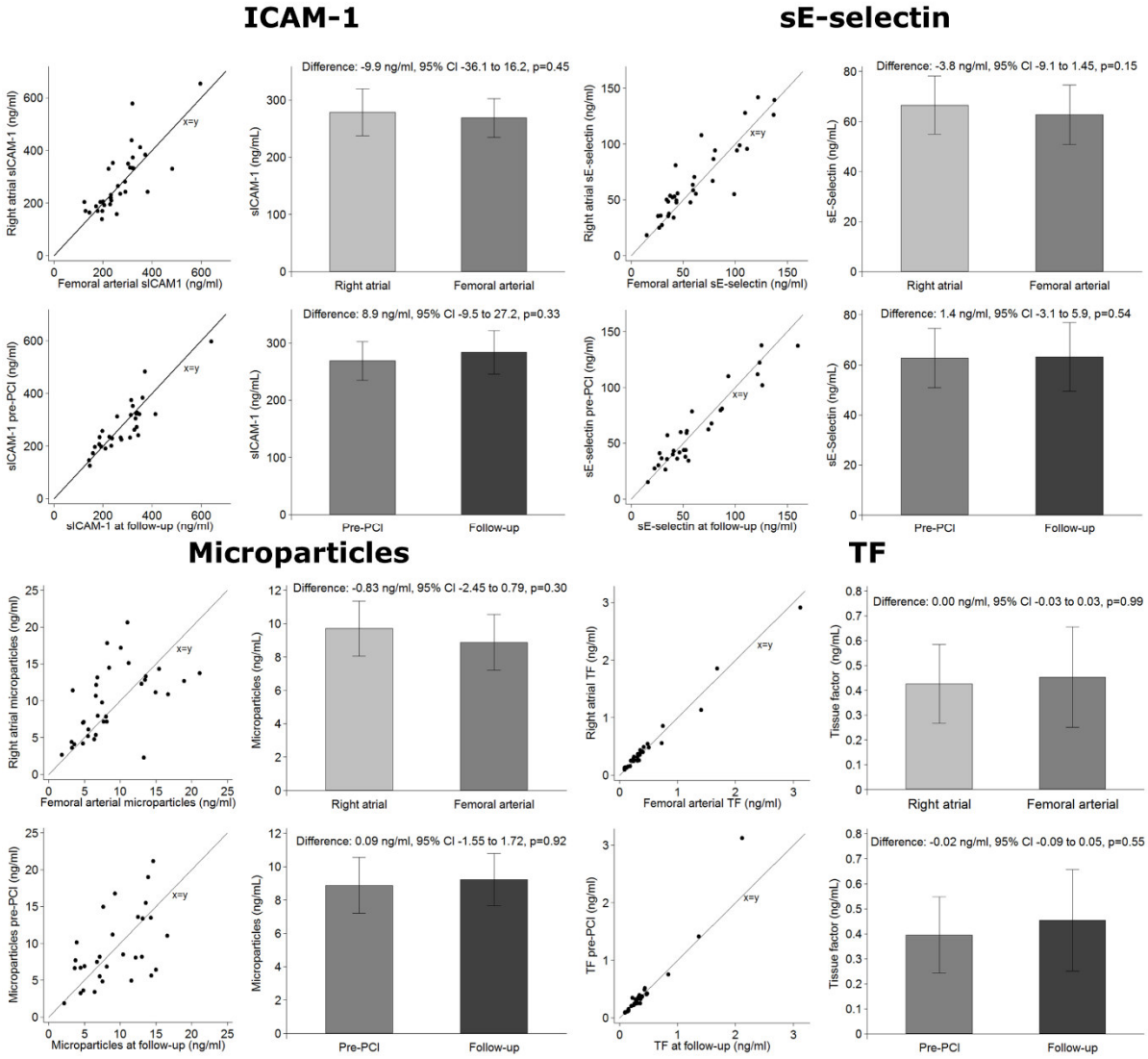

Figure 6.1 Comparison of right atrial with femoral arterial biomarker levels pre-PCI, and pre-PCI biomarker levels (Femoral arterial) with levels at follow-up (peripheral venous). Top left: sICAM-1, top right: sE-selectin, bottom left: microparticles, bottom right: tissue factor (TF).

Figure 6.2 shows the relationship between pre-procedural right atrial biomarker levels and measures of collateral perfusion. Although we did not demonstrate a relationship between any of the biomarkers and measures of collateralisation taken distal to the point of occlusion prior to recanalisation, we did demonstrate a significant correlation between the change in baseline non-target vessel flow and levels of sICAM-1, sE-selectin and microparticles. We found no correlation between any biomarker and measures of collateral pathway resistance R_{coll} (sICAM-1 r=0.10, p=0.57; sE-selectin r=0.14, p=0.46; microparticles r=0.16, p=0.39; TF r=-0.06,

p=0.75) or the resistance of the collateral recipient vessel downstream of the collateral supply R_P (sICAM-1 r=0.09, p=0.65; sE-selectin r=-0.18, p=0.33; microparticles r=0.09, p=0.64; TF r=-0.15, p=0.44).

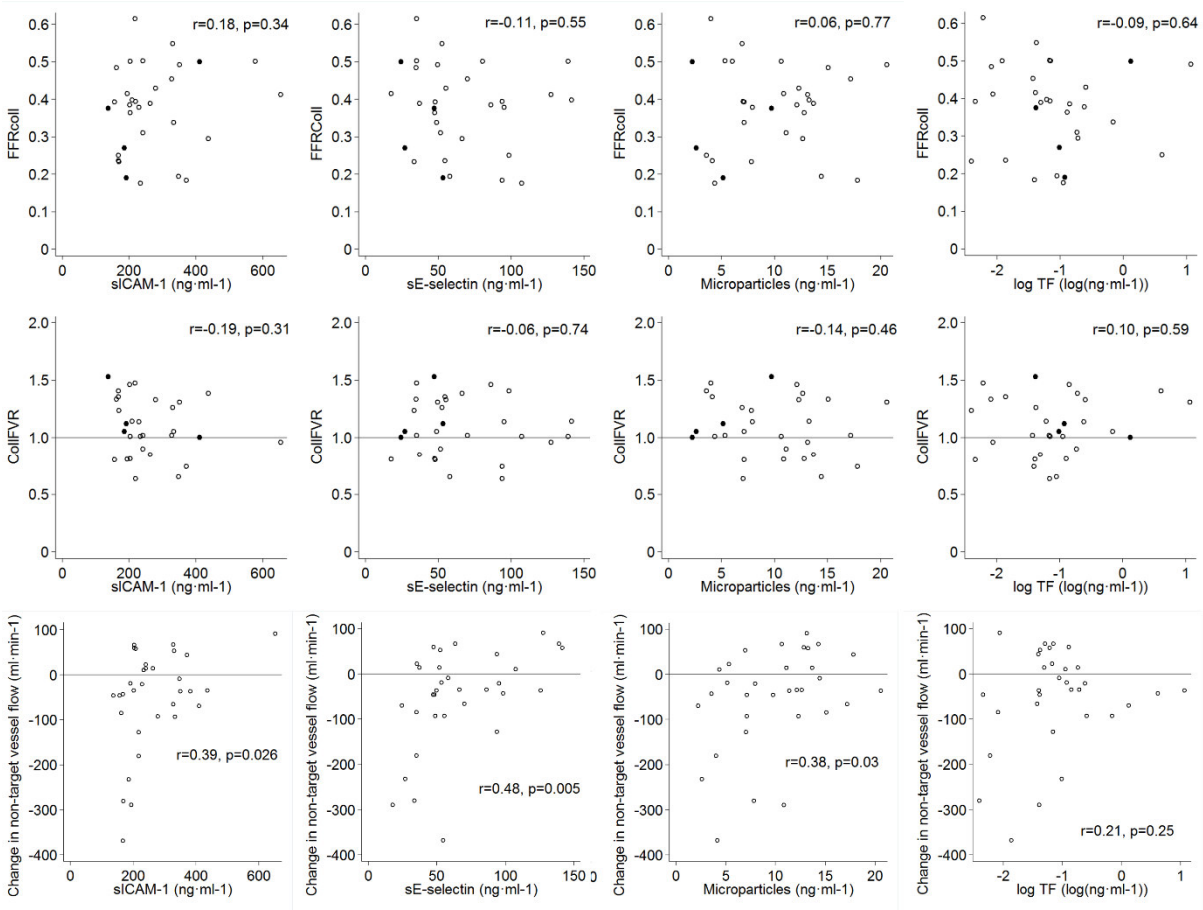

Figure 6.2 Relationships between biomarkers levels measured in the right atrium prior to PCI: sICAM-1, sE-selectin, microparticles and tissue factor (TF); and measures of collateral perfusion: fractional collateral flow reserve (FFRcoll, n=31), Collateral flow velocity reserve (CollFVR, n=30), and summed change in absolute baseline flow, adjusted for rate pressure product after CTO PCI (n=32). TF levels are transformed logarithmically. Solid markers represent measures taken by a retrograde approach.

6.5 Discussion

6.5.1 Summary of findings

In a population with established extensive collateral networks, with a median duration of coronary occlusion in excess of 1 year, I have demonstrated no significant reduction in systemic sICAM-1, sE-selectin, microparticle or TF levels at 6-8 weeks after CTO recanalisation. I have also not demonstrated a gradient in levels of these biomarkers between the right atrial and femoral arterial circulation when the collateral network was

still present and 'active'. In addition, we have found no association between established guidewire based indices of collateral perfusion (FFRcoll and CollFVR) and biomarker levels. We have however demonstrated an association between change in summed non-target CTO vessel flow and levels of sICAM-1, sE-selectin and microparticles, which may represent an association between retrograde collateral absolute flow prior to CTO recanalisation and each biomarker.

6.5.2 The absence of a veno-arterial gradient

We found no significant difference in any biomarker levels if they were sampled in the right atrium compared with samples taken remote from the heart through the femoral arterial sheath. This suggests peripherally taken samples at follow up are representative of levels in the right atrium. A limitation of the current study is that we did not take samples from the coronary sinus, or beyond the occluded segment. It is possible that levels of biomarkers taken in this way may have been higher than in the systemic circulation.

6.5.3 No reduction in biomarker levels at follow-up

In previous studies which have demonstrated relationships between collateral perfusion (whether functional or angiographic) and biomarker levels[133–136,139,151], including some of the biomarkers measured here[151], a more heterogeneous patient population was included than in this study. In those studies which included patients with non-occlusive disease, lesions of differing physiological severity must have been included[134–136,139,151]. A difference in basic fibroblast growth factor has been identified in coronary occlusions of <3 months duration compared with those of ≥3 months in duration (what we would now consider to be a CTO[1]). It is conceivable that other growth factors implicated in the arteriogenic process might show a similar relationship with duration of occlusion. The population included in this study all had a well developed collateral circulation with Rentrop grade 2-3 and CC grade 1-2 angiographic collateralisation in all patients and

coronary occlusion which had been present for at least 3 months (median 53 weeks). If biomarker levels related directly to extent of collateralisation, then one would expect that after the chronically occluded vessel is recanalised, that biomarker levels would fall. This was not the case in any of the four biomarkers that we measured.

Extent of collateralisation does not necessarily reflect level of arteriogenic activity. In fact, a less well developed collateral circulation may reflect more recent coronary occlusion or progression of flow limitation caused by a coronary stenosis. The various differences in biochemical activity that have been reported between individuals of differing degrees of collateralisation may reflect an active arteriogenic process in the less well collateralised patients. Our results, showing no difference in biomarker levels 6-8 weeks after recanalisation in a population with well established collaterals would be in keeping with, but are not necessarily supportive of that hypothesis. By the same token, the absence of a fall in biomarker levels on recanalisation of a CTO with an established collateral network, does not mean that biomarker was not important during the arteriogenic process.

In ICAM-1 and sE-selectin we chose to investigate two biomarkers which appear to have a clear role in arteriogenesis[130,150]. Both are involved in the adhesion of macrophages to shear-stress activated collateral endothelium, an important step in the arteriogenic process and ICAM-1 expression has been shown to be stimulated by increased shear stress[149]. In animal models, arteriogenesis is reduced in the presence of ICAM-1 deficiency[150]. Both sICAM-1 and sE-selectin have been shown to be at higher levels in patients with a lower angiographic grade of collateral[151]. Our finding that their level did not decrease after CTO recanalisation would suggest that any relationship between extent of collateralisation does not relate to the presence of an established collateral circulation, but more likely the ability to form one.

6.5.4 Biomarker levels: relationship with collateral flow

We were unable to demonstrate a relationship between invasively derived measures of collateral perfusion and resistance and any of the four biomarkers we tested (**figure 6.2**). One might expect functional measures of collateralisation to be more reliable than angiographic measures and a relationship therefore easier to identify[90]. However, the number of patients in our study was small and even excluding measures in which the collateral dependent vessel was accessed via retrograde collaterals; antegrade access was gained by a dissection re-entry approach in a sizeable proportion of patients, which may have altered measures of collateral perfusion. Regardless of approach to CTO recanalisation, collateral flow diminishes rapidly after restoration of antegrade flow[63,64]. The change in flow in the other major epicardial arteries should represent an approximation of retrograde collateral flow in the presence of a CTO. Given the limitations of multiple testing, the correlations that we have identified between change in donor vessel flow and levels of sICAM-1, sE-Selectin and microparticles can only be viewed as hypothesis generating. Nevertheless, the presence of a similar correlation between three of the four biomarkers that we have studied is interesting and is consistent with previous published findings[151].

6.5.5 Markers of endothelial health: a possible explanation

Arteriogenesis is a complex, endothelium dependent process and it is perhaps too much to expect a single biomarker to reflect arteriogenic activity or capacity. The levels of biomarkers that we have investigated have all been shown to be related to what could be considered to be endothelial health[142–146,152], lower levels of each being associated with a more 'healthy endothelium'. Angiographic extent of collateralisation has previously been shown to be associated with endothelial function in recanalised segments of CTOs[124]. This may well extend to an association between global coronary (and possibly systemic) endothelial health and the ability for collateral maturation. The various studies (including this one)

which have demonstrated an association between various biomarkers levels and extent of collateralisation, may be indirectly describing the same link between endothelial health and arteriogenic capacity.

6.5.6 Limitations

This is a single centre study, with a small number of patients. However, the population all had well developed collateral networks and we made comparison to biomarker levels both in the presence and absence of a collateral network supplying an occluded coronary segment and also to coronary haemodynamics associated with that change.

It is possible that biomarker levels taken from the coronary sinus or occluded coronary segment (through a microcatheter) may have been higher than in the right atrium or systemic circulation. However a number of studies have related systemic levels of biomarker to collateral development[134–136,151], so these measures remain pertinent. In addition, without systemic measures of biomarkers, it would not have been possible to take samples at follow-up for comparison.

An important limitation is that we tested for biomarker correlations with multiple physiological indices and these correlations should therefore be interpreted with caution. The significant correlations we have identified are related to one another however and have a plausible unifying biological explanation. Finally, our estimate of retrograde collateral flow ignores any antegrade bridging collaterals; this might have resulted in an underestimation of collateral flow in some patients.

6.5.7 Conclusions

In patients with established collateral circulations supplying viable myocardium distal to CTOs, systemic levels of ICAM-1, sE-selectin, microparticles and TF do not reduce at follow up after recanalisation of the CTO. Keeping in mind the limitations of multiple testing, levels of ICAM-1, sE-selectin, microparticles may be related to estimated total retrograde

collateral flow prior to recanalisation, which could be explained by an association with the ability to develop a collateral supply.

A possible biological explanation is that those with greater endothelial health have a greater capacity for arteriogenesis, however further studies would be required to examine this hypothesis further.

7.0 Synthesis

7.1 Summary of findings

The aim of this series of studies was to provide further guidance on revascularisation strategy in patients found to have a CTO. I have demonstrated an association between superior long-term clinical outcomes and CTO revascularisation over a conservative strategy. The alternative approach of comparing elective medical therapy with CTO PCI instead of PCI success versus failure[18,22] adds further weight to the suggestion that recanalisation of a CTO is of prognostic benefit on a population level, pending the results of randomised trials.

In addition, I have demonstrated the haemodynamic consequences of CTO revascularisation and the implications for optimal revascularisation strategy. Recanalisation of a CTO results in haemodynamic changes in vessels donating collaterals to the occluded segment. We are limited in the extent to which that can be predicted, but it would seem that it is only in lesions with an FFR close to the established treatment threshold of 0.80[56,58] that best revascularisation strategy might be altered. The behaviour of the recently recanalised chronically occluded segment can present a challenge to achieve an optimal result post PCI[123,124]. If physiological lesion assessment is employed for optimisation, I have shown that measurements taken under baseline conditions such as the iFR[167] are likely to result in over-estimation of lesion severity given the (most likely transient) conditions of reduced microvascular resistance in the immediate aftermath of CTO recanalisation.

Finally, I have explored four potential biomarkers of collateralisation and endothelial function in patients with a CTO. Identification of biomarkers associated with functional extent of collateralisation and subsequent pharmacological or mechanical stimulation of arteriogenesis is an attractive goal with the potential for real clinical utility, particularly in the setting of refractory angina unsuitable for revascularisation, or even with the more modest ambition of enlarging collaterals sufficiently to facilitate a retrograde attempt at PCI to a CTO. Few studies have investigated the change in biomarker levels at prior to recanalisation and at follow-up, and

the demonstrated finding of no change suggests that for the tested biomarkers at least, any associations between functional collateralisation and levels are likely to represent an individual's ability to form collaterals rather than signifying the level of arteriogenic activity.

7.2 An over-arching theme

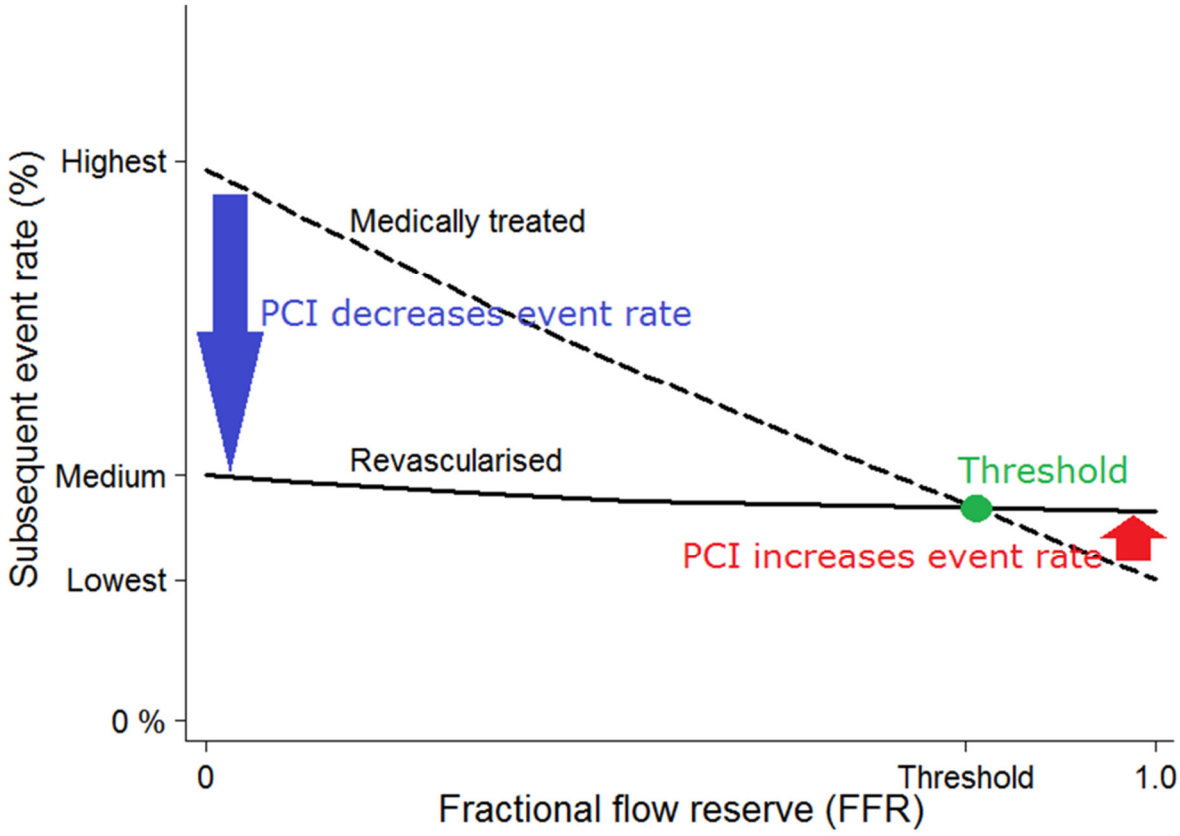

Figure 7.1 Adapted from Johnson et al[188], conceptual plot of risk of events in relation to baseline FFR as a measure of extent of ischaemia for medically treated (dashed line) and revascularised (solid line) individuals.

A large part of this thesis relates to the principle illustrated in **figure 7.1** and proposed by Johnson et al[188]. If the presence of myocardial ischaemia is related to adverse outcomes and it is incrementally associated with a worse prognosis[39,42,43], then as extent of ischaemia increases (demonstrated in the figure by a lower FFR) the benefit of revascularisation should also increase. However, there is a threshold at which, on a population level at least, the risks associated with revascularisation (by PCI or CABG) outweigh any benefits. At present the practiced threshold for FFR

is 0.80, however this was not derived for this purpose. It was the selected threshold for treatment in the FAME trial[56], above the 'grey-zone' (0.75-0.80) where the threshold value for FFR to predict ischaemia by non-invasive testing lies[55,189]. The actual optimal treatment threshold may differ from this and may not be the same between individuals. Johnson et al performed a study level meta-regression and individual patient data Cox-regression analysis using data from studies reporting outcomes amongst patients in whom FFR was measured and looked at groups treated by revascularisation and those treated medically. The regression lines they demonstrated were very similar to the conceptual plot in **figure 7.1**[188]. Hachamovich et al demonstrated similar crossing regression lines in an observational study comparing extent of myocardial ischaemia measured by SPECT, where below a threshold of ischaemic extent, medical therapy appears to be associated with superior clinical outcomes compared with revascularisation[42]; the reverse being true in patients with a greater extent of ischaemia.

7.3 The benefit of reduced ischaemia applied to CTOs

The haemodynamic studies conducted as part of this thesis, consistent with previous studies[45,92,121], have shown that in a population of patients with viable myocardial territory distal to a CTO inducible myocardial ischaemia is universal. In the 31 patients in whom FFR_{coll} was measured, mean FFR_{coll} was 0.37±0.12, with a range of 0.18 to 0.61. The mean FFR of revascularised stenoses (which were largely non-occlusive) in the FAME study was 0.60±0.14. If the extent of reduction of ischaemia is what drives any benefit in survival between revascularisation and medical therapy, then one would expect revascularisation of CTOs to have a greater benefit than revascularisation of non-occlusive disease.

I have demonstrated an association between reduced death and MI at follow-up in patients treated by CTO PCI compared with medical therapy, and a larger associated benefit in the sub-group in whom the CTO was revascularised in all patients in one group and in no patients in the other.

This supports the concept that a reduction in ischaemia is associated with prognostic benefit.

Whether it is the ischaemia necessarily that is associated with adverse outcomes or that the presence of ischaemia tends to co-exist with a more vulnerable coronary circulation to de novo coronary occlusion is difficult (if not impossible) to separate. The presence of a concomitant CTO is associated with adverse outcomes in STEMI[37,38], indeed increased angiographic complexity is associated with adverse outcomes at follow-up whether a CTO is present or not[190]. Complete revascularisation is associated with improved long-term outcomes[7,191]. These findings could be explained by either a reduction in ischaemia or vulnerability to subsequent infarction. The International Study of Comparative Health Effectiveness with Medical and Invasive Approaches (ISCHEMIA trial) Clinicaltrials.gov identifier: NCT01471522 should shed further light on whether reducing myocardial ischaemia carries a prognostic benefit by randomising patients with significant myocardial ischaemia to medical therapy or revascularisation, but it seems unlikely that it will demonstrate whether it is ischaemia itself that is harmful.

7.3.1 Similar comparisons in the literature

Although much of the literature investigating the relationship between CTO recanalisation and clinical outcomes compares successful versus failed CTO PCI[18,22], recently, in addition to the work included in this thesis other studies using an alternative approach have been published.

Jang et al compared patients with a CTO and Rentrop grade 3 collaterals in whom their CTO was revascularised by CABG or PCI (n=502) with those in whom the CTO was treated medically (n=236). They found a significant associated reduction in cardiac death in the CTO revascularisation group by multi-variate Cox regression analysis, a very similar finding was reported on comparison of 215 propensity matched pairs[179]. Kim et al compared revascularisation strategy in 2024 patients

with ≥2 CTOs and found medical therapy to be associated with adverse outcomes[192].

7.4 Avoiding unnecessary revascularisation: do no harm

The long-term clinical outcomes reported in this thesis go some way to supporting the concept that the revascularisation of ischaemic myocardium improves prognosis, and the shifting of an individual from the less favourable medical therapy curve on the left of **figure 7.1** to the more favourable revascularisation curve. The haemodynamic studies presented in chapters 4 and 5 are based upon one of the major principles of the concept illustrated in **figure 7.1**. That principle is that the benefit of revascularisation of lesions which are not haemodynamically flow limiting is outweighed by the risk.

In chapter 4 I have demonstrated the change in collateral donor FFR associated with PCI of a CTO and that in patients with a collateral donor vessel FFR close to the established treatment threshold of ≤0.80, some consideration of whether that vessel is likely to require treatment should be given. In chapter 5 I have described the conditions of microvascular resistance in the target vessel shortly after CTO PCI and have demonstrated that iFR measurement would be erroneously low (ischaemic) which could lead to unnecessary further treatment if used to guide further PCI.

Unnecessary revascularisation is not benign. Both PCI and CABG are associated with peri-procedural risk. PCI also carries with it a long-term risk of stent thrombosis[193], which can be catastrophic; as well as in-stent restenosis requiring revascularisation[194]. Bypass grafts placed on non-flow limiting lesions have a higher occlusion rate than when placed haemodynamically significant lesions[81]. The low shear conditions generated by the competitive 'see-saw' flow prior to graft occlusion on a grafted, unobstructed native vessel may even be detrimental. This is an area which requires further investigation, but shear stress is likely to be reduced under these conditions, which could in turn result in the more rapid progression

of native vessel disease[195,196] that we often see on grafted coronary arteries.

The FAME trial demonstrated a clinical outcome benefit for the use of FFR to guide PCI rather than angiography alone[56]. It might be inferred that this was due to minimising the treatment of stenoses that were not flow limiting (FFR ≤0.8) in the FFR guided group, which is supported by a mean number of stents in the FFR guided group of 1.9, compared with 2.7 in the angiographically guided group ($p<0.001$). If a stenosis is not severe enough to cause ischaemia, then treating it would be of no benefit, but would still expose the patient to a small risk. Whilst patients with a CTO were excluded from the FAME trial, it seems incongruous that the same principle of potential harm associated with treating non flow-limiting stenoses would not also apply to a population of patients with a concomitant CTO.

7.5 Biomarkers of collateralisation

The extent of functional collateralisation in patients with coronary disease is related to prognosis[131] and ultimately, revascularisation is not possible in all patients. Arteriogenesis is a complex process and the idea that a single biomarker would represent activity or that a single agent could be supplemented to augment collateralisation is a little fanciful. Thus far, pharmacological attempts to stimulate collateral growth have met with limited success[141], and the prospect of any clinical application in the near future seems slim. Exercise has been shown to stimulate invasively measured collateral function with a 'dose-response' relationship[197], however a translation into any clinical benefit is yet to be demonstrated.

The findings presented in this thesis with respect to biomarkers can only be considered hypothesis generating. I have demonstrated a relationship between estimated retrograde collateral flow and three biomarkers that have been shown to be associated with endothelial health. If the endothelium were able to be modified, or made more 'healthy', this

could represent an alternative avenue worth exploring with a view to therapeutically improving collateralisation.

7.6 Conclusions

The revascularisation of CTOs is associated with improved long-term clinical outcomes. Pending the results of randomised trials and on the basis of the findings reported in this thesis and in the literature[18,22,179,192], patients with a CTO should be considered for revascularisation on the grounds of prognosis. Revascularisation of a CTO is associated with specific haemodynamic changes, which if not considered could result in unnecessary treatment of either the target vessel or collateral donor vessel.

7.7 Further work

The haemodynamic work making up chapters 4.0 and 5.0 was carried out within two hours following CTO recanalisation. Changes to the previously occluded segment do occur in the weeks following CTO PCI[123], with vasodilatation and the return of normal vasomotion. Further work examining changes in FFR in the (previous) collateral donor vessel at an interval of several weeks after CTO recanalisation would clarify whether there is any further change of clinical significance over and above what I have demonstrated early after PCI. Similarly, a study at several time intervals after CTO recanalisation would clarify the duration of increased microvascular dilatation under baseline conditions in the previously occluded segment and the time-scale for the return of 'normal' coronary physiology in that segment.

With respect to further investigation of a clinical outcome benefit of CTO PCI over medical therapy, this can only be answered by a randomised trial. Two trials are currently underway to this end. Whether a mortality benefit is demonstrated or not, one might expect these trials to demonstrate a reduction in angina in the CTO PCI group, which would support the current major indication for CTO PCI; that is for symptomatic relief of angina.

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Appendices

Appendix I

Hull and East Yorkshire Hospitals NHS Trust

Participant Information Sheet for the Fractional Flow Reserve and Recanalisation of CTOs Study Version 3. 27/09/2012.

A research project investigating the effect of re-opening a blocked vessel that supplies the heart on the flow down the other arteries that supply the heart.

Study Title:

Impact of Recanalization of Chronic Total Coronary Occlusions on Donor Artery Fractional Flow Reserve and the Association with Collateral Function

You are being invited to take part in a research study. Before you decide whether to participate or not it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

A coronary chronic total coronary occlusion (CTO) is long-standing blockage of a heart artery. When an artery is blocked, small branches called 'collaterals' may form from one of the two other heart arteries and provide blood and oxygen to the heart muscle that was originally supplied by the blocked vessel. Often, more than one of the three blood vessels supplying the heart is diseased (meaning it is narrowed or blocked). To evaluate the significance of narrowings in those arteries, it is commonplace to pass a specialised 'pressure wire' through the blockage. This wire measures pressure and can tell us whether narrowings are severe enough to warrant treatment with balloons and stents or bypass surgery. Using these specialised wires to measure the importance of a narrowing in an artery, lots of factors affect the outcome, some related to the narrowing (such as severity and length), but also the amount of heart muscle that it supplies. There are well established 'cut-offs' in our measurements which guide us as to whether treatment with balloons and stents or bypass surgery is advantageous for the patient. If a vessel gives off 'collateral branches' to heart muscle which was originally supplied by another vessel, it supplies more heart muscle than if it does not. If a blocked vessel is reopened with a stent, these collateral branches close. The resultant reduction in the amount of heart muscle that the artery supplies will potentially change the outcome of our measures using the specialised pressure wire to assess the narrowing; the extent of change is presently unknown but might be sufficient to alter the need for treatment with balloons and stents or bypass surgery. In this study, we will measure the size of the change in the importance of narrowings in a patient's other heart arteries assessed by a specialist 'pressure wire' before and after a blocked vessel is treated with angioplasty.

We are also looking for potential markers, identified by blood tests that might help us assess how well these small collateral branches work and also if new ones are developing.

Why have I been chosen?

We would like to study patients who have a vessel which has been blocked for some time, and are due to undergo angioplasty and stenting to that vessel.

Participant Information Sheet for the Fractional Flow Reserve and Recanalisation of CTOs Study
Version 3. 27/09/2012.

Do I have to take part?

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

What does it involve?

The study will take place at Castle Hill Hospital. It will involve 3 visits.

Visit 1: This will happen at the time of pre-assessment for your angioplasty. It will involve a meeting with a research doctor. The doctor will go over what the study will involve with you. If you are happy to go ahead with the study you will be asked to sign a consent form. You will be given a signed copy to keep.

Visit 2: This will take place within 6 weeks and will last between one and three hours. Those willing to take part will have a date for angioplasty (opening the blocked vessel with balloons and metal scaffolds called 'stents') within approximately 6 weeks of their angiogram. The experience of the angioplasty procedure would be very close to the experience, if you were not involved in the study.

The doctor performing the procedure would place an arterial sheath (tube) in either your femoral artery (at the top of your leg) or radial artery (in your wrist). Two tubes would be inserted to allow dye injection via another artery as the one being treated at the same time. This is common practice during angioplasty of blocked vessels. You would also have a tube placed in the vein at the top of your leg. This tube in the vein would be used to infuse the drug Adenosine when required and also a catheter (long tube) would be inserted through it into the heart to measure the pressure, which is required for some of our calculations. Although it is common practice to insert a tube into the vein at the top of the leg during angioplasty, we do not always need to do it. For the purposes of this study however, it is necessary and therefore may be additional to the treatment you would receive if you did not take part.

Initially, prior to attempting angioplasty of the blocked vessel, a specialised wire would be passed sequentially into both vessels not being treated by angioplasty and after the injection of the drug Nitroglycerin into the vessel in question (which is frequently done during angioplasty) we would take measurements during infusion of a drug called Adenosine. We would then commence the angioplasty procedure. As for any angioplasty of a blocked vessel, the blockage would be crossed with a specialised wire. Once crossed, a small tube would be passed over the wire into the part of the vessel beyond the blockage, as is standard practice during the angioplasty of blocked vessels. A specialised wire would be then passed though the tube and measurements taken. The specialised wire has the capacity to measure pressure and speed of blood flow in the artery. At this point measures of pressure and flow would be taken both at rest and during drug induced stress which would be used for calculations after the procedure. The drug used to stress the heart is called Adenosine, it would be infused via the tube in the vein at the top of the leg. Adenosine's effect lasts

for two to three minutes, it is widely used in the cardiac catheterisation laboratory in routine practice when pressure sensor tipped wires are used to assess the importance of the narrowing of an artery. Once the measures for calculation of collateral function have been taken, the angioplasty will continue as it would for any other patient not included in the study. Once the artery is open and the stents are placed, the specialised wire will be positioned in the same position it was in at the beginning of the procedure for one final recording (flow measurement). The angioplasty procedure will be completed as for any other. The measurements in the vessels not being treated will be taken again sequentially. Data collection will then be complete and the procedure will be finished as for any other. We will aim to keep you in hospital overnight and send you home the following day; this would also be the case if you opted not to take part.

Visit 3: Between 6 and 8 weeks after your angioplasty, you will come back for your routine follow-up appointment after your angioplasty. This will predominantly be a normal clinic visit to see how you are getting on, but we will also take a blood test at the time

If you choose to participate in the study, the additional procedures you would undergo would be:

-You would have a tube placed in the vein at the top of your leg, which may, or may not be necessary if you chose not to participate.

-You would receive the drug Adenosine, which is safe and commonly used in the cardiac catheterisation laboratory, but would not be necessary if you chose not to take part.

-We would take some blood samples during the procedure. They would not involve any additional needles.

-As we would need to position the specialised wires to take measurements, you would receive a very small additional amount of X-ray radiation and the procedure would take slightly longer. We would expect that the additional measurements would add approximately 30-40 minutes to the procedure.

At your routine follow up appointment 6-8 weeks after the procedure, we would take a further blood test.

What do I have to do?

If you do decide to participate in the study, you should be able to do all the things you would normally do.

You should continue to take your regular medication.

You should avoid caffeine for 24 hours prior to the procedure.

Participant Information Sheet for the Fractional Flow Reserve and Recanalisation of CTOs Study
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Which drugs will be used?

Adenosine

Adenosine is a naturally occurring substance. It causes relaxation of the blood vessels in the heart, causing the blood vessels to dilate.

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

Adenosine may cause flushing or headache. It can slow the heart rate, and may also cause some wheeze. It may give some patients chest tightness. Adenosine is very short-acting, therefore any side effects will wear off within 2 or 3 minutes.

If you suffer these or any other symptoms you should report them. If you are affected by side effects, you will not receive the planned second dose of adenosine. If you become in any way concerned please contact the lead investigator (see below).

What are the possible disadvantages and risks of taking part?

The risk of the angioplasty procedure itself would be no greater than if you were not taking part in the research. As you will have been informed, there is a small risk for anyone undergoing angioplasty of a blocked vessel.

This procedure is carried out in a very controlled and well equipped environment, ready to deal with any potential untoward circumstances.

The main disadvantages of taking part are:

-Your angioplasty will take slightly longer than it would do otherwise, by approximately 30-40 minutes.

-You will receive a small additional amount of X-ray radiation

What are the possible benefits of taking part?

Although you are unlikely to benefit directly from the study, the information we get may help us to improve treatment for patients like you in the future.

What if something goes wrong?

We do not expect you to suffer any health problems as a result of taking part in this study.

If you have any complaint about the way that you have been treated you should feel free to discuss this with any member of the research team.

You could also discuss any concerns with the Patient Advice and Liason Service (PALS). They can be contacted in the following ways:

Participant Information Sheet for the Fractional Flow Reserve and Recanalisation of CTOs Study
Version 3. 27/09/2012.

Telephone: 01482 623065
Fax: 01482 622252
E-mail: pals.hey@hey.nhs.uk

If you wished to formally complain about your treatment you could contact the Complaints Department directly at the address below:

Head of Complaints
Complaints Department
5th Floor
Alderson House
Hull Royal Infirmary
Anlaby Road
Hull
HU3 2JZ
Tel: 01482 605284
Email: pals.hey@hey.nhs.uk

Will I be paid for participating*?*

No.

Will my taking part in this study be kept confidential?

All the information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it.

However, we are required to notify your GP about your participation in the study, your permission will be sought with respect to this.

What will happen to the results of the research study?

The results will be published in a scientific journal. They are likely to be published in 18 to 24 months' time. A copy of the published results could be obtained from the Cardiology Department at Castle Hill Hospital. You will not be identified in any report or publication.

Who is organising and funding the research?

The research is being organised by the Department of Cardiology at Castle Hill Hospital, part of the Hull and East Yorkshire Hospitals NHS Trust.

The Hull and East Riding Cardiac Trust has funded the research.

Contact for Further Information

Please feel free to contact the investigators for any further information.

Participant Information Sheet for the Fractional Flow Reserve and Recanalisation of CTOs Study Version 3. 27/09/2012.

Principal Investigator:
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Thank you for reading this information sheet and considering participating in the study.

You will be given a copy of the information sheet and a signed consent form to keep.

Participant Information Sheet for the Fractional Flow Reserve and Recanalisation of CTOs Study Version 3. 27/09/2012.

Appendix II

Hull and East Yorkshire Hospitals NHS Trust

Version 3. 27/09/2012

Consent Form for the Fractional Flow Reserve and Recanalisation of CTOs Study

A research project investigating the effect of re-opening a blocked vessel that supplies the heart on the flow down the other arteries that supply the heart

Participant ID:	Initials:
Participant's date of birth:	Principal Investigator: Dr Andrew Ladwiniec

	Please initial after each question
1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.	
2. I understand that participation is voluntary and that I am free to withdraw at any time without my care or any legal rights being affected.	
3. I agree to give blood samples for research and understand that on each occasion, my permission will be asked.	
4. I give permission to store my blood samples and to use them for medical research that has research ethics committee approval.	
5. I understand that my medical record may be looked at by the researcher to collect data for the purposes of the study.	
6. I understand that relevant sections of data collected during the study may be looked at by individuals from Hull York Medical School, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	
7. I understand that if I withdraw from the above study, the data collected from me will not be used in analysing the results of the study. I understand that my identity will remain anonymous.	
8. I agree to allow any information or results arising from this study to be used for healthcare and/or medical research purposes. I understand that my identity will remain anonymous.	
9. I agree that my GP and other clinicians treating me at the practice will be notified of my participation in the study.	
10. I agree to take part in the study.	

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

Please take 3 copies. 1 for participants to keep, 1 for the research file and 1 for the hospital notes.